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RESEARCH ARTICLE

Phenotypic plasticity of *Platanus acerifolia* (Platanaceae): morphological and anatomical trait variations in response to different pollution levels in Rome

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Abstract

Platanus acerifolia (Aiton) Willd was generated spontaneously around the beginning of the 1600s in Spain between *Platanus orientalis* L. and *Platanus occidentalis* L. The study was carried out on *P. acerifolia* trees in different sites in Rome in the period April-July 2019. Trees comparable for size were selected in A sites (historical parks), B sites (high traffic density streets) and in C sites (high traffic density avenues along the Tevere River characterized by a large water availability). At the morphological level, leaf area (LA) was significantly higher in A and C sites and leaf tissue density (LTD) in C sites. At anatomical level, the total leaf thickness (LT), the palisade parenchyma thickness (Pt), the spongy parenchyma thickness (St) and the abaxial epidermis thickness (Abet) were significantly higher in A sites, while the adaxial cuticle thickness (Adct) and the adaxial epidermis thickness (Adet) in B sites. The ratio between palisade and spongy parenchyma thickness (P/S) was significantly higher in C sites. The plasticity index (PI, 0.42) was calculated on all the considered leaf traits. Among the morphological traits, LTD had the highest plasticity (0.49) and among the anatomical traits, trichomes density (TD) and Adet (0.74 and 0.54, respectively). Overall, the results highlight the large adaptability of *P. acerifolia* to grow in different sites in Rome through several changes in leaf morphological and anatomical traits.

Keywords: *Platanus acerifolia*, plasticity index, urban area, leaf morphology, leaf anatomy

Introduction

Platanus acerifolia (Aiton) Willd (syn. *Platanus* × *hispanica* Mill. ex Münchh.) was generated spontaneously around the beginning of the 1600s in Spain between *Platanus orientalis* L. native to South-Eastern Europe-Asia Minor, and *Platanus occidentalis* L. native to North America. *P. acerifolia* has wide adaptability to various types of soils even those strongly alkaline (Pignatti, 1982) and it is particularly resistant to stress factors, including pollution (Pourkhabbaz et al., 2010 Gratani & Varone, 2007). It can adapt even at low temperatures and has a higher growth rate than its ancestors (Cennamo & Cafasso, 2002). For these characteristics, starting from the middle of the 1600s, *P. acerifolia* was imported in England and widely used in gardens, along with irrigation canals and roadsides with the name of the "London plane" (Cennamo & Cafasso, 2002) and then widely distributed throughout Europe. In Italy, this species has been largely used to adorn squares and avenues (Pignatti, 2017) since the beginning of the 20th century (Gratani & Varone 2007). *P. acerifolia* has high longevity (about 500 years) and it can reach 40 m in height. The stem has a monopodial growth, tapered in the apical part, main branches mighty, more or less twisted, with an angle of insertion to the stem of 45°. The crown has an expanded globular-oval shape. The root system is characterized by the main root deepening into the soil with numerous branches and secondary roots expanded horizontally and more superficially; the deciduous leaves have a palmate lamina with 3-7 obtuse lobes and a central lobe with 1-3 obtuse teeth on the edge (Pignatti, 2017). A discriminating character of *P. acerifolia* concerning *P. orientalis* is the central lobe of the leaf having the same ratio between length and width, with 1-3 obtuse or full margin teeth (Pignatti, 2017). *P. acerifolia* has candelabra form trichomes restricted to major veins on the abaxial surface (Carpenter et al., 2005). Stomata are confined to small areolar regions on the abaxial leaf surface (Carpenter et al., 2005). The flowers form roundish and pendulous inflorescences with a diameter of 2-3 cm, often located on the same branch, anteriorly the female inflorescences of a reddish color and posteriorly the male yellowish-green inflorescences; flowering period is from April to June (Pignatti, 2017). The fruits are conical achenes, each with a single seed and a tuft of hair that facilitates its anemophilous dispersion. They are gathered in globose infructescence, 2.5-4 cm in diameter (Portal to the Flora of Italy, 2019). Since the 1970s, a serious fungal disease, the colorful cancer of the plane tree, has compromised the *P. acerifolia* heritage of Italy.

In such a context, the main objective of this research was to analyze anatomical and morphological leaf trait variations of *P. acerifolia* in response to different environmental conditions in Rome. Some reports highlight the importance of phenotypic plasticity in local adaptation (Ramírez-Valiente et al., 2010; Bonito et al., 2011; Pesoli et al., 2003; Gratani, 2014). Phenotypic plasticity is the change in the phenotypic expression of a genotype in response to environmental factors (Bradshaw, 1965) and is one of the major means by which plants can cope with environmental factors variability (Gratani, 2014). Since phenotypic plasticity influences environmental tolerance, different plastic responses may contribute to differences in the range of environments that a species inhabit (Ackerly et al., 2000). In particular, the environment can induce changes at morphological, anatomical and physiological level, and such changes may be crucial to surviving in variable conditions (Schlichting & Levin, 1986; Gratani, 1996; Ghalambor et al., 2007; Zunzunegui et al., 2011, Gratani et al., 2018) and to outcompete the existing vegetation (Murray et al., 2002). Adaptation to global change could require the evolution of different traits that may be constrained by the correlation between them (Etterson & Shaw, 2001). Thus, it is necessary to identify those traits in which plasticity is likely to be a determinant in plant response to environmental stress factor variations including climate change, thus contributing to predict species distribution changes and shifts (Nicotra et al., 2010). We analyzed the phenotypic plasticity of *P. acerifolia* to identify those traits involved in its adaptive strategy to different environmental conditions.

