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ORIGINAL PAPER

Nutrient Interaction in the Soil-Plant System and Tree Physiological Functional Traits in an Urban Green Infrastructure

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Abstract
Soil-plant indicators are useful to select tree species suitable for the urban conditions and to maximize the benefits provided by green infrastructures (GE). To identify effective indicators for GE, soil-plant nutrient interaction and related physiological responses were assessed in evergreen (*Cupressus sempervirens* L.) and deciduous (*Acer opalus* Mill., *Acer rubrum* L., *Tilia platyphyllos* Scop., *Ulmus* 'Plinio') tree species, in a novel urban GE (Florence, Italy). Soil and leaf nutrient contents and the soil enzyme stoichiometry were applied as indicators of plant nutrient status and bioavailability. Gas exchange and stable isotopes of carbon (C) and nitrogen (N) were used as indicators of tree physiological status and resource-use strategies, respectively. The soil was suitable for tree growth, however, the enzyme activities estimated N limited condition. Trees differed in leaf nutrient composition and stoichiometry. *Acer rubrum* and *A. opalus* leaves had manganese concentration below and above the plant optimal range, respectively, leading to alteration in the nutrient uptake and on the leaf stoichiometry between C, N and phosphorus (C: N:P), with consequence for tree health status. *Tilia platyphyllos* and *Ulmus* 'Plinio' had the best photosynthetic performance, while photosynthesis in *A. rubrum* was severely impaired. Interspecific differences in N- and water-use strategies were observed. *Tilia platyphyllos* showed the highest water-use efficiency, leaf C: P and N: P compared to the other species. Tree nutritional and physiological traits gave insights into soil-plant nutrient interaction and may be proposed as useful indicators for choosing the most suitable species to improve GE management in urban environments.

Keywords Leaf stoichiometry · Resource-use strategy · Nature-based solutions · Soil-plant indicators · Stable isotopes · Plant nutrition

1 Introduction

Urban trees are subject to several environmental stressors that can limit their ability to mitigate key environmental and social concerns (Makhinya et al. 2020). Healthy trees can provide a range of ecosystem services in urban environments, such as local cooling, stormwater absorption, mitigation of climate change, improvement of air quality, attenuation of noise level, promotion of citizens' health and support to biodiversity (Pataki et al. 2021). On the other hand, tree health status and growth are strongly dependent on urban soil properties. Pouyat et al. (2010) defined urban soil as the "brown infrastructure" that, together with the urban vegetation (i.e., the so-called "green infrastructure"), provides several life-sustaining ecosystem services making cities livable and more resilient to climate change. Urban soils are generally subjected to contamination, salinization, nutrient unbalance, compaction and sealing, which cause profound

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alterations of soil properties and functioning (Sager 2020). In particular, in urban areas, the nutrient bioavailability could be reduced by soil alkalization due to lime-rich construction waste materials, with consequences for tree health status (Kleiber et al. 2019).

From a perspective of green infrastructure feasibility, the choice and use of stress-tolerant plant species are crucial to pursue climate change resilience and positively contribute to maximizing ecosystem services in urban areas (Vannucchi et al. 2021). The study of plant strategy adopted to cope with urban conditions (e.g., urban soil) through the selection of suitable plant-soil indicators is crucial for a proper plant selection. The soil macronutrient ratios (e.g. Ca, Mg, K) and enzymatic stoichiometry can be useful indicators of the nutritional status and plant nutrient uptake limitations in soil (Vannucchi et al. 2022; Kunito et al. 2024). Leaf nutrients have been widely used for assessing the health status in urban trees (Petrova et al. 2014). Kleiber et al. (2019) related the nutritional composition of lime and horse chestnut trees to plant health in alkaline urban soil, detecting a negative effect of high leaf Mn concentration on plant health status. Zukswert et al. (2021) also analyzed the foliar chemistry and nutrient imbalance related to urban soil properties, while Su et al. (2021) measured morphological and nutrient traits of several common woody plants along a continuous urban–rural gradient. Nutritional physiology analyzes the link between nutrient uptake and plant physiology, including nutritional effects on photosynthesis and resource-use efficiency. Hence, plant physiological traits are valuable tools to assess the plant responses and the health status of vegetation under urban conditions, contributing to the effective development of ecological performance indices (Calfapietra et al. 2015). Among these, plant nutrient stoichiometry and leaf-level physiological responses related to photosynthetic performance allow to better understand the capability of tree species to grow and thrive in urban areas. Leaf gas exchanges have been widely used to evaluate (i) the interspecific differences in photosynthetic performance; (ii) the health status and the water-use efficiency (WUE) in urban street trees (Gillner et al. 2015); (iii) the CO_2 absorption capacity of trees in urban parks (Tor-Ngern and Leksungnoen 2020); (iv) the effects of urbanization and air pollutants on photosynthetic performance in urban and peri-urban tree species (Huarancca Reyes et al. 2022). In addition, carbon and nitrogen stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) have been measured to trace the pollution sources and to study the physiological responses of urban vegetation to pollution and environmental constraints (Trammell et al. 2020; Scartazza et al. 2023). As cities are hotspots of N deposition, $\delta^{15}N$ in leaves can be used as valuable biomarker of nitrogen oxides (NO_x) emitted by road transport and as indicator of N cycle and leaf uptake of atmospheric N pollution (Pereira et al. 2022). Conversely, $\delta^{13}C$ is mainly dependent on isotopic fractionations occurring in the photosynthetic CO_2 uptake and, subsequently, in the plant-soil system interplay (Brüggemann et al. 2011). Leaf $\delta^{13}C$ is a valuable proxy of time-integrated intrinsic WUE and can be useful for evaluating interspecific differences in water consumption strategies in urban environment (Cui et al. 2022). Overall, $\delta^{15}N$ and $\delta^{13}C$ in the soil-plant system can be valuable indicators of urbanization degree and soil–plant–atmosphere interaction in an urban context (Scartazza et al. 2023).

In the framework of LIFE AIRFRESH project (LIFE19 ENV/FR/000086), a novel urban green infrastructure inside the city of Florence (Italy) was developed to enhance air quality, and thus species known for their high capacity of air pollutant removal were planted (Manzini et al. 2023). Tree species with different leaf economic spectrum and ecology were used, including both evergreen coniferous and deciduous broadleaf species with conservative and acquisitive strategy (Fernandez et al. 2022). The main objective of this study was to evaluate how some functional indicators of soil-plant nutrient interaction and tree physiological status can be effectively used to select the most suitable species to be planted in urban green infrastructures. We hypothesized that the potential success of green infrastructure depends on interspecific differences in soil-plant interactions and strategies to optimize resource acquisition and use adopted by tree species living in the same resource-poor and stressful urban environment. To test this hypothesis, we measured several soil properties (physical, chemical, and biological) in association with leaf nutritional status and physiological parameters in different urban tree species used in afforestation program. The research questions are the following: (i) are there interspecific differences in leaf nutritional status in trees growing in the same urban soil conditions and nutrient limitations? (ii) how can the different nutritional composition and stoichiometry of leaves influence the physiological performance and health status of urban tree species? (iii) are carbon (C) and Nitrogen (N) stable isotopes valuable proxies of interspecific differences in nitrogen and water-use strategies in urban environment?

2 Materials and Methods

2.1 Site Description

The test area is located in a Mediterranean climate zone (Csa, Köppen climate classification) within the western suburb of Florence in San Bartolo a Cintoia (43° 46’ 38” N, 11° 11’ 25” E). It has an extension of approximately 0.55 ha and is part of a wider park (4 ha) composed of scattered rows of mature trees. The soil was characterized by a sandy loam texture. The planting was accomplished from January to February 2022 using a total of 170 young trees belonging to

the following species: *Tilia platyphyllos* Scop., *Acer rubrum* L., *Acer opalus* Mill., *Cupressus sempervirens* L. and the hybrid *Ulmus* 'Plinio'. These tree species were selected for their different ecology and high performance in removing air pollutants such as O_3 and NO_2 (deciduous broadleaves) or particulate matters (*C. sempervirens*), according to Manzini et al. (2023). Moreover, *C. sempervirens* and *Ulmus* 'Plinio' were clones developed by the Institute for Sustainable Plant Protection of the Italian National Research Council (IPSP-CNR) and patented to be resistant to the following fungal disease: *Ophiostoma ulmi* (Buisman) Nannf and *Seiridium cardinale* (Wagener) Sutton et Gibson, respectively. The young trees were three years old at the time of planting. *Tilia platyphyllos*, *A. rubrum*, and *A. opalus* were obtained from the nursery "Vivai Guagno" (Comacchio, Italy), while *C. sempervirens* and *Ulmus* 'Plinio' were cultivated in pots at the nursery "Umbraflor" (Spello, Italy). All trees had a height and diameter between 2 and 4 m and 2–3.5 cm, respectively. They were constantly watered, during the summer, via an automatic irrigation system. Meteorological data of air temperature (°C) and relative humidity (%) were hourly recorded by Airqino sensors (https://www.airqino.it) installed inside the new green area (Fig. S1).

