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Original article

From lava to leaf: Physiological responses and trace element mobility in *Tilia cordata* L. trees grown in volcanic ash amended urban soil

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ABSTRACT

This study investigates the potential of utilizing volcanic ash (VA), classified as special waste, as an inorganic soil amendment to enhance tree growth and resilience in urban areas near volcanic regions. Lime trees were transplanted into pots filled with urban soil (Cnt) or amended with 10 % VA. Tree's physiological traits were monitored over the growing season. Notably, VA-treated trees showed improved net CO_2 assimilation (P_n) and stomatal conductance (g_s) during the leaf senescent stage. The analysis of ash, and Cnt and VA soils, showed low concentrations of trace elements. In addition, trace element accumulation in the leaves of VA trees was not observed. In summer, during a 12-day drought stress test, VA-stressed trees exhibited enhanced water absorption, reduced lipid peroxidation, and higher P_n and g_s values in the initial days compared to control-stressed trees. Importantly, the VA also promoted a 33 % larger tree root system, potentially enhancing drought resilience. This could offer an important advantage for trees, especially during the tree's critical establishment phase. Thus, VA could be a promising amendment for urban soils to bolster tree tolerance to drought.

1. Introduction

Volcanic ash (tephra particles ø < 2 mm) emissions during volcanic eruptions dramatically impact on urban and extra-urban infrastructure networks, resulting in damage to buildings, reduced street viability and closure of airports due to volcanic ash accumulation on airport runways. Currently, volcanic ash is considered waste and is disposed of in landfills, representing high costs for municipalities closely located to volcanic areas (Belfiore et al., 2020). There is no clear law on how to dispose ore recycle volcanic ash. The European Union defines volcanic ash as 'Municipal residues', and, in the specific, as "street-cleaning residues" (Cultrone et al., 2022). This kind of waste is designed as "unsuitable for reuse" and must be sent to authorized landfills. Moreover, gathering costs must be added to the disposal costs due to their rapid accumulation along the city streets during a volcanic eruption (Hayes et al., 2015; Jenkins et al., 2015). Recently, Catania, an Italian municipality, which faces this issue annually, has decided to attribute the European Waste Catalogue (EWC) relating to the type of "land and rocks" (EWC 170504), also to the ashes of Etna volcano resulting from the cleaning of extra-urban areas. This decision effectively paves the way for their reuse as inert materials. Several works have attempted to find innovative alternatives to reduce the disposal of ash in landfills, including the conversion of volcanic material into building blocks, adsorbent materials, manufacture or ceramic products (Belfiore et al., 2020; Belviso et al., 2021; Cultrone, 2022). Another potential alternative involves the use of raw volcanic ash as an inorganic soil amendment, as it can ensure soil fertility in areas located near volcanic zones (Fiantis et al., 2019). However, since volcanic ash may contain a plethora of elements (e.g., Si, Fe, Na, Zn, Cu, Cd, Cr, V, Hg, As) that can interact with the environment (e.g., water, soil) once deposited, is

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necessary to investigate the potential risks to human health (Bardelli et al., 2020; Barone et al., 2021). Moreover, the elemental composition of volcanic ash can vary from one volcano to another and even from one eruption to another (Cultrone, 2022; Fiantis et al., 2019).

Urban soils are usually of scarce quality, e.g. strongly alkaline and rich in trace elements (especially Cu, Zn, As, and Hg) due to the incorporation of building materials (Nehls et al., 2013; Yang and Zhang, 2015), soil compaction leading to a reduction in gas exchanges and water infiltration (Jim, 1993; Moore, 2012; Sax et al., 2017). These factors can inhibit the growth of urban shrubs and trees when transplanted in such soils, making them more prone to both biotic (insects, fungal parasites) and abiotic stressors (such as drought), especially for newly planted trees (Moore, 2012; Roman et al., 2015). The establishment phase is crucial for trees in urban areas because of their limited root dimensions and the scarce maintenance by municipalities (e.g., lack of pruning, irrigation, and fertilization) (Roman et al., 2015; Smith, 2019). In the Mediterranean region, drought is commonly encountered by plants during the summer period. Under drought conditions, plants may decrease the rate of CO_2 assimilation by stomata closure to minimize evapotranspiration and save water (Grassi and Magnani, 2005). This condition can be detrimental to photosynthetic processes, as excessive light energy absorbed by chlorophyll pigments can increase the risk of damage to the photosynthetic machinery, resulting in reduced growth rate and plant development (Cotrozzi et al., 2017; Guidi et al., 2019). Furthermore, if extended, drought conditions can be fatal for newly planted trees, which are already threatened by the unfavourable urban environment conditions (Haase and Hellwig, 2022; Moore, 2012).