Materials and Methods

Study area and climate

The study was carried out on *P. acerifolia* trees largely distributed in Rome (41°53'N12°29'E) in the period April-July 2019. Trees comparable for size were selected in different sites (tree plants for each site) (Fig. 1).

Sites A (historical parks):

A1=Doria Pamphilj Historical Park (41°53'N; 12°27'E) extending over 184 ha in the southwest of the city.

Figure 1. Map of the considered sites: A sites (A1=Doria Pamphilj Historical Park, A2=Borghese Historical Park, A3=Caffarella Valley Park); B sites (B1=Passeggiata del Gianicolo Street, B2=Castro Pretorio Street, B3=Policlinico Street, B4=Quinto Cecilio Square, B5=Oppio Hill, B6=Celio Hill); C sites (C1=Lungotevere Aventino, C2=Lungotevere Melini, C3=Lungotevere Testaccio, C4=Galvani Street). From Google Earth.

A2=Borghese Historical Park (41°55'N; 12°29'E) extending over 80 ha in the city center.

A3=Caffarella Valley Park (41°50'N; 12°33'E) extending over 190 ha inside the Appia Antica Archeological Park, in the southeast of the city.

Sites B (high traffic density streets):

B1=Passeggiata del Gianicolo Street (41°53'N; 12°27'E) extending from Trastevere district to Garibaldi Square, running alongside the Bambino Gesu' Hospital and characterized by an all-day high traffic density.

B2=Castro Pretorio Street (41°54'N; 12°30'E) positioned on the side of the Termini Station, delimited by buildings on both sides and characterized by an all-day high traffic density.

B3=Policlinico Street (41°54'N; 12°30'E) positioned in front of the Policlinico Hospital and characterized by an all-day high traffic density (58 cars min^{-1}).

B4=Quinto Cecilio Square (41°52'N; 12°27'E) a residential area characterized by a low traffic density.

B5=Oppio Hill (41°53'N; 12°29'E) extending for 11 ha in the city center and characterized by an all-day high traffic density.

B6=Celio Hill (41°53'N; 12° 29'E) extending over 2 ha in the city center and characterized by an all-day high traffic density.

Sites C (high traffic density streets along the Tevere River, characterized by a large water availability):

C1=Lungotevere Aventino (41°53'N; 12°28'E) characterized by an all-day high traffic density.

C2=Lungotevere Melini (41°53'N; 12°28'E) characterized by an all-day high traffic density (72 cars min^{-1}).

C3=Lungotevere Testaccio (41°52'N; 12°28'E) characterized by an all-day high traffic density.

C4=Galvani Street (41°52'N; 12°28'E) delimited by buildings on both sides and characterized by an all-day high traffic density.

Rome is under a Mediterranean type of climate. The average total year rainfall is 841 mm, most of it distributed in autumn and winter. The average maximum air temperature of the hottest months (July and August) is 31.9 ± 0.4°C and the average minimum air temperature of the coldest month (January) is 4.8 ± 1.0°C. The mean yearly air temperature is 16.7 ± 6.6°C (data provided by the Lazio Regional Agency for Development and Agricultural Innovation, Meteorological Station of Rome, Lanciani Street, for the period 2008-2018).

Leaf morphology

Fully expanded sun leaves were collected at the beginning of July 2019 from the external portion of the tree crown (10 leaves per each tree and site). Leaf samples were stored in polyethylene bags and transferred immediately to the laboratory. The following morphological parameters were measured: leaf area excluding the petiole (LA, cm^2), obtained by the Image Analysis System (Delta-T Devices, UK) and leaf dry mass (DM, mg), determined to dry leaves at 80°C to constant mass. The leaf mass per unit of leaf area (LMA, mg cm^{-2}) was calculated by the ratio between DM and LA (Larcher, 2003) and leaf tissue density (LTD, mg cm^{-3}) by the ratio between DM and total leaf thickness (LT, μm) (Wright & Westoby, 2002).

Leaf anatomy

Leaf anatomy was analyzed on fully expanded sun leaves collected at the beginning of July from the external portion of the tree crown (5 leaves, respectively, per each tree and site). Leaf sections were hand-cut from fresh leaves and analyzed by light microscopy using the Image Analysis System (Axiovision, AC software). Measurements were restricted to free-vein areas, according to Chabot & Chabot (1977). The following parameters were measured: total leaf thickness (LT, μm), palisade parenchyma thickness (Pt, μm) and spongy parenchyma thickness (St, μm), thickness of the adaxial and of the abaxial epidermis (Adet and Abet, respectively, μm), thickness of the adaxial and of the abaxial cuticle (Adct and Abct, respectively, μm), parenchyma palisade cell length (Pcl, μm), parenchyma palisade cell width (PCW, μm), parenchyma spongy diameter cells (Dsc, μm), adaxial epidermis cell length (Aecl, μm), adaxial epidermis cell width (Aecw, μm). The ratio between palisade and spongy thickness (P/S) was calculated. Stomata density (SD, number mm^{-2}) and stomatal length (SL, μm) were measured from nail varnish impressions of the abaxial lamina, according to Sack et al. (2003). The number of stomata was counted on separate impressions of the leaf blade, each of 0.5 × 1.0 cm. Stomatal area index (SAI) was calculated by the product of stomatal length and SD, according to Ashton & Berlyn (1994). The density of trichomes on the abaxial epidermis (TD, number mm^{-2}) was counted.