2.2 Soil Sampling and Analysis

Nine soil samples (3 composite samples x 3 plots) were collected in September 2022 at both 0–15 and 15–30 cm depth. The urban park was divided into three equally spaced plots in which the five species were planted (Fig. S2). In each plot, three composite soil samples were collected at about 1 m from the collar of the plants. Each composite soil sample was a bulk of three subsamples. Bulk density (BD) was assessed using the core method (Blake and Hartge 1986). Soil samples were air-dried, and 2 mm sieved. Electrical conductivity (EC) and pH were determined on water extract, 1:5 and 1:2.5 (w: v), respectively, using specific electrodes (EC: Orion conductivity meter; pH: SevenMulti, Mettler Toledo) (ASA-SSSA 1996). Soil texture was determined through size distribution of aggregates (0.02–2000 μm), using a laser granulometer Mastersizer 2000 (Malvern Panalytical Ltd., UK) equipped with a wet sample dispersion unit.

β-glucosidase (BG; EC 3.2.1.21), acid phosphatase (AP; EC 3.1.3.2), N-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.14) hydrolytic enzyme activities were analysed at 0–15 cm soil depth, according to fluorometric method proposed by Marx et al. (2001) and Vepsäläinen et al. (2001). The enzyme activities (BG, AP and NAG) were normalized to total organic carbon (TOC) and log-transformed. The microbial nutrient limitations for N (MNL), P (MPL) and C (MCL), were calculated following the threshold models of nutrient use efficiency-based proposed by Cui et al. (2023). Specifically, $MNL = \ln(1.5 * (n_0 / EEA_{C:N}))$, $MPL = \ln(1.5 * (p_0 / EEA_{C:p}))$ and $MCL = \ln((EEA_{C:N} * EEA_{C:P})/(2.25 * (n_0 * p_0)))$, where $EEA_{C:N}$ is the ratio amongst enzymes acquiring C and N, while $EEA_{C:P}$ is the ratio amongst enzymes acquiring C and P. Instead, $n_0 = e^{intercept}$ was calculated in the regressions for ln(BG) vs. ln(NAG) and $p_0 = e^{intercept}$ in the regression for ln(BG) vs. ln(AP). MNL, MPL and MCL values above 0 represent N, P and C limitations, respectively.

2.3 Leaf Sampling and Leaf Mass Per Area Detection

The leaf sampling for isotopic and nutrient analyses was conducted towards the end of the first growing season in September 2022, before the onset of the senescence phase. Three fully-expanded leaves exposed to light were sampled from nine trees per species (3 trees × 3 plot). The leaves were randomly selected from the tree crown to cover all directions against the sun at the same height (about two meters) and did not show any visible disease or symptoms of senescence. In the laboratory, the samples were washed for 60 s with deionized water under stirring. Then, they were stored in an oven at 70 °C for five days. Subsequently, the dry-leaf samples were pulverized with a pestle by using liquid nitrogen and excluding the central leaf vein.

To assess the broadleaved species-specific Leaf Mass per Area (LMA), five leaf sub-samples of known area (0.50 cm^2; Ø 0.8 cm) were obtained by a leaf punch (Fujiwara Scientific Company Co., Ltd.,Tokyo, Japan) for each sampled leaf ($n = 3$ leaves × 9 trees). For the squamiform *C. sempervirens* conifer, 3 sun-exposed twigs × 3 trees were collected, and the projected twig area of the samples was assessed by Easy Leaf Area application (Easlon and Bloom 2014). The leaf discs or twigs were oven-dried at 70 °C until constant weight and then weighed by an analytical balance (Sartorius, Germany, sensitivity: ± 0.01 g). The species-specific LMA (g m^{-2}) was calculated as the ratio between the average dry leaf discs or twigs biomass and the relative area.

2.4 Soil and Leaf Nutrient Assessment

The total phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni), lead (Pb) concentrations were determined. Well-homogeneous powder samples (0.3 g) were digested in a solution of nitric acid (HNO_3 65%) and hydrogen peroxide (H_2O_2 30%) (2:1, v/v) in a closed-vessel microwave-assisted digestion system (Milestone Ethos 900, Bergamo, Italy) using EPA Method 3052. The concentrations of Na, K, Mg, Ca, Fe, Mn, Cu, Zn, Cr, Ni, Pb were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES 5900, Agilent, Santa Clara, California, USA)

(EPA 2007). The total P concentration was performed following the molybdate blue ascorbic acid method (Murphy and Riley 1962). An aliquot of the dry powder of leaf (about 3–5 mg) and soil (10–60 mg) samples was used to determine Carbon (C) and nitrogen (N) concentration (% Dry Weight) using an elemental analyzer (Model NA 1500, Carlo Erba, Milan, Italy), after conversion of samples into gases (CO_2 and N_2) by combustion.

2.5 Leaf Nitrogen and Carbon Stable Isotope Analyses and WUE Determination

About 0.5 mg and 2.0 mg of leaf dry material were used to analyze C and N isotope compositions ($\delta^{13}C$ and $\delta^{15}N$), respectively. Samples were quantitatively combusted into an elemental analyzer (Model NA 1500, Carlo Erba, Milan, Italy) and the gaseous obtained (CO_2 and N_2) were admitted in helium stream to an isotope ratio mass spectrometer (Isoprime Ltd., Cheadle, UK). Isotope ratios of C ($R = {}^{13}C/{}^{12}C$) and N ($R = {}^{15}N/{}^{14}N$) were measured in order to calculate $\delta^{13}C$ and $\delta^{15}N$ referring to the VPDB and atmospheric N_2 standards respectively, as: $\delta^{13}C$ or $\delta^{15}N = R_{sample} / R_{standard} - 1$. Anchoring was pursued on the VPDB and atmospheric N_2 scales by means of IAEA standards. The precision of sample measurements was better than 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Leaf C isotope discrimination (D) of different tree species was determined as: $\Delta = (\delta_a - \delta_p)/(1 + \delta_p)$ (1) where δ_p and δ_a represent $\delta^{13}C$ of leaf dry matter and of tropospheric CO_2, respectively. We assumed $\delta_a = -8.5‰$ based on local measurements. The D values were then used to determine the ratio between atmospheric and intercellular CO_2 concentrations (C_i/C_a) using the model of Farquhar et al. (1982) as: $C_i/C_g = (\Delta - a)/(b - a)$ (2) where a is the isotopic fractionation during diffusion of CO_2 in air (4.4‰) and b is the isotopic fractionation associated with the enzymatic carboxylation (27‰). The C_i/C_a values determined through Eq. 2 by the Δ values were then applied to evaluate the canopy-integrated values of intrinsic water-use efficiency (iWUE, the ratio between net photosynthetic rate and stomatal conductance) for the different tree species using the following equations: $iWUE = A/g_s = C_a(1 - C_i/C_a)/1.6$, where C_a was assumed to be 410 µmol mol^{-1} and the value 1.6 is the ratio of the stomatal conductance of water vapor to that of CO_2. The instantaneous water use-efficiency (WUE, the ratio between net photosynthetic rate and transpiration rate) was calculated dividing iWUE by the leaf-to-air difference in water vapor concentration. The value of C_a was assumed to be 410 µmol mol^{-1} and ν was estimated, based on local micro-meteorological measurements, according to Scartazza et al. (2014).

2.6 Gas Exchange Measurements

Gas exchange measurements were carried out in situ using portable infrared gas analyzer systems (LI6800, Li-Cor, Lincoln, USA) equipped with a cuvette for broadleaves with a circular opening of 6 cm^2. Instantaneous light-saturated measurements of eco-physiological parameters such as photosynthesis (A), stomatal conductance (g_s), transpiration (E), leaf internal and ambient CO_2 concentration (C_i and C_a, respectively) were performed during summer (June 2022) from 9:00 to 12:00. The following instrument parameters were set: 1500 µmol m^{-2} s^{-1} for the photosynthetic photon flux density (PPFD), 410 µmol mol^{-1} as ambient CO_2 concentration (C_a) and 25 °C as leaf temperature. Six trees per species were selected (total = 30 trees) and measurements were performed on one fully-expanded leaf exposed to sunlight for each tree. As cypress is a conifer, parameters returned by the instrument were re-proportioned to the actual leaf surface placed in the cuvette. The effective leaf area of cypress twigs was obtained using the Easy Leaf Area application (Easlon and Bloom 2014).