In spite of the frequently mentioned ecosystem functions, such as enhancing air quality, capturing CO_2, mitigating the urban heat island effect and the related benefits for citizens (Klingberg et al., 2022; Solecki et al., 2005), there is still limited discussion within our administrations regarding the relationship between tree health and their ecosystem service efficacy. One potential approach to enhance urban soil properties involves the application of organic soil conditioners such as green composts, biochar, and composted sewage sludges (Ceccanti et al., 2022; Lo Piccolo et al., 2022; Sæbø and Ferrini, 2006; Sax et al., 2017). Additionally, inorganic soil conditioners can improve soil texture and positively affect porosity, thereby increasing soil permeability and aeration. However, research efforts aimed at assessing the actual benefits of inorganic soil conditioners on degraded urban soils and plants remain limited, often primarily focused on their potential role as heavy metal immobilisers (Huang et al., 2017; Sloan et al., 2012). Furthermore, to the best of our knowledge, there are no reports focused on volcanic ash as a potential soil conditioner for urban soils. Therefore, following two linked experimental trials, this study aimed to i) examine the physiology of a frequently planted tree in Italy (i.e., *Tilia cordata* L.) to the application of volcanic ash at a 10 % in an urban soil throughout an entire vegetative season; ii) evaluate the concentrations of elements in volcanic ash and amended soil, their leaching into water, and their possible translocation into tree leaves (experimental trial 1); iii) test the response of ash-amended trees to drought stress (experimental trial 2). This two-step experiment offers new perspectives on how the introduction of volcanic ash in urban soil influences both tree physiological responses and soil characteristics.

2. Materials and methods

2.1. Plant material, soil characteristics and experimental framework

Forty lime trees (*Tilia cordata* L.), each three years old and measuring 100 cm in height, were acquired from a plant nursery (Umbraflor, Perugia, IT). The trials were conducted under uncontrolled field conditions at the Department of Agriculture, Food, and Environment, University of Pisa, situated at coordinates 43°42′N; 10°25′E. Monthly average temperatures and precipitation levels during the experimental period can be found in Figure S1. The soil used was a sandy loam type, and it was collected in January 2022 near a construction site located at the Department of Agriculture, Food, and Environment, University of Pisa. Soil and volcanic ash characteristics are reported in Table 1. The experimental design was set up according to Lo Piccolo et al. (2022), with some modifications. In February, prior to the onset of leaf emergence, all trees were transferred into pots (9.5 L; ø 24 × H 24 cm). Then

Table 1
Volcanic ash, pure urban soil (Cnt) and amended with 10 % of volcanic ash (VA) physical and chemical attributes. The showed values represent the mean ± SD of three samples. An unpaired *t*-test was used to determine statistical differences between treatments (*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$). Abbreviations: Lq, limit of quantification; EC, electrical conductivity; CEC, cation exchange capacity.