Traffic density

Traffic density (number of cars min^{-1}) was monitored in the selected B and C sites in June 2019 (mean of the first 10 days of June) from 7:30 to 10:00 a.m. (Gratani & Varone, 2005; Gratani & Varone, 2013).

Plasticity index

The plasticity index was calculated for each of the considered morphological (PI_m) and anatomical (PI_a) leaf traits as the difference between the minimum and the maximum mean value divided by the maximum mean value per each of the measured leaf traits, according to Valladares et al. (2000) and Valladares et al. (2006). The

Plasticity index scales from 0 to 1. The plasticity index of the species (PI) was calculated by averaging the plasticity for all the considered morphological and anatomical leaf traits, according to Valladares et al. (2000).

Statistical analysis

All statistical tests were performed using a statistical software package (Statistica, Statsoft, USA). Data were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey test for multiple comparisons to detect significant differences among leaves collected in A, B, and C sites. Simple regressions analysis was carried out to analyze the relationships among the considered leaf traits. A Principal component analysis (PCA) was carried out using the leaf morphological (LA, DM, LMA, LTD) and anatomical (LT, Pt, St, Pcl, PCW, Dsc, Adct, Adet, Abct, Abet, Aecl, Aecw, Sl, P/S, SD, TD, SAI) traits.

Results and Discussion

Traffic density

The monitored traffic density in B and C sites are shown in Tab. 1. The highest traffic density was monitored at Celio Hill (B6 site=96 ± 3 cars m^{-1}) and Galvani street (C4 site=75 ± 3 cars m^{-1}).

Leaf morphology

The results of leaf morphology are shown in Tab. 2. LA was significantly higher in A and C sites (206.95 ± 14.75 cm^2, mean value) decreasing by 21.76% in B sites. DM did not show significant differences between A and C sites (1.18 ± 0.12 g, mean value) while it was significantly higher than in B sites (0.86 ± 0.05 g). LMA showed no significant differences among the considered A, B and C sites. LTD was significantly higher in C sites (74.47 ± 3.32 mg cm^{-3}, mean value) than in A and B sites (49.66 ± 6.90 mg cm^{-3}, mean value).

The regression analysis highlights the significant and positive correlation among the considered morphological leaf traits (Fig. 2). In particular, LA was significantly correlated to DM and to LTD, DM was significantly correlated to LTD.

Leaf anatomy

The results of the anatomical analysis are shown in Tab. 3. The LT, Pt, St, Pcl, PCW Dsc and SL were significantly higher in A sites (198.65 ± 5.59 µm, 81.31 ± 3.86 µm, 80.18 ± 3.47 µm; 76.20 ± 3.53 µm, 14.64 ± 0.57 µm, 30.32 ± 1.09 µm, and 39.61 ± 0.85 µm, respectively, mean value) decreasing by 13.32%, 18.42%, 16.80%, 17.64%, 15.23%, 21.60%, and 10.38%, respectively, in B and by 19.19%, 20.67%, 28.71%, 17.24%, 11.54%, 17.41%, and 6.29%, respectively, in C sites. On the contrary, Adct and Adet were significantly higher in B sites (4.77 ± 0.09 µm; 22.52 ± 0.70 µm, respectively, mean value) decreasing by 8.18% and 11.86%, respectively, in C sites and by 31.24% and 15.81%, respectively, in A sites. The P/S ratio was significantly higher in C sites (1.13 ± 0.11 µm, mean value) decreasing by 8.85% in A sites and by 12.39% in B sites.

The regression analysis highlights significant correlations among the considered leaf traits (Fig. 3). In

Table 1. Traffic density for the considered B and C sites.

	Site	Traffic density (car min^{-1})
B 1	Passeggiata del Gianicolo Street	65
B 2	Castro Pretorio Street	59
B 3	Policlinico Street	58
B 4	Quinto Cecilio Square	32
B 5	Oppio Hill	60
B 6	Celio Hill	96
C 1	Lungotevere Aventino	74
C 2	Lungotevere Melini	72
C 3	Lungotevere Testaccio	68
C 4	Galvani Street	75

Table 2. Morphological leaf traits of *P. acerifolia* in the considered A sites (A1=Caffarella Valley Park, A2=Borghese Historical Park, A3=Doria Pamphjli Historical Park); B sites (B1=Passeggiata del Gianicolo Street, B2=Castro Pretorio Street, B3=Policlinico Street, B4= Quinto Cecilio Square, B5=Oppio Hill, B6=Celio Hill); C sites (C1=Lungotevere Aventino, C2=Lungotevere Mellini, C3=Lungotevere Testaccio, C4=Galvani Street). LA (leaf area), DM (dry mass), LMA (leaf mass per unit of leaf area), LTD (leaf tissue density). Different letters indicate significant differences for the considered species among A, B and C sites (p<0.05). Mean values ± standard deviation are shown.