2.7 Statistical Analysis

A principal component analysis (PCA) was carried out using the 'prcomp' R function (R Core Team 2022) for the leaf concentrations of Ca, Cu, Fe, K, Mg, Mn, Na, Zn, N, C, and P determined on the nine leaf samples for each of the five tree species analyzed. The 'fviz_pca' R function of the factoextra R package (Kassambara and Mundt 2020) was used to visualize the PCA in a biplot of individuals and variables. We also inspected pairwise Pearson's correlations across each of these variables across the leaves of the five tree species. All variables were scaled and centered before conducting the analyses. The Shapiro–Wilk test was used to assess data normality. One-way analysis of variance (ANOVA) was applied to compare mean values of leaf nutrient concentrations and physiological traits (i.e., stable isotope compositions, gas exchange parameters, ISO-WUE) of each tree species ($n = 9$). Separation of means was performed by Fisher's least significance difference (LSD) test at a significance level of $P \leq 0.05$.

3 Results

3.1 Soil Properties

Table 1 shows the soil properties at each layer depth. No significant differences were observed among the sampling areas, so the average values for the individual parameters are reported. The soil in the studied park

Table 1 Soil properties and nutrient ratios (±SD, $n=9$) detected at two soil depths in the study urban park

	0–15 cm	15–30 cm	Reference values		Literature references
BD (g cm^{-3})	1.3 ± 0.1	1.5 ± 0.1	< 1.7		Hamza and Anderson (2005)
pH	8.1 ± 0.0	8.2 ± 0.1			
EC (dS m^{-1})	0.93 ± 0.03	0.82 ± 0.02	< 2		Vasenev et al. (2017)
TOC (%)	1.11 ± 0.13	0.74 ± 0.09			
TN (%)	0.16 ± 0.01	0.10 ± 0.01			
Ca (g kg^{-1})	15.1 ± 3.2	16.4 ± 4.3			
K (g kg^{-1})	4.4 ± 0.5	3.6 ± 0.8			
Mg (g kg^{-1})	4.1 ± 0.2	4.0 ± 0.2			
Fe (g kg^{-1})	19.3 ± 1.6	18.6 ± 2.0			
TP (mg kg^{-1})	1023 ± 73	1096 ± 100			
Na (mg kg^{-1})	222 ± 7	212 ± 12			
Mn (mg kg^{-1})	1053 ± 71	993 ± 96			
Cr (mg kg^{-1})	63.0 ± 1.6	58.6 ± 2.79			
Cu (mg kg^{-1})	61.6 ± 14.2	49.3 ± 3.0	< 120		D.Lgs Decreto legislative 152/2006
Zn (mg kg^{-1})	82.4 ± 2.2	78.4 ± 2.5	< 150		
Ni (mg kg^{-1})	43.1 ± 0.7	41.6 ± 1.7	< 120		
Pb (mg kg^{-1})	41.8 ± 5.6	51.3 ± 9.5	< 100		
TOC: TN*	8.5 ± 3.6	9.1 ± 4.4	14.3 ± 0.3		Cleveland and Liptzin (2007)
TOC: TP*	28.8 ± 10.0	18.9 ± 8.5	186 ± 13		
TN: TP*	3.6 ± 1.0	2.2 ± 0.9	13.1 ± 0.8		
Ca: Mg	3.6 ± 0.7	4.1 ± 1.0	< 1	Ca deficiency	Vannucchi et al. (2022)
			1–2	Low level of Ca to Mg	
			2–5	Ideal	
			> 5	Mg deficiency	
Mg: K	1.0 ± 0.1	1.2 ± 0.2	< 1	Mg deficiency	
			1–3	Acceptable	
			3	Ideal	
			3–18	Acceptable	
			> 18	K deficiency	
Ca: K	3.4 ± 0.8	4.9 ± 1.6	< 30	Suitable	
			> 30	K deficiency	
(Ca + Mg): K	4.4 ± 0.8	6.1 ± 1.7	< 40	K suitable	
			> 40	K deficiency	
MNL	2.9 ± 0.06		> 0	N limitation	Cui et al. (2023)
MPL	0.3 ± 0.17		> 0	P limitation	
MCL	−1.8 ± 0.44		> 0	C limitation	

*atomic ratio; *BD* bulk density, *EC* electrical conductivity, *TOC* total organic carbon, *TN* total nitrogen, *TP* total phosphorus, *MNL* microbial nitrogen limitation, *MPL* microbial phosphorous limitation, *MCL* microbial carbon limitation

was characterized by a sandy loam texture (clay = 16%; silt = 27%; sand = 57%) with an alkaline pH (~ 8), BD higher than 1 and EC below 1 dS/m. The ratios amongst macro-nutrients (Ca: Mg; Mg: K; Ca: K; (Ca + Mg): K) did not reveal any possible nutrient deficiencies for plant growth. Instead, the TOC: TN, TOC: TP and TN: TP atomic ratios were below the reference values for terrestrial ecosystems. The ecoenzyme stoichiometry revealed microbial N and P limitations.

3.2 Tree Nutritional Status and Nutrient Interaction

The PCA analysis (Fig. 1) and the average nutrient concentrations (Table 2) highlighted significant interspecific differences in micro- and macro-nutrient leaf chemical composition and nutrient ratios across the five tree species used for urban afforestation. The five species were well clustered in the PCA biplot, underlining the interspecific variability. *Cupressus sempervirens* showed higher LMA and lower nutrient concentrations than the other deciduous tree species,

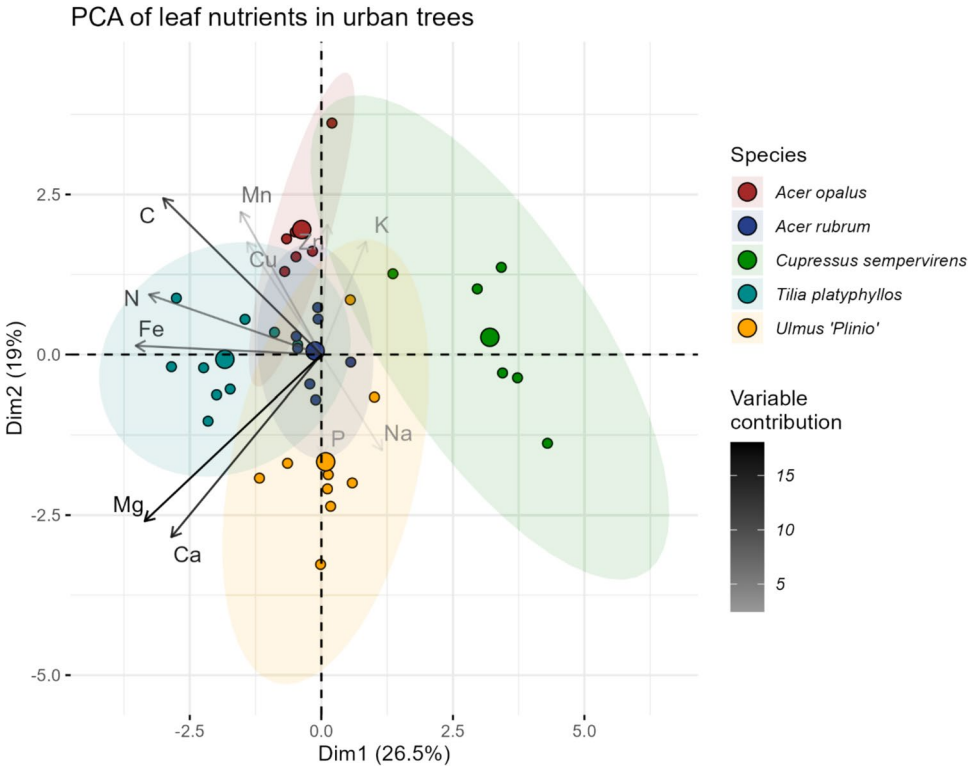

Fig. 1 Biplot of plant nutrient variables sampled across five tree species. Arrows represent the coordination of each variable with each dimension of the PCA. Their color represents the variable contribution to the PCA. Each small point represents a measurement of a given species. Ellipses are drawn for each species, with larger points indicating the centroids of the ellipses

Table 2 Leaf mass per area (LMA), macro- and micro-nutrient concentrations and C and N isotope compositions ($\delta^{13}C$, $\delta^{15}N$) in leaves of the different tree urban species. Values shown are means ± SE ($n = 9$). Data were analyzed independently by one-way ANOVA. For each parameter, data followed by different letters in the same line are significantly different ($P \leq 0.05$, Fisher's LSD) for the significance level indicated (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$)