Parameters	Volcanic ash alone	Cnt	VA	
Sand (%)	–	72	–	
Silt (%)	–	19	–	
Clay (%)	–	9	–	
pH	7.88 ± 0.08	8.31 ± 0.01	8.34 ± 0.01*	
EC (mS cm^{-1})	0.43 ± 0.05	0.99 ± 0.01	0.83 ± 0.04**	
CEC (cmol$^{(+)}$ kg^{-1})	<lq	8.78 ± 0.10	7.80 ± 0.30**	
P (mg kg^{-1})	0.66 ± 0.04	7.58 ± 0.19	5.32 ± 0.03***	***Assimilable***
Ca (g kg^{-1})	3.27 ± 0.08	0.61 ± 0.00	0.61 ± 0.00	
K (g kg^{-1})	0.10 ± 0.00	0.02 ± 0.00	0.05 ± 0.04	
Mg (g kg^{-1})	0.14 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	
Na (mg kg^{-1})	70.90 ± 0.60	8.03 ± 0.32	8.20 ± 0.36	
Ca (g kg^{-1})	11.56 ± 0.30	28.61 ± 0.10	21.49 ± 0.10***	***Total***
K (g kg^{-1})	5.46 ± 0.04	1.93 ± 0.22	2.19 ± 0.08	
Mg (g kg^{-1})	4.62 ± 0.06	5.43 ± 0.13	4.42 ± 0.08***	
Na (g kg^{-1})	4.23 ± 0.08	0.32 ± 0.03	0.70 ± 0.04***	
Cd (mg kg^{-1})	<lq	<lq	<lq	
Cr (mg kg^{-1})	<lq	45.07 ± 0.05	25.93 ± 0.95***	
Cu (mg kg^{-1})	54.60 ± 3.98	44.27 ± 0.94	57.77 ± 1.88***	
Fe (g kg^{-1})	16.51 ± 0.34	14.81 ± 0.44	15.34 ± 0.28	
Mn (g kg^{-1})	0.40 ± 0.00	0.62 ± 0.012	0.61 ± 0.00	
Ni (mg kg^{-1})	<lq	78.34 ± 1.25	35.87 ± 3.74***	
Pb (mg kg^{-1})	<lq	21.11 ±1.92	25.29 ± 1.91	
V (mg kg^{-1})	68.68 ± 2.74	18.60 ± 1.05	22.17 ± 2.59	
Zn (mg kg^{-1})	86.48 ± 1.64	65.94 ± 1.10	80.66 ± 0.87***	
Hg (mg kg^{-1})	0.53 ± 0.03	<lq	<lq	
As (mg kg^{-1})	5.04 ± 0.06	4.05 ± 0.10	3.90 ± 0.09	
Cd (mg kg^{-1})	<lq	<lq	<lq	***H_2O***
Cr (mg kg^{-1})	<lq	<lq	<lq	
Cu (mg kg^{-1})	2.10 ± 0.10	<lq	<lq	
Fe (mg kg^{-1})	866.20 ± 8.51	<lq	<lq	
Mn (mg kg^{-1})	46.80 ± 1.05	<lq	<lq	
Ni (mg kg^{-1})	<lq	<lq	<lq	
Pb (mg kg^{-1})	<lq	<lq	<lq	
V (mg kg^{-1})	0.70 ± 0.10	<lq	<lq	
Zn (mg kg^{-1})	2.33 ± 0.15	<lq	<lq	
Hg (mg kg^{-1})	0.02 ± 0.00	<lq	<lq	
As (mg kg^{-1})	0.01 ± 0.00	<lq	<lq	

trees were assigned to two different group treatments (twenty trees per treatment): not amended (Cnt) and amended with 10 % volcanic ash (*v/v*; VA). Volcanic ash was taken from an eruption of Mt Etna (37° 59' 35" N; 15° 04' 54" E) that fell in Mascalucia (Catania, Italy) area on 20 November 2021. Throughout the trial, all the trees were well-irrigated without the application of fertilization. Two experimental trials were carried out simultaneously to investigate the physiological evaluation of volcanic ash amendment during the three-growth stages of plant trees (experimental trial 1), and the response of volcanic ash amended trees to drought stress (experimental trial 2).

In experimental trial 1, physiological analyses (i.e., gas exchange, chlorophyll a fluorescence and chlorophyll content on leaves) were conducted at the young (leaf emergence, April), mature (July), and senescent (October) leaf stages. Subsequently, destructive leaf analyses were performed at the leaf senescent stage (October). Samples of Cnt and VA soils were sampled before the start of the experiment (February). At the conclusion of the experimental trial 1 (November), a portion of the plants was allocated for destructive analyses (i.e., tree biomass). In experimental trial 2, the drought stress trial was executed during the latter part of July, recognised as Italy's hottest and driest month (Fig. S1). The pots were water-saturated, after which the irrigation was withheld for a continuous period of twelve days in 8 trees per treatment, Cnt-S and VA-S, representing the stressed treatments for the control and volcanic ash amended treatment, respectively. Physiological assessments were conducted at regular intervals (3-day intervals) until the twelfth day. Leaf samples for the analysis of lipid peroxidation were gathered at the final drought stress period (T4) at midday, swiftly frozen in liquid nitrogen, and preserved at −80 °C until analyses. In experimental trial 1, sub-samples of soil were gathered from twelve pots (depth of up to 20 cm). Subsequently, four sub-samples were combined to achieve homogeneous soil samples (n = 3).

2.2. Biomass parameters

In November, the tree height was measured, while measurements of stem diameter were taken at the root collar level, 50 cm, and 100 cm from the ground, for both the Cnt and VA trees (n = 5) with a calliper. The plant materials, including stem, branches, and root components, were individually separated and their fresh weight (FW) was determined (n = 5). Subsequently, these plant portions were subjected to a drying process using an electric thermostatic oven (Memmert UN30, Germany) at a temperature of 105 °C until a constant weight was reached, which took approximately 4 days. The dry weight (DW) was then quantified.