Site	LA (cm^2)	DM (g)	LMA (g m^{-2})	LTD (g cm^{-3})
A1	216.48 ± 8.1	1.23 ± 0.19	4.67 ± 2.09	64.8 ± 10.1
A2	190.23 ± 9.3	1.14 ± 0.2	7.13 ± 1.44	51.5 ± 5.5
A3	190.49 ± 6.2	1.09 ± 0.22	4.35 ± 0.82	46.4 ± 6.9
Mean value	199.06 ± 0.9 a	1.15 ± 0 a	5.39 ± 0.36 a	54.23 ± 1.36 a
B1	180.62 ± 35.83	0.94 ± 0.4	5.32 ± 2.15	49.1 ± 8.8
B2	174.02 ± 13.86	0.72 ± 0.1	4.19 ± 0.57	40.6 ± 2.9
B3	163.43 ± 12.36	1.08 ± 0.22	6.6 ± 1.07	42.8 ± 1.4
B4	174.47 ± 5.75	0.77 ± 0.07	4.45 ± 0.47	49.1 ± 5.6
B5	153.78 ± 21.44	0.92 ± 0.15	6.05 ± 1.15	52.3 ± 2.4
B6	153 ± 34.09	0.75 ± 0.23	5.02 ± 1.37	50.3 ± 6.4
Mean value	166.55 ± 4.73 b	0.86 ± 0.05 b	5.27 ± 0.37 a	47.36 ± 1.87 a
C1	222.78 ± 34.65	1.37 ± 0.38	6.15 ± 1.36	79.4 ± 25.9
C2	195.74 ± 43.23	1.23 ± 0.34	6.3 ± 1.02	79.4 ± 11.5
C3	209.17 ± 30.69	1.22 ± 0.14	5.91 ± 0.73	70.7 ± 2.8
C4	223.78 ± 31.98	1 ± 0.17	4.47 ± 0.44	68.4 ± 5
Mean value	212.87 ± 7.63 a	1.2 ± 0.08 a	5.71 ± 0.48 a	74.47 ± 3.32 b

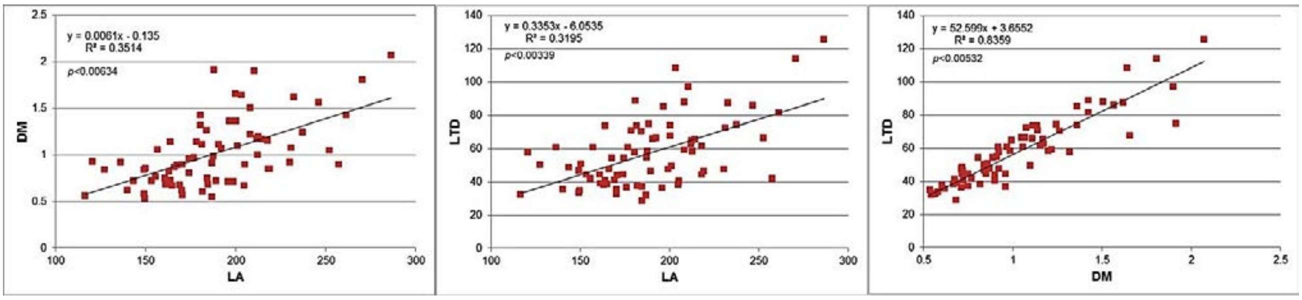

Figure 2. Regression analysis between leaf area (LA) and leaf dry mass (DM), LA and leaf tissue density (LTD), DM and LTD. The regression equations, the determination coefficient (R^2) and *the p*-value are shown (n=91).

Table 3. Anatomical leaf traits of *P. acerifolia* in the considered A sites (A1=Caffarella Valley Park, A2=Borghese Historical Park, A3=Doria Pamphjli Historical Park); B sites (B1=Passeggiata del Gianicolo Street, B2=Castro Petronio Street, B3=Policlinico Street, B4=Quinto Cecilio Square, B5=Oppio Hill, B6= Celio Hill); C sites (C1=Lungotevere Aventino, C2=Lungotevere Mellini, C3=Lungotevere Testaccio, C4= Galvani Street). LT (total leaf thickness), Pt (palisade layer thickness), St (Spongy layer thickness), Pcl (palisade cell length), PCW (palisade cell width), Dsc (diameter spongy cells), Adct (adaxial cuticle thickness), Adet (adaxial epidermis thickness), Abct (abaxial cuticle thickness), Abet (abaxial epidermis thickness, Aecl (adaxial epidermis cell length), Aecw (adaxial epidermis cell width), SL (stomatal length), P/S (ratio between palisade and spongy thickness), SD (stomatal density), TD (trichomes density), SAI (stomatal area index). Different letters indicate significant differences for the considered species between A, B and C sites (p<0,05). Mean values ± standard deviation are shown.