	Acer opalus	*Acer rubrum*	*Tilia platyphyllos*	*Ulmus 'Plinio'*	*Cupressus sempervirens*	*P* value
LMA (g m^{-2})	105.7 ± 1.6 c	90.3 ± 3.1 cd	82.2 ± 2.1 d	131.8 ± 5.3 b	318.5 ± 12.4 a	***
C (g kg^{-1})	473.7 ± 6.0 a	466.2 ± 7.0 a	485.7 ± 4.1 a	369.0 ± 9.2 b	357.4 ± 26.2 b	***
N (g kg^{-1})	19.4 ± 0.6 b	14.2 ± 0.8 c	22.7 ± 1.0 a	17.3 ± 1.2 b	11.5 ± 1.0 c	***
P (g kg^{-1})	3.48 ± 0.53 b	3.23 ± 0.36 b	1.51 ± 0.07 c	5.66 ± 0.53 a	1.68 ± 0.19 c	***
K (g kg^{-1})	6.57 ± 0.91 a	3.79 ± 0.16 b	5.68 ± 0.52 ab	6.23 ± 0.93 a	6.92 ± 0.76 a	*
Ca (g kg^{-1})	10.2 ± 0.7 d	12.1 ± 0.4 c	14.5 ± 0.6 b	17.1 ± 0.7 a	6.58 ± 0.92 e	***
Mg (g kg^{-1})	2.12 ± 0.19 c	3.13 ± 0.05 b	3.80 ± 0.22 a	3.59 ± 0.17 a	1.16 ± 0.09 d	***
Na (mg kg^{-1})	79.3 ± 7.6 c	92.2 ± 7.8 bc	184.1 ± 19.1 ab	176.5 ± 29.0 abc	218.6 ± 68.2 a	*
Fe (mg kg^{-1})	146.9 ± 9.8 b	145.9 ± 11.0 b	213.3 ± 17.5 a	125.1 ± 11.8 bc	93.1 ± 19.0 c	***
Mn (mg kg^{-1})	187.9 ± 19.7 a	6.45 ± 0.86 d	77.7 ± 6.1 b	57.3 ± 8.7 b	18.8 ± 3.6 c	***
Cu (mg kg^{-1})	13.4 ± 1.7 ab	19.6 ± 5.1 a	8.63 ± 1.02 bc	7.48 ± 0.56 bc	4.49 ± 0.70 c	**
Zn (mg kg^{-1})	13.9 ± 2.1	19.0 ± 4.3	9.48 ± 0.86	11.8 ± 3.0	11.1 ± 1.7	n.s.
C: N	24.5 ± 0.9 b	33.7 ± 2.1 a	21.7 ± 0.8 b	22.1 ± 0.7 b	31.1 ± 2.6 a	***
C: P	153.8 ± 20.5 c	157.7 ± 13.8 c	326.0 ± 12.6 a	69.2 ± 5.5 d	225.4 ± 21.3 b	***
N: P	6.19 ± 0.72 bc	4.91 ± 0.64 cd	15.13 ± 0.67 a	3.20 ± 0.26 d	7.29 ± 0.79 b	***
$\delta^{15}N$ (‰)	0.81 ± 0.24 bc	0.05 ± 0.76 c	1.95 ± 0.32 b	4.74 ± 0.30 a	4.93 ± 0.82 a	***
$\delta^{13}C$ (‰)	−27.7 ± 0.4 b	−29.3 ± 0.4 c	−26.3 ± 0.2 a	−29.4 ± 0.1 c	−27.9 ± 0.4 b	***

except for K and Na (Table 2; Fig. 1). Compared to the other tree species, *Ulmus* 'Plinio' and *T. platyphyllos* leaves showed higher concentrations of Ca and Mg, as highlighted in the PCA biplot (Fig. 1), while the lowest concentrations of these elements were detected in *C. sempervirens* followed by *A. opalus* and *A. rubrum* (Table 2). Moreover, *A. rubrum* showed lower leaf K concentration than other species. It is worth noting the large interspecific variability in leaf Mn

concentration (Table 2), with leaves of *A. rubrum* and *A. opalus* showing the lowest and the highest Mn concentration, respectively (Table 2). Conversely, *C. sempervirens* and the other deciduous trees (i.e., *T. platyphyllos* and *Ulmus* 'Plinio') showed intermediate Mn values (Table 2). The highest Cu and Fe concentrations were observed in leaves of *A. rubrum* and *T. platyphyllos*, respectively. In particular, Fe, N and C and partially Ca and Mg concentrations in leaves contributed to interspecific variability, discriminating the plant species in three groups: *C. sempervirens*; *A. opalus*, *A.* rubrum and *Ulmus* 'Plinio'; and *T. platyphyllos* (Fig. 1). The concentration of Zn did not show any significantly interspecific difference. *Acer opalus*, *A. rubrum* and *T. platyphyllos* showed higher leaf C concentrations than *Ulmus* 'Plinio' and *C. sempervirens*. The highest leaf N concentration was observed in *T. platyphyllos*, while *A. rubrum* and *C. sempervirens* showed the lowest N concentration and *Ulmus* 'Plinio' as well as *A. opalus* intermediate values. As regards P, the highest leaf concentration was observed in *Ulmus* 'Plinio' while the lowest in *T. platyphyllos* and *C. sempervirens*. An interspecific variability of C: N ratio was found, with *A. rubrum* and *C. sempervirens* leaves showing higher leaf C: N ratio compared to the other tree species, mainly due to the much lower N concentration in their leaf tissues (Table 2). The highest and lowest C: P and N: P ratios were observed for *T. platyphyllos* and *Ulmus* 'Plinio' leaves respectively, whereas the other species showed intermediate values (Table 2).

The pairwise correlation matrix illustrating the relationships among leaf nutrient variables of the five tree species is reported in Fig. 2. A strong positive correlation between Ca and Mg concentrations was observed across the leaves

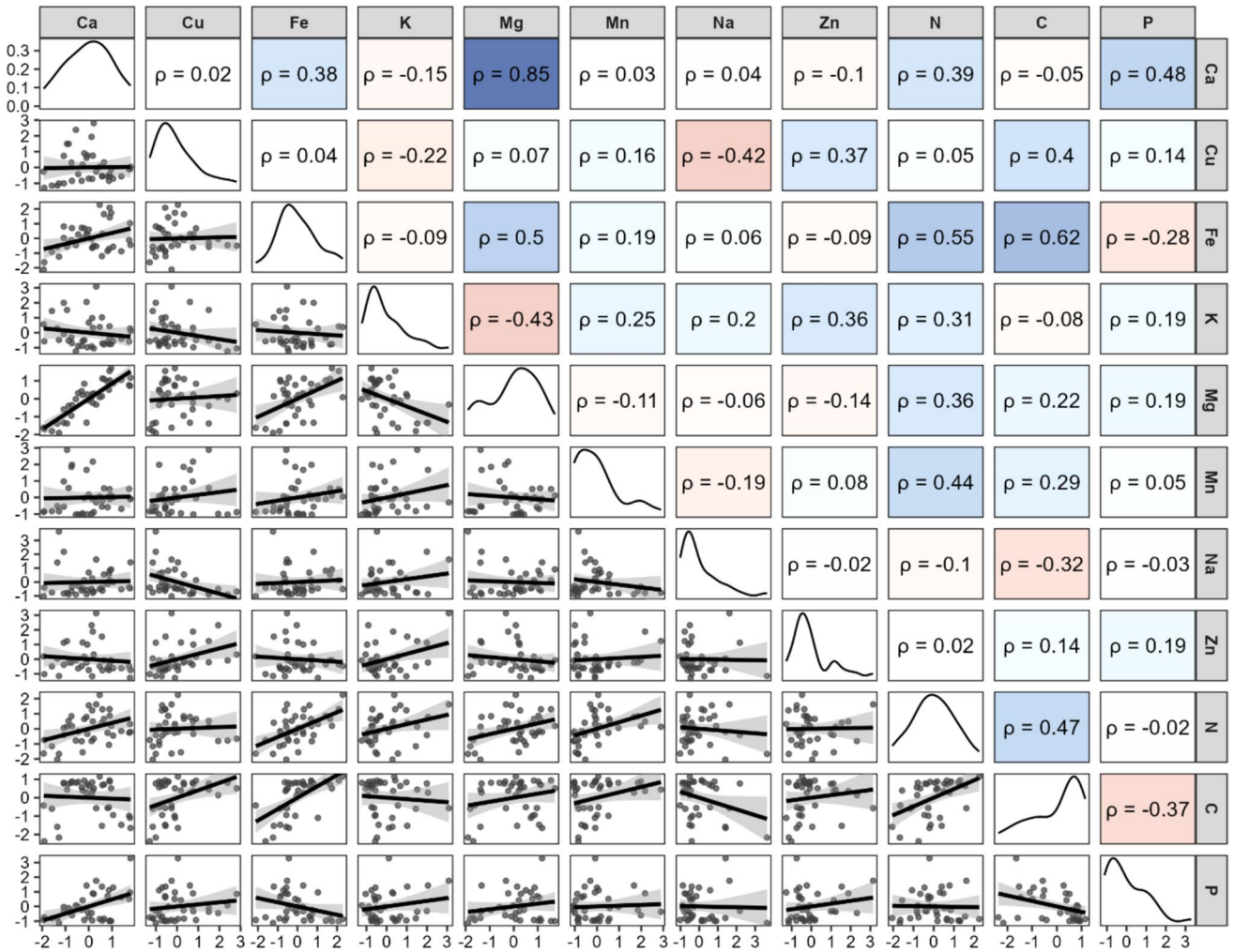

Fig. 2 Visualization of the pairwise correlation matrix illustrating the relationships among leaf nutrient variables of the five tree urban species. The diagonal represents the distribution (density plot) of each nutrient variable. The top-right panel displays pairwise Pearson's correlation values (dark blue color = + 1; dark red color = − 1). The bottom-left panel displays the scatterplots of the observations; each line represents a linear model estimate and the shaded areas are 95% CI