SITE	LT (μm)	Pt (μm)	St (μm)	Pcl (μm)	Pcw (μm)	Dsc (μm)	Adct (μm)	Adet (μm)	Abct (μm)	Abet (μm)	Aecl (μm)	Aecw (μm)	SL (μm)	P/S	SD (n mm^{-2})	TD (n mm^{-2})	SAI
A 1	186.46 ± 12.25	78.12 ± 8.26	64.37 ± 5.19	71.8 ± 6.58	15.84 ± 0.82	30.26 ± 1.50	3.96 ± 0.14	20.26 ± 1.57	3.74 ± 0.26	16.96 ± 0.69	54.72 ± 0.86	34.72 ± 1.32	35.37 ± 0.96	1.21 ± 1.58	218.14 ± 21.26	10.61 ± 3.98	7.72 ± 0.28
A 2	221.17 ± 5.62	95.76 ± 4.06	89.39 ± 4.93	89.14 ± 2.73	13.85 ± 1.03	26.43 ± 1.44	3.33 ± 0.17	23.20 ± 0.56	3.31 ± 0.14	18.49 ± 1.82	64.85 ± 3.68	35.61 ± 2.27	41.46 ± 1.26	1.07 ± 0.82	163.12 ± 23.21	14.47 ± 2.14	6.76 ± 0.36
A 3	188.31 ± 1.93	70.05 ± 2.68	86.79 ± 2.50	67.66 ± 5.54	14.23 ± 1.07	34.27 ± 1.60	2.56 ± 0.14	13.41 ± 0.27	2.92 ± 0.14	18.31 ± 0.95	61.46 ± 2.58	48.19 ± 3.62	41.99 ± 0.48	0.80 ± 1.07	143.82 ± 17.75	21.47 ± 3.74	6.04 ± 0.28
Mean value	198.65 ± 5.59 a	81.31 ± 3.86 a	80.18 ± 3.47 a	76.20 ± 3.53 a	14.64 ± 0.57 a	30.32 ± 1.09 a	3.28 ± 0.15 a	18.96 ± 1.06 a	3.32 ± 0.12 a	17.92 ± 0.70 a	60.34 ± 1.72 a	39.51 ± 1.97 a	39.61 ± 0.85 a	1.03 ± 0.22 a	175.03 ± 1.59 a	15.52 ± 0.57 a	6.84 ± 0.31 a
B 1	178.92 ± 2.82	75.25 ± 1.47	65.74 ± 1.27	64.57 ± 1.29	11.72 ± 0.43	23.76 ± 0.78	5.12 ± 0.10	19.27 ± 0.42	3.51 ± 0.10	14.95 ± 0.36	51.54 ± 1.24	36.03 ± 1.29	36.28 ± 0.65	1.14 ± 1.15	186.93 ± 25.52	12.78 ± 5.50	6.63 ± 0.38
B 2	173.86 ± 3.96	61.91 ± 2.42	65.96 ± 2.22	58.04 ± 2.29	12.69 ± 0.33	21.71 ± 1.05	4.29 ± 0.27	29.16 ± 0.77	2.43 ± 0.10	17.56 ± 0.92	66.44 ± 3.01	36.63 ± 1.71	35.63 ± 1.06	0.93 ± 1.08	111.96 ± 19.44	6.27 ± 1.60	4.53 ± 0.18
B 3	194.80 ± 4.21	73.72 ± 3.34	82.14 ± 2.30	73.18 ± 3.54	12.91 ± 0.81	27.03 ± 1.72	4.77 ± 0.33	24.7 ± 1.44	3.03 ± 0.14	16.00 ± 0.50	64.52 ± 1.94	41.89 ± 1.39	33.94 ± 1.57	0.89 ± 1.45	169.88 ± 30.82	17.11 ± 2.27	5.38 ± 0.42
B 4	156.37 ± 2.00	61.60 ± 1.28	59.91 ± 1.98	60.45 ± 1.10	10.92 ± 0.20	21.31 ± 0.74	4.64 ± 0.13	21.16 ± 0.77	3.24 ± 0.11	15.79 ± 0.54	60.56 ± 2.05	38.43 ± 1.45	35.36 ± 0.48	1.02 ± 0.65	148.64 ± 10.05	19.30 ± 1.98	5.53 ± 0.26