of the five tree species, while Mg was inversely related to K and positively to Fe (Fig. 2). However, this fact was particularly evident for *Ulmus* 'Plinio' and *T. platyphyllos* (Fig. 1). A positive correlation between K and other elements, such as Mn and Zn, was observed (Fig. 2). Leaf Cu concentration was negatively correlated with Na and positively with both Z and C. A positive correlation between Fe and C was observed. Leaf P concentration was positively correlated with Ca and negatively with both Fe and C (Fig. 2). Leaf N concentration was positively correlated with C, Ca, Fe, K, Mg and Mn concentrations (Fig. 2).

3.3 Leaf Stable Isotope Composition

A wide interspecific variability in $\delta^{15}N$ and $\delta^{13}C$ across the urban trees was observed (Table 2; Fig. 3), suggesting different resource-use strategies among the five tree species. The lowest $\delta^{15}N$ values were observed in *A. rubrum* and *A. opalus*, while leaves of *Ulmus* 'Plinio' and *C. sempervirens* showed higher leaf $\delta^{15}N$ values with respect to the other trees (Table 2; Fig. 3a). A positive significant relationship between $\delta^{15}N$ and N concentration was observed across leaves of *A. rubrum*, *A. opalus* and *T. platyphyllos*, with *A. rubrum* showing the lowest values of both $\delta^{15}N$ and N concentration (Fig. 3a).

The highest leaf $\delta^{13}C$ values were found in *T. platyphyllos* and the lowest in *A. rubrum* and *Ulmus* 'Plinio', while *A. opalus* and *C. sempervirens* showed intermediate values (Table 2). A positive relationship between leaf $\delta^{13}C$ and N concentration was found (Fig. 3b), although the evergreen coniferous *C. sempervirens* showed a shift of this relationship compared to the broadleaved deciduous tree species.

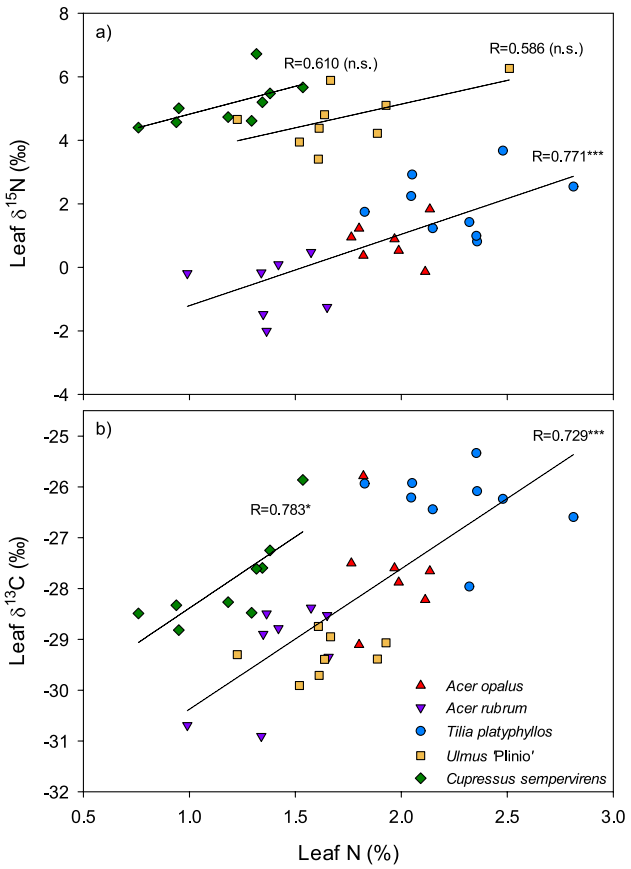

Fig. 3 Linear relationships between leaf N concentration with **a** nitrogen isotope composition ($\delta^{15}N$); **b** carbon isotope composition ($\delta^{13}C$) in leaves of the different urban tree species. Each point value of the linear relationship represents a single tree; The Pearson correlation coefficient (R) and the significance level (*P*) of the linear regressions are shown (***$P < 0.001$; *$P < 0.05$; n.s., not significant)

3.4 Effects of Nutritional Status on Photosynthesis and Water-Use Efficiency in Urban Trees

The interspecific variability in photosynthetic gas exchange parameters is shown in Fig. 4. *Tilia platyphyllos* and *Ulmus* 'Plinio' showed the highest CO_2 uptake capacity, while the lowest CO_2 assimilation rate was recorded in *A. rubrum* (Fig. 4a). The reduced A in this species was associated with low g_s (Fig. 4b) and E (Fig. 4c) values, while C_i (Fig. 4d) was not significantly different compared to the other deciduous species. Trees of *A. opalus* and *C. sempervirens* showed intermediate values of A, E and g_s. Moreover, *C. sempervirens* showed higher intercellular CO_2 concentration (C_i) compared to the other species (Fig. 4d).

An interspecific variability in intrinsic and instantaneous WUE, estimated through leaf D values, among the target species was observed, with *T. platyphyllos* showing the highest value, while *A. rubrum* and *Ulmus* 'Plinio' the lowest one (Fig. 5a, b). Highly significant relationships between WUE with both N: P and C: P ratio across the five tree species were detected (Fig. 5c, d).

4 Discussion

Plant growth and development largely depend on physical, chemical and biological soil properties. The urban soil resulted suitable for plant growing, even though the alkaline pH could negatively affect the bioavailability of nutrients (Kleiber et al. 2019). The soil had low salinity and BD value ideal for root growth (Vasenev et al. 2017), and no deficiency for Mg, Ca and K (Rodelo-Torrente et al. 2022). The Ca, Mg and K ratios showed values that reached the ideal values for plant growth according to Rodelo-Torrente et al. (2022). In addition, the trace element concentrations (Pb, Ni, Cu, Zn) were below the law limits allowed for residential areas (D.Lgs Decreto legislative 152/2006). The TOC and TP content was in line with values detected in other urban areas (Yang et al. 2021; Du et al. 2022) as well as the TN