B 5	163.31 ± 2.32	58.67 ± 1.16	58.19 ± 1.11	54.78 ± 1.13	12.9 ± 0.32	23.29 ± 0.63	4.82 ± 0.12	23.78 ± 0.40	2.90 ± 0.06	22.43 ± 0.61	67.02 ± 2.16	36.32 ± 0.63	36.78 ± 0.88	1.00 ± 1.04	117.76 ± 26.12	13.75 ± 4.61	4.33 ± 0.36
B 6	165.87 ± 3.28	66.84 ± 1.52	68.36 ± 1.68	65.56 ± 1.29	13.34 ± 0.69	25.55 ± 1.09	4.95 ± 0.25	17.08 ± 1.11	3.96 ± 0.17	16.08 ± 0.80	54.71 ± 1.78	34.86 ± 3.66	35.05 ± 1.59	0.97 ± 0.90	190.15 ± 21.86	20.02 ± 6.85	6.26 ± 0.44
Mean value	172.19 ± 2.29 b**	66.33 ± 1.24 b**	66.71 ± 1.39 b**	62.76 ± 1.19 b**	12.41 ± 0.23 b**	23.77 ± 0.51 b**	4.77 ± 0.09 b**	22.52 ± 0.70 b**	3.18 ± 0.08 a	17.13 ± 0.46 a	60.8 ± 1.22 a	37.36 ± 0.82 a	35.5 ± 0.45 b**	0.99 ± 0.03 a	154.22 ± 13.84 a	14.87 ± 2.08 a	5.37 ± 0.35 a
C 1	151.96 ± 2.84	57.38 ± 1.44	59.83 ± 1.89	58.25 ± 1.64	12.82 ± 0.81	24.61 ± 1.24	4.72 ± 0.21	18.57 ± 0.82	3.75 ± 0.18	14.46 ± 0.63	50.47 ± 2.80	29.68 ± 1.31	35.18 ± 1.13	0.95 ± 0.76	172.77 ± 26.98	23.88 ± 4.61	6.46 ± 0.38
C 2	146.89 ± 2.90	55.95 ± 1.08	56.09 ± 3.40	57.02 ± 0.93	12.22 ± 0.27	25.78 ± 0.48	4.01 ± 0.09	14.44 ± 0.52	2.51 ± 0.08	13.25 ± 0.24	65.51 ± 3.36	37.09 ± 1.60	39.57 ± 1.22	0.99 ± 0.31	179.53 ± 13.98	15.68 ± 6.44	6.32 ± 0.19
C 3	168.18 ± 7.77	74.86 ± 4.73	53.02 ± 2.87	70.03 ± 5.98	13.83 ± 1.01	22.17 ± 2.05	4.43 ± 0.33	24.12 ± 2.27	3.22 ± 0.33	13.35 ± 1.15	65.22 ± 1.97	41.81 ± 3.02	36.36 ± 1.09	1.41 ± 1.64	206.56 ± 28.36	22.20 ± 5.63	7.51 ± 0.39
C 4	175.09 ± 4.79	69.83 ± 2.86	59.68 ± 3.32	66.94 ± 1.46	12.93 ± 1.05	27.59 ± 0.67	4.35 ± 0.38	22.25 ± 1.20	3.63 ± 0.25	16.34 ± 0.95	53.74 ± 2.72	35.22 ± 0.56	37.37 ± 1.88	1.17 ± 0.86	129.34 ± 22.21	10.85 ± 1.91	5.12 ± 0.33
Mean value	160.53 ± 3.23 c**	64.50 ± 2.07 bc**	57.16 ± 1.48 c**	63.06 ± 1.85 b**	12.95 ± 0.41 b**	25.04 ± 0.70 b**	4.38 ± 0.14 c*	19.85 ± 0.96 a	3.28 ± 0.14 a	14.35 ± 0.45 b**	58.74 ± 1.83 a	35.95 ± 1.20 a	37.12 ± 0.71 b**	1.13 ± 0.11 b*	172.05 ± 18.47 a	18.15 ± 3.47 a	6.35 ± 0.48 a