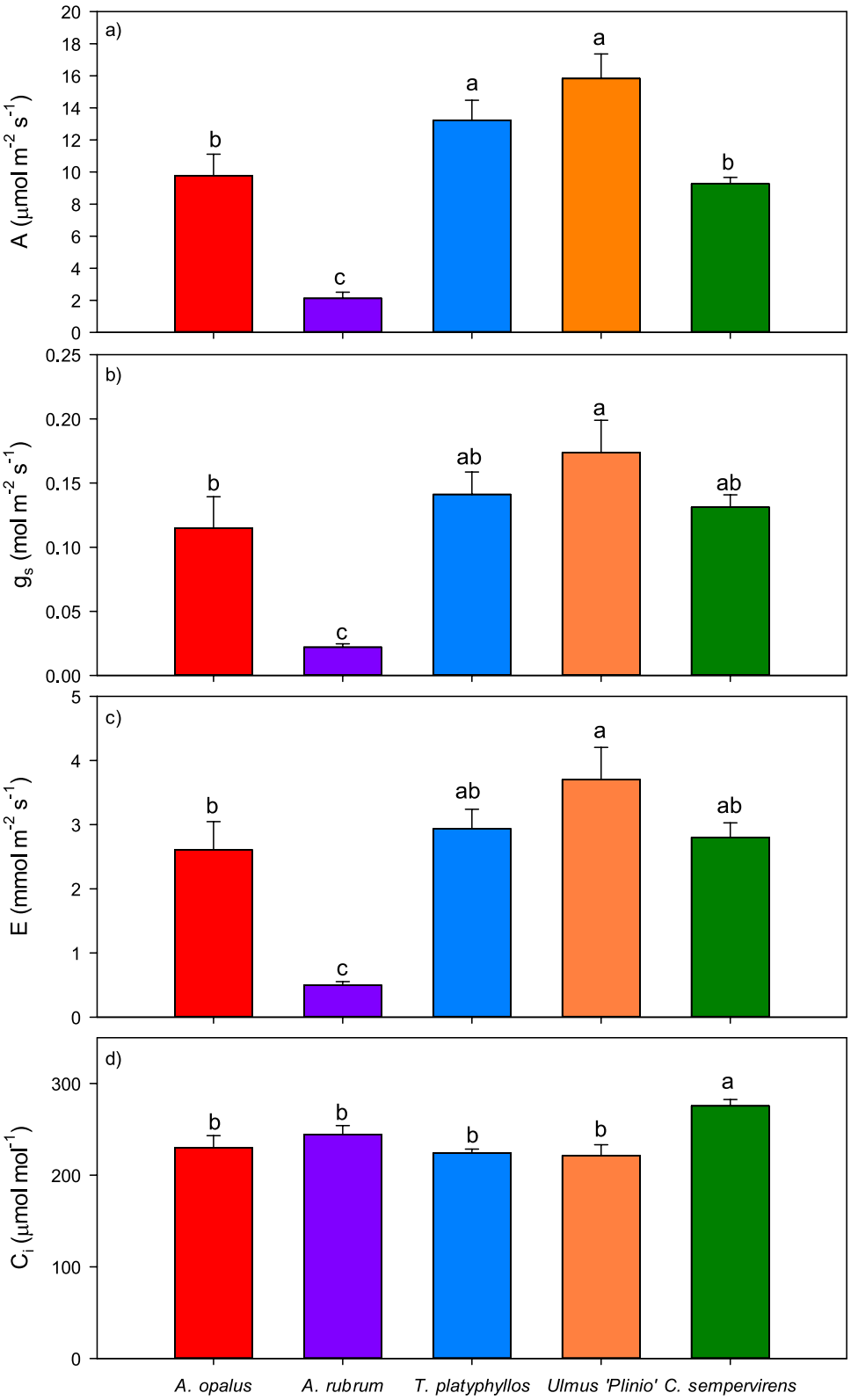

Fig. 4 Gas exchange parameters in leaves of the different urban tree species. Values shown are means ± SE ($n = 9$). Data were analyzed independently by one-way ANOVA. For each parameter, data followed by different letters are significantly different ($P \leq 0.05$, Fisher's LSD). **a** net CO_2 assimilation rate (A); **b** stomatal conductance (g_s); **c** transpiration rate (E); **d** intercellular CO_2 concentration (C_i)

content with young parks (Lu et al. 2022). Instead, alterations for C and nutrient (i.e., N, P) ratios occurred. The C: N, C: P and N: P were lower than the mean values detected for terrestrial ecosystem (Cleveland and Liptzin 2007). Lower ratios can occur in urban areas, as a consequence of limited organic matter input and P enrichment due to anthropogenic

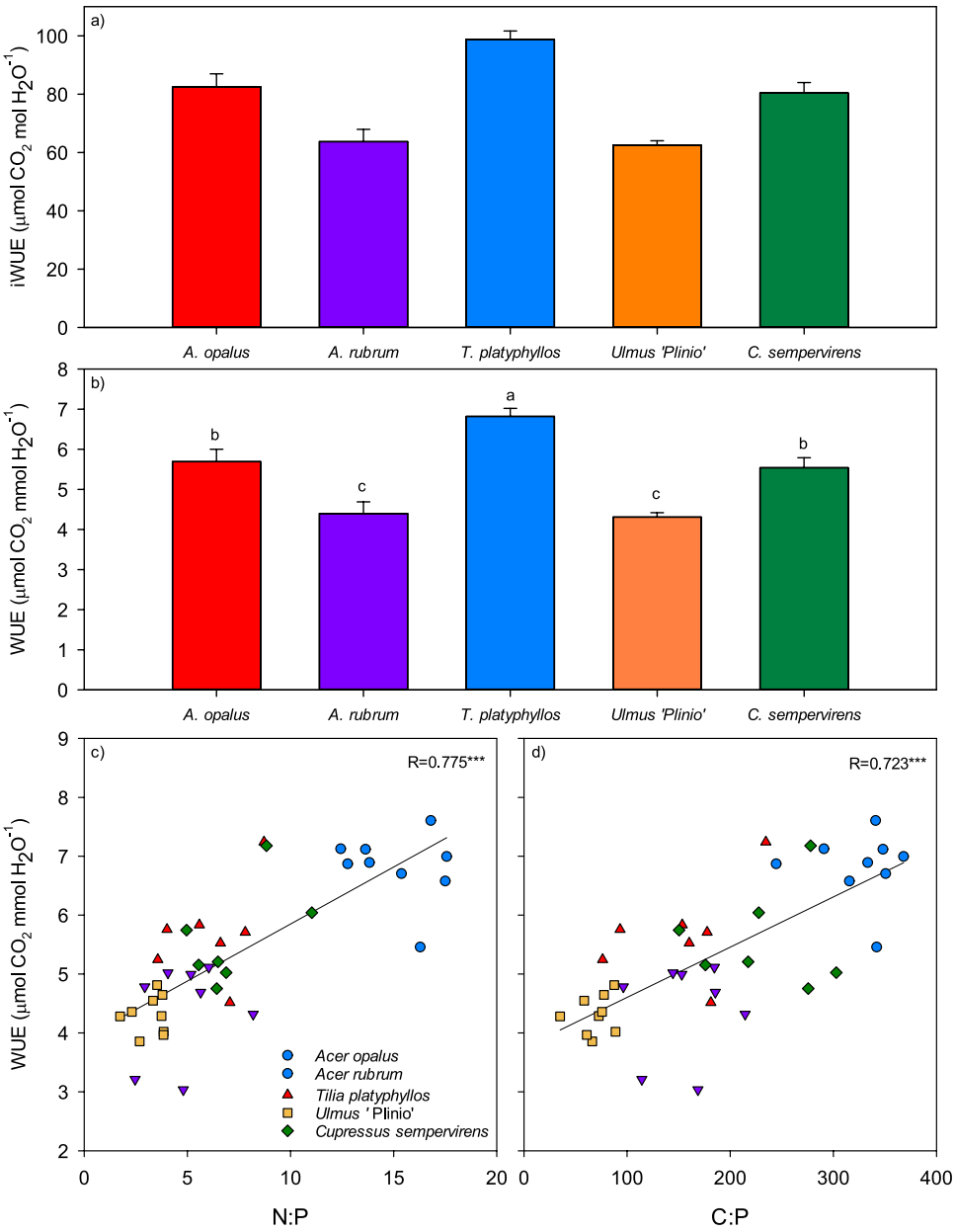

Fig. 5 Interspecific variability of intrinsic iWUE, **a** and instantaneous WUE, **b** water-use efficiency estimated by carbon isotope discrimination method and linear relationships between WUE with N: P **c** and C: P **d** ratio in leaves of the different urban tree species. Values of ISO-WUE are means ± SE. Data were analyzed independently by one-way ANOVA. Data followed by different letters in a) are significantly different ($P \leq 0.05$, Fisher's LSD). Each point of the linear relationships represents a single tree. The Pearson correlation coefficient (R) and the significance level (*P*) of the linear regressions are shown (***$P < 0.001$)

waste deposition (Hu et al. 2011). The nutrient ratio alternation also occurred for soil microbial community that resulted N and P limited, in fact the ecoenzymatic stoichiometry revealed a more microbial resource allocation for the production of enzymes for the acquisition of N and P. As plant and microorganisms use the same N pools, the microbial N limitation revealed a N limitation also for plants. In fact, the plant nutrient uptake can reduce when the microbial nutrient availability is limited (Kunito et al. 2024). Indeed, plants respond to fluctuations of nutrients availability, such as N, in a complex network that involves the plant ecological strategy and the interactions with soil biota and neighboring plants (Fernandez et al. 2022). In this context, it is crucial to select tree species able to maximize their nutrient acquisition abilities while protecting against the accumulation of excess nutrients, which can be toxic to the plant. The five tree species used for urban afforestation showed a clear interspecific difference in leaf nutrient composition and balance. It is also worth noting that some soil metals (i.e., Pb, Ni, Cr) were found in leaves under the detectable limits, suggesting that their availability for plant roots was strongly reduced by the alkaline soil pH (Kicińska et al. 2022). All plant species were not affected by the content of Cu or Zn, as highlighted by their lack of significance in PCA analysis. The discrimination of *C. sempervirens* in the right of the PCA biplot, compared to the other deciduous tree species associated to a generally lower leaf nutrient concentrations, especially for Fe and N, suggested a contrasting plant economic strategy

and resource-use efficiency between evergreen conifers and deciduous broad-leaves species (Zhang et al. 2020). The low leaf N and P concentrations, together with high leaf dry mass investment (as suggested by higher LMA) and relatively low photosynthetic rate, are characteristics of the conservative leaf strategy of evergreen species, such as *C. sempervirens* (Wright et al. 2004). Linear relationships between shoot Ca and Mg concentrations have been previously observed in several angiosperm species growing under the same environmental conditions (White et al. 2018), which was also confirmed by Mg and Ca positive correlation in PCA. The interspecific variability in Ca and Mg concentration is due to differences in chemical composition and cation exchange capacity of the cell walls within each functional group (White et al. 2018). Moreover, Ca stabilizes plant cell walls and membranes playing a key role in enhancing stress tolerance (Gupta et al. 2023), hence the interspecific differences in this element deserve more attention for evaluating possible implications on tree adaptability in urban environmental conditions. Conversely, the negative correlation between Mg and K concentrations across leaves of the five tree species suggests an antagonism between these elements (Xie et al. 2021). A positive interaction between K and other elements, such as Mn and Zn, can be inferred by the correlation analysis. Accordingly, it was found that K supply increased Zn absorption and translocation in plants (Dibb and Thompson 1985), while a low level of Mn compromised the plant ability to take up soil K (Xu et al. 2007). Alejandro et al. (2020) suggested that Mn deficiency can represent a serious nutritional disorder in dry, calcareous and organic-rich soils, due to a sharp decrease of its bioavailability below the minimum threshold for normal plant growth. This is a typical condition for urban environment, where Mn uptake can be severely reduced due to the high frequency of alkaline calcareous soils (Kleiber et al. 2019). Leaves of *A. rubrum* showed Mn concentrations far below the minimum threshold required for normal plant growth, while in *A. opalus* it exceeded the maximum threshold (i.e., from 10 to 25 to 80 mg kg^{-1}, according to Nikolic and Pavlovic 2018). These results suggest that *A. opalus* was able to uptake and accumulate excessive amounts of Mn despite the alkaline soil, while *A. rubrum* suffered from Mn deficiency which, negatively affecting K uptake (Xu et al. 2007), it was also associated with K deficiency. Lambers et al. (2015) suggested that Mn concentration in mature leaves acts as an indicator of rhizosphere carboxylate concentrations and proposed it as a promising functional trait for plants growing in alkaline soils, where carboxylate release could represent an effective strategy to release P and mobilize Mn. Therefore, the interspecific variability in leaf Mn concentration could indicate a species-specific ability to mobilize Mn into the rhizosphere. A concentration of Mn higher than the maximum threshold was found with possible toxicity effects even in alkaline urban soils (Kleiber et al. 2019), as detected in our study for *A. opalus*. Excessive Mn can hinder the uptake and translocation of other essential elements, such as Ca and Mg (e.g., Blamey et al. 2015). Heenan and Campbell (1981) showed that the reduced concentration of Mg in the leaves of soybeans was due to effects of K and Mn on the rate of Mg root absorption. In addition, *A. opalus* leaves exhibited a low Fe: Mn ratio (~0.78) and a Fe: Mn ratio below 1.50 has been associated with an increasing Mn toxicity risk (Horuz et al. 2013). Overall, the relatively higher concentrations of Mn and K in *A. opalus*, associated with lower Ca, Mg and Fe: Mn ratio compared to optimal leaf values, suggest a possible Mn toxicity risk which could negatively affect the stress tolerance capacity in this species. This may be, at least in part, responsible for the trans-planting mortality rate (20%) observed in *A. opalus* compared to other tree species.

The positive interaction between leaf P and Ca has been attributed to the synergistic effects of these elements in sustaining the nutritional requirements for plant growth, being both elements involved in cell division processes (White et al. 2018; Yan et al. 2019). In contrast, the negative correlation between leaf P and C could be due to the role of this element in promoting the phloem export of non-structural carbohydrates from leaves to other plant tissues (Lambers et al. 2008) and the accumulation of mineral elements (Poorter and Bergkotte 1992). Moreover, previous works reported that P excess can induce Fe chlorosis in calcareous soils, suggesting the existence of a negative crosstalk between Fe and P nutrition (Lucena et al. 2018).

The positive correlations between leaf N concentration with C, Ca, Fe, K, Mg and Mn, highlight the existence of positive interactions among these elements. The positive correlation between C and N agrees with previous findings on broadleaf species (Liang et al. 2018). The high leaf N concentration in *T. platyphyllos* can be due to the high level of Mg and Fe, which could affect N uptake and metabolism as noticed also in the PCA biplot; the biplot also revealed an opposite trend for N, Mg and Fe concentration for *C. semperivirens* leaves. In detail, Fe plays a crucial role in N uptake, transport and translocation acting as metal cofactor of fundamental enzymes of the reductive assimilatory pathway, such as nitrate reductase (NR), nitrite reductase (NiR) and glutamate synthase (GOGAT) (Nasar et al. 2022). In addition, a decrease of N transport from roots to mature leaves under Mg deficiency was observed, while Mg supply enhanced N metabolism (Zhang et al. 2023). On the other hand, the low level of Mn and K could partly explain the low N concentrations in leaves of *A. rubrum*. Tao et al. (2023) found that Mn deficiency restricted uptake and transport of NO_3^- and inhibited the activities of several N-metabolism-related enzymes, such as NR and GOGAT. Moreover, leaf K content is strictly related to N metabolism, possibly due to a synergistic co-translocation effect between these elements

(Raddatz et al. 2020). Overall, our data suggest that Mn and K deficiency in *A. rubrum* led to a reduced N uptake and metabolism, explaining the relatively low leaf N concentration (Table 2) and the positive interaction of N with both Mn and K (Fig. 2).

Nutrient stoichiometry has been related to forest health status (Mcnulty et al. 1996). In particular, C:N: P stoichiometric ratios can mechanistically connect growth strategy, cellular processes, biochemical mechanisms and ecosystem functioning (Vrede et al. 2004). The C: N and C: P ratios are proxies of the plant efficiency in assimilating C when simultaneously adsorbing N and P, hence the interspecific variability in C: N and C: P suggests differences in N- and P-use efficiency (He et al. 2006). The N: P ratio has been widely used to investigate nutrient limitation. Values of N: P ratios below the minimum threshold of 10 and above the maximum threshold of 20 have been proposed to detect plant N and P limitations, respectively (Güsewell 2004). Our data showed N: P ratios far below 10 in *A. opalus*, *A. rubrum*, *Ulmus* 'Plinio' and *C. sempervirens*, indicating that all these species were N limited. Conversely, *T. platyphyllos* showed a value between 10 and 20, highlighting a co-limitation between N and P and a more balanced nutritional status.

The interspecific differences in leaf nutrient composition and stoichiometry affected the selected tree species' physiological performance and resource-use strategies, as suggested by the analysis of C and N stable isotopes. The variability in leaf $\delta^{15}N$ indicated possible differences in N source preferences and/or N metabolism among tree species (Evans 2001). Nitrogen can be absorbed mainly as nitrate (NO_3^-), ammonium (NH_4^+) and/or organic forms, depending on plant species (Templer and Dawson 2004). These N forms are generally isotopically different, being NH_4^+ generally ^{15}N enriched compared to NO_3^- due to N fractionation during the nitrification process. Subsequently, after N root uptake, the first step of nitrate assimilation is the reduction to nitrite (NO_2^-) by nitrate reductase activity. Hence, in addition to the possible fractionation associated with NO_3^- uptake itself, $\delta^{15}N$ in plant tissue can be affected by the fractionation associated with nitrate reduction (Tcherkez and Hodges 2008; Evans 2001). The positive relationship between $\delta^{15}N$ and N concentration across leaves of *A. rubrum*, *A. opalus* and *T. platyphyllos* suggests a ^{15}N enrichment with increasing N assimilation by leaves (Evans 2001). Moreover, Evans et al. (1996) found that tomato leaves were consistently enriched in ^{15}N compared to roots in plants growing with NO_3^- as N source due to the relative NO_3^- assimilation in leaves versus roots. The reduced concentration of both Mn and K in leaves of *A. rubrum* could severely impair the uptake and metabolization of NO_3^- (Tao et al. 2023), leading to the lowest values of both $\delta^{15}N$ and N concentration observed in its leaves. Therefore, the positive relationship between leaf $\delta^{15}N$ and N concentration in these species could be related to their different ability to uptake, translocate and reduce NO_3. The relatively higher leaf $\delta^{15}N$ values in *Ulmus* 'Plinio' and *C. sempervirens* suggest that these species can rely on ^{15}N-enriched sources (e.g., N depositions) or that their roots uptake stable organic N or inorganic N at greater soil depths (Evans 2007). Furthermore, the influence of adjacent N-fixing species and different contributions of N transferred to plants through mycorrhizal fungi can contribute to differentiating the N sources. Overall, these data highlight that $\delta^{15}N$ analysis can provide useful information on plant N economics and support the role of interspecific differentiation in N source exploitation as plant strategy to escape from competition with neighbors at community level (Fernandez et al. 2022).