particular, ST was significantly correlated to LT and PT, Pcl to PT and ST; Dsc to PCW and ST.

Principal component analysis (PCA)

The PCA (Fig. 4) returned two axes of variation with a percentage of explained variance of 26.62% and 24.42% for PC1 and PC2, respectively. In particular, LA, DM, LMA, LTD, P/S, SD, TD, Abct, and SAI were positively related to PC1 and negatively related to PC2. Moreover, LT, PT, ST, Pcl, PCW, Dsc, Aecw and SL were positively related to PC1 and PC2 while Adet, Abet, and Aecl were negatively related to PC1 and positively related to PC2. Adct was negatively related to PC1 and PC2. The projection of the sites in PC1-PC2 planes clustered the sites in three different groups:

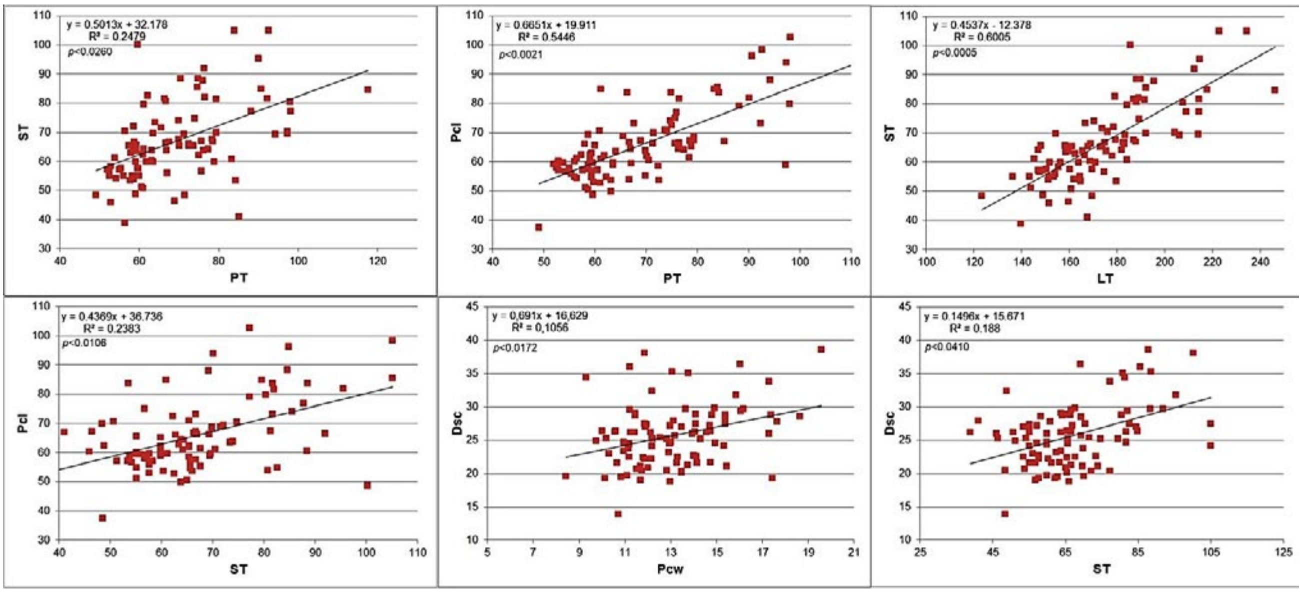

Figure 3. Regression analysis between palisade parenchyma thickness (PT) and spongy parenchyma thickness (ST); PT and palisade cell length (Pcl); total leaf thickness (LT) and ST; ST and Pcl; palisade cell width (PCW) and diameter spongy cells (Dsc); ST and Dsc. The regression equations, determination coefficient (R^2) and *p*-value are shown (n=91).

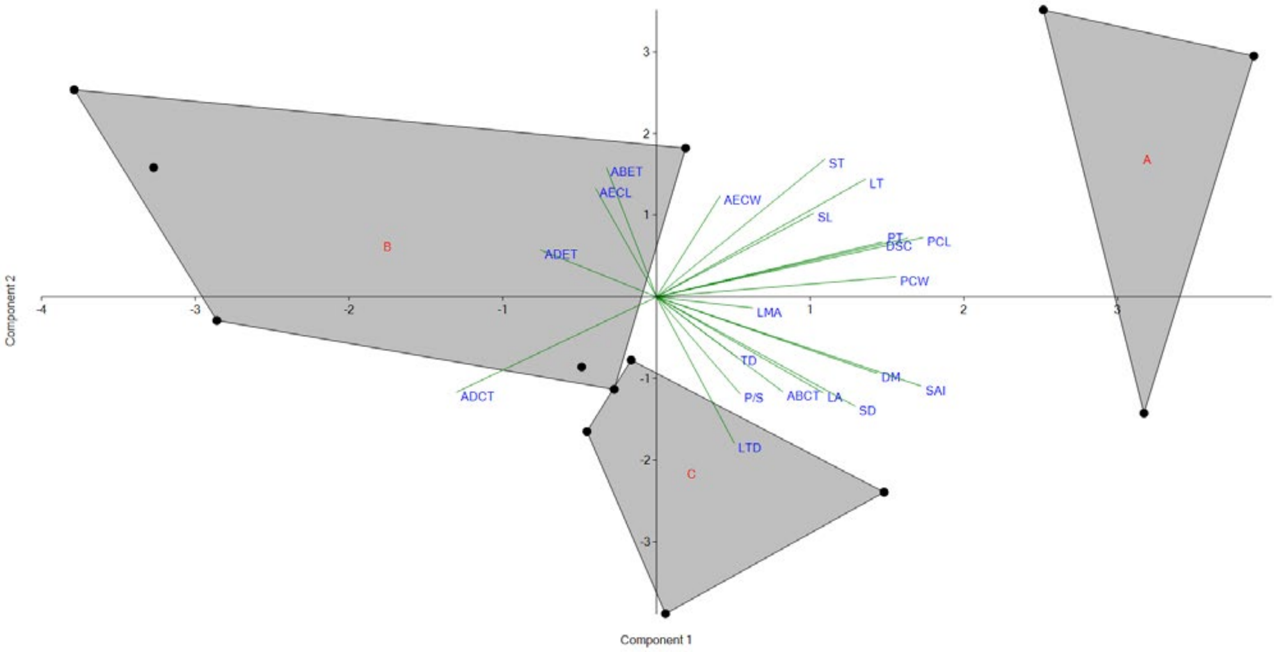

Figure 4. Principal component analysis (PCA) performed considering as input variables the morphological and anatomical leaf traits: LA (leaf area), DM (dry mass), LMA (leaf mass per unit of leaf area), LTD (leaf tissue density), LT (total leaf thickness), Pt (palisade parenchyma thickness), St (spongy parenchyma thickness), Pcl (palisade cell length), PCW (palisade cell width), Dsc (diameter spongy cells), Adct (adaxial cuticle thickness), Adet (adaxial epidermis thickness), Abct (abaxial cuticle thickness), Abet (abaxial epidermis thickness), Aecl (adaxial epidermis cell length), Aecw (adaxial epidermis cell width), SL (stomatal length), P/S (ratio between palisade and spongy thickness); SD (stomatal density), TD (trichomes density), SAI (stomata area index).

sites A in the right of the plot with positive PC1 values, and sites B in the left of the plot with PC1 negative values and PC2 positive values. This separation highlights the influence of vehicular traffic on B sites. In particular, the streets on the side of the Tevere River (C sites) were separate from A and B sites in the lower of the plot (negative PC2 values) justified by the influence of water availability.

Plasticity index

The results of the plasticity index are shown in Tab. 4. *P. acerifolia* had a plasticity index (PI) of 0.42, and among the considered traits LTD, TD and Adet had the highest plasticity (0.49, 0.74 and 0.54, respectively). The high PI compared to other species (Gratani et al., 2006; Gratani, 2014; Larcher et al., 2015; Catoni, 2015; Vasheka et al., 2019) highlight its capability to grow in different environmental conditions.

Plants are exposed to the heterogeneity of the environment where new stress factors (e.g. emission of greenhouse gases, air temperature increases, and land-

Table 4. Plasticity index for *P. acerifolia* morphological and anatomical leaf traits. Leaf area (LA), dry mass (DM), leaf mass per unit of leaf area (LMA) leaf tissue density (LTD); total leaf thickness (LT), palisade parenchyma thickness (Pt), spongy parenchyma thickness (St), palisade cell length (Pcl), palisade cell width, (PCW), diameter spongy cells (Dsc), adaxial cuticle thickness (Adct), adaxial epidermis thickness (Adet), abaxial cuticle thickness (Abct), abaxial epidermis thickness (Abet), adaxial epidermis cell length (Aecl), adaxial epidermis cell width (Aecw), stomatal length (SL), ratio between palisade and spongy thickness (P/S), stomatal density (SD), trichomes density (TD); stomata area index (SAI), mean value of leaf morphological trait plasticity ($PI_{m)}$, mean value of leaf anatomical trait plasticity (PI_a), *P. acerifolia* plasticity index (PI).

Morphological leaf traits	Plasticity index
Leaf area	0.31
Dry mass	0.47
Leaf mass per unit of leaf area	0.41
Leaf tissue density	0.49
Mean value	**0.42**
Anatomical leaf traits	
Total leaf thickness	0.34
Palisade parenchyma thickness	0.42
Spongy parenchyma thickness	0.41
Palisade cell length	0.39
Palisade cell width	0.31
Diameter spongy cells	0.38
Adaxial cuticle thickness	0.50
Adaxial epidermis thickness	0.54
Abaxial cuticle thickness	0.39
Abaxial epidermis thickness	0.41
Adaxial epidermis cell length	0.25
Adaxial epidermis cell width	0.38
Stomatal length	0.19
The ratio between palisade and spongy parenchyma thickness	0.43
Stomata density	0.49
Trichomes density	0.74
Stomata area index	0.48
Mean value	0.41
P. acerifolia plasticity index	0.42

use change) are introduced and where inter and intra-species differences may reflect resource limitation. The magnitude of human-induced changes to the environment has accelerated since the second half of the last century (Steffen et al., 2004; Doblas-Miranda et al., 2017) especially in urbanized areas (Gratani et al., 2016; Gratani et al., 2019). The interaction of these factors and the associated feedback effects are likely to represent one of the largest uncertainties in projections of future biodiversity change (Thuiller, 2007). Studies that measure a variety of traits across the phenotype and match these to variation in fitness contribute the most to our understanding of how selection operates and how correlated traits evolve (Arntz & Delph, 2001). Leaf traits variation among plant species occurs in a coordinated way (Villar et al., 2013) resulting in groups of co-varying traits (Maire et al., 2013). Thus, it is important to analyze the correlation among traits and relate these traits to plant species strategies (Maire et al., 2013). Ghalambor et al. (2007) suggested that phenotypic plasticity confers greater tolerance to different environmental conditions (Ghalambor et al., 2007). Thus, differences in phenotypic plasticity among species influence how they will respond to environmental conditions changing (Gratani, 2014) including climate change (Chapin, 2003).

Conclusion

The literature on urban forests, pollution, and sustainability promotes the positive contribution of trees in maintaining environmental quality. If an urban forest can be promoted to policymakers and citizens as means of mitigating pollution within the parameters of urban sustainability, then they can be used to improve human quality of life throughout the cities of the world. The results on the whole highlight the large capability of *P. acerifolia* to grow in sites characterized by different environmental conditions in Rome, in particular, historical parks (A sites), high traffic density sites (B sites) and sites characterized by a high traffic density but with a larger water availability (C sites, avenues along the Tevere River), through several adaptations at leaf morphological and anatomical level. At the morphological level, *P. acerifolia* shows a 19.13% and 26.92% larger LA and DM, respectively, in A and C sites (mean value) than in B sites. Moreover, P/S and LTD are 10.62% and 31.89% larger, respectively, in C sites than in A and B sites (mean value). Such results can be explained by the better soil conditions in A sites, associated with larger water availability in C sites due to the Tevere River. The influence of water availability on plasticity may be of particular importance. The larger LT in A sites is mainly due to the 18.42%, 16.80%, 17.64%, and 15.23% larger Pt, St, Pcl and PCW, respectively, than in B sites. On the contrary, the 31.23% and 15.80% larger Adct and Adet, respectively, in B sites than in A sites, is due to the high pollution level by high traffic density. Plants growing under stress conditions increase the presence of trichomes on leaves. Thus, the increased TD in B sites can be induced by a high traffic level. The trichomes act as a physical barrier for the plant's pollutants absorption, thus contributing to air amelioration quality. The results, on the whole, highlight the leaf traits which are indicators of the species performance to grow in different sites in Rome.

The high phenotypic plasticity (0.42) of *P. acerifolia* respect to other species explains its wider ecological distribution and might be advantageous in conditions of environmental changes, including climate change.

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