Plant strategies may adjust to fluctuating resource availability by altering morphophysiological traits. Among these traits, a key role has been played by photosynthesis and WUE. Carbon isotope composition ($\delta^{13}C$) has been widely used to estimate the intrinsic WUE at plant and ecosystem level and to reveal differences in WUE among coexisting plant species and functional types (Scartazza et al. 2014). On the other hand, it has been shown that $\delta^{13}C$ can effectively represent a valuable proxy of seasonal changes in leaf stoichiometry (Du et al. 2021). The positive relationship between $\delta^{13}C$ and leaf N concentration has been previously observed in several other studies and linked to the major role of N on the photosynthetic capacity (Liang et al. 2020) leading to a decrease of C_i/C_a ratio and, hence, of C isotope discrimination during photosynthetic CO_2 uptake. The shift of the relationship in the evergreen *C. sempervirens* compared to the deciduous tree species, could be due to the different leaf economic spectrum and N partitioning between the photosynthetic apparatus and other leaf structural components (e.g., cell wall proteins) in high LMA leaves of coniferous species, lowering the N investment towards the photosynthetic apparatus (Zhang et al. 2020). The conservative strategy of *C. semprevirens* was supported by leaf gas exchanges. In fact, the relatively lower assimilation rate, associated with higher C_i, in *C. sempervirens* is possibly due to lower within-leaf CO_2 diffusion rates and thicker mesophyll cell walls (Warren and Adams 2004) that are typical of conservative species with high LMA and low leaf N concentration (Wright et al. 2004). Concerning the deciduous species, the differences in photosynthetic performance can be partly ascribed to altered leaf nutritional composition and balance. The lowest CO_2 uptake capacity was recorded in *Acer opalus* and, especially *A. rubrum*. It is worth noting that, notwithstanding the strong reduction of stomatal opening observed in *A. rubrum*, C_i was not significantly different from the other tree species, suggesting also a reduced CO_2 uptake capacity. Chen et al. (2018) found a reduction of net CO_2 photosynthetic rate in N-, P-, and K-deficient leaves of *Eustoma grandiflorum* mainly due to

stomatal closure, while N-deficient leaves showed also an increase of C_i. Hence, the lowest concentrations of both N and K in leaves of *A. rubrum* could contribute to its reduced photosynthetic capacity due to a combination of both stomatal and non-stomatal limitations. However, also the very low Mn concentration can play a major role in reducing CO_2 assimilation rate in this species. It is well known that Mn is essential as cofactor for the oxygen-evolving complex of the photosynthetic apparatus, catalyzing the water-splitting reaction in photosystem II (PSII), therefore Mn deficiency is generally associated with reduced photosynthetic activity (Alejandro et al. 2020). Conversely, the highest photosynthetic CO_2 uptake in *Ulmus* 'Plinio' and *T. platyphyllos* suggests an optimal nutrient balance at the leaf level to sustain their photosynthetic activity. This could be partly due to the high leaf Mg concentration found in their leaves, because of the dominant role of this element in photosynthesis. In fact, Mg is involved in chlorophyll formation and in key chloroplast photosynthetic enzymes, such as ATP synthase and ribulose-1,5-bisphosphate carboxylase/oxygenase (Tränkner et al. 2018). Moreover, Wang et al. (2018) proposed that Mg can enhance photosynthetic N- and P-use efficiency, highlighting a possible co-regulation of photosynthetic capacity by the interaction among N, P and Mg nutrition. It has been reported that a balanced N and Mg supply could promote chlorophyll synthesis, increase photosynthesis and regulate N metabolism, while an imbalanced N and Mg supply is generally reflected in a decreased photosynthetic capacity (Hauer-Jákli and Tränkner 2019). Calcium nutrition also affects both photosynthesis and N absorption and metabolism, by regulating stomatal movements and several photosynthetic proteins (Wang et al. 2019). Hence, the lower CO_2 assimilation rate in *A. opalus* than in *Ulmus* 'Plinio' and *T. platyphyllos* could be partly due to an unbalanced leaf nutrient composition due to an excessive Mn content associated with deficiency in both Mg and Ca. Another crucial element in regulating N absorption and CO_2 assimilation is P, which is an essential component of P-rich molecules, such as ATP and NADP, and is involved in the ribulose-1,5-bisphosphate (RUBP) regeneration affecting C uptake and N allocation to photosynthetic processes (Wang et al. 2018). Hence, leaf C: N:P stoichiometry is considered a functional trait related to photosynthetic performance and WUE (Sardans et al. 2012). Carbon isotope discrimination ($\Delta^{13}C$), calculated by leaf $\delta^{13}C$, has been extensively used to determine WUE through the well-known negative correlation between $\Delta^{13}C$ and WUE at plant and ecosystem level (Scartazza et al. 2014). Moreover, it has been shown that leaf stoichiometry and WUE, estimated by D, can be closely coupled (Du et al. 2021). One of the main advantages of using leaf D is due to the possibility of obtaining a time-integrated estimate of C_i/C_a and intrinsic WUE, weighted by net CO_2 assimilation rate, over different temporal scales compared to point measurements of leaf gas exchanges. In fact, the D values of structural C determined on dry matter of mature leaves allow to obtain an estimate of the average WUE over the entire growing season (Scartazza et al. 2014). Intrinsic WUE, which depends mainly on physiological variables, is considered a useful functional leaf trait for comparing plants across the world, while instantaneous WUE is influenced by the leaf-to-air vapor pressure difference but is a more direct quantification of the trade-off between carbon and water at leaf level. The interspecific differences in WUE were related to changes C: N:P stoichiometry. Previous research found an increase of plant WUE with increasing N and P concentration in plant biomass (e.g., Raven et al. 2004; while in other studies measurements of $\delta^{13}C$ indicated variations in WUE as a function of N availability, but not as a function of P availability (Garrish et al. 2010). Matimati et al. (2014) found that N: P ratio exhibits a strong relationship with hydraulic processes explaining 42% and 53% of the WUE variation, respectively. Moreover, Yan et al. (2016) showed that WUE was positively and negatively correlated with the N: P ratio and P concentration, respectively, when the plants were growing under N limitation conditions. Hence, the correlations found in the present study provided strong support for the hypothesis of a relationship between leaf N: P ratio and WUE. However, this relationship was stronger in the trees and under conditions that included a balanced N and P supply, partly supporting our results. *Tilia platyphyllos* was the only species showing a N: P value included between 14 and 16, indicating a co-limitation condition characterized by balanced N and P supply (Güsewell 2004). Accordingly, it was shown that when plants grow under co-limitation conditions for these nutrients, the relationship between N: P ratio and WUE is strong, with WUE explaining 34.01% of the variation in the N: P ratio (Yan et al. 2016).

5 Conclusions

This study, which links nutrient interaction and stoichiometry in the soil-plant system with plant physiological traits, allowed to define the strategies adopted by the five tree species to optimize resource acquisition and use under the same urban microclimatic and soil conditions (e.g., soil properties and alkaline pH), as well as their suitability as green infrastructure and ecosystem services provisioning. Leaf Mn concentration and C: N:P stoichiometry, together with C and N isotope composition, were found to be effective leaf traits related to plant nutritional and physiological status and can be proposed as useful indicators of soil-plant nutrient interaction and tree ecological strategy in species selection for optimal design, development and management of green infrastructure in urban areas. Specifically, *Ulmus* 'Plinio' and *T. platyphyllos* showed a more balanced nutritional

status and a better physiological performance than the other species in the tested local urban area. Conversely, *A. rubrum* and *A. opalus* were less adapted to the soil urban conditions and showed an altered leaf Mn nutrition that was reflected on the uptake of other essential elements, on N metabolism and, finally, on C: N:P stoichiometry. However, it is worth noting that our results were obtained on very young trees in the first growing season after planting and therefore reflect the short-term transient performance of the selected tree species in urban soil and microclimate conditions. Indeed, depending on the ecological strategies adopted by plants, some trees may take time to build their ecological niche depending on environmental changes and bidirectional biotic interactions among actors participating in the complex soil-plant system network. Therefore, further studies are needed to verify the long-term ecological strategies of these tree species to urban soil and microclimate conditions and the dynamic interactions in a complex system perspective.

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Declarations

Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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