2.3. Physiological analyses

Gas exchange analyses (n = 10 for experimental trial 1; n = 7 for experimental trial 2) were conducted, using an infrared gas analyzer (Li-cor 6400; Li-cor, Lincoln, NE, USA), between 11:00 and 14:00 (GMT) on randomly chosen leaves (one per plant) with a light intensity of 1600 µmol m^{-2} s^{-1}. A constant CO_2 concentration of 400 µmol mol^{-1} inside the leaf chamber was maintained, and the flow rate was set at 500 µmol s^{-1}. Once a steady state was attained, various parameters, including the net CO_2 assimilation rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and intrinsic water use efficiency (WUE_{int}), were analysed. Chlorophyll *a* fluorescence parameters were measured using a Plant Efficiency Analyzer fluorimeter (Handy PEA, Hansatech Ltd, Norfolk, UK). Measurements (n = 5) were conducted on randomly chosen leaves (one per plant), simultaneously with gas exchange analysis according to Lauria et al. (2023).

The chlorophyll content (Chl) in leaves was determined utilising a leaf clip sensor DUALEX® (Force-A, Orsay, France). In experimental trial 1 (April to October), six trees were randomly chosen, and thirty randomly selected leaves (5 per plant) were measured at each leaf stage at 12:00 (GMT). In the Exp. 2, the same number of leaves were analysed at each sampling time.

2.4. Soil water loss and leaf water potential

In experimental trial 2, soil water loss was determined for each treatment (n = 3) by weighing plants at each sampling time. Midday water potential (Ψ_{MD}) was assessed on fully expanded leaves (one per plant; n = 4). For the analyses, a Scholander pressure chamber (model 600, PMS Instrument, Albany, OR, USA) was used.

2.5. Physico-chemical characterization of soil

The soil samples were sieved through a 2 mm mesh and further homogenized and analysed for soil characterization. Physical and chemical properties of the soil (pH, texture, CEC) were determined by standard methods (SSSA, 1996).

2.6. Element concentrations in soil and leaves of trees grown in volcanic ash amended soil

Micro and macro-element concentration were determined in volcanic ash, in control and volcanic ash amended soil, and in leaves.

A total of 0.5 g dried soil or leaf samples (two leaves per sample; n = 3) were powdered and mineralized by microwave-assisted digestor (90 min at 200 ∘C; Milestone Start D, Sorisole (BG), Italy) using a solution of HNO_3 (70 %; v/v) and H_2O_2 (30 %; v/v) (2.5:1; v-v). The elements (Ca, K, Mg, Na, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn, As, Hg) were determined using an ICP-EOS 5900 Agilent (Agilent Technologies, Santa Clara, USA). Data are expressed as mg element per kg FW.

Available (in soil samples) and total (in soil and leaf samples) P was determined colorimetrically by the modified molybdenum blue method on acid extracts (SSSA, 1996).

The evaluation of elements solubility was determined using ad ICP-EOS 5900 Agilent (Agilent Technologies, Santa Clara, USA) after a water extraction with a soil: extractant ratio of 1:5 and stirring time of 3 h step (Petruzzelli et al., 2004). The extracts were analyzed after centrifugation and filtration.

2.7. 2-thiobarbituric acid reactive substances (TBARS) assay

The TBARS assay (n = 3) was based on the procedures reported by Landi, (2017). For the extraction, 200 mg of leaves were homogenized with 5 mL of 0.1 % (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 5500 rpm for 10 min at 4 °C. After centrifugation, the supernatant was separated from the pellet and used for the determinations.

For the analysis, leaf extracts (1 mL) were put into a vial containing 1 mL of 80 % (v/v) aqueous ethanolic solution with the addition of 20 % (w/v) TCA, or the same mixture with the addition of 0.5 % (w/v) TBA (w/v). Every solution underwent incubation at 90 °C for a duration of 30 min, with the reaction being stopped by placing the vials on ice. Malondialdehyde (MDA) equivalents were determined by measuring spectrophotometric absorbance at 532 nm, with any potential interference from other compounds assessed at 440 and 600 nm. The calculation of MDA equivalents took into account the MDA extinction coefficient of 157,000 M^{-1} cm^{-1}.

2.8. Statistical analysis

The normality of the data was assessed using the Shapiro-Wilk test, and the homoscedasticity was examined using Bartlett's test. Data collected from physiological analyses and water balance assessments were subjected to two-way ANOVA, with volcanic ash treatments and time as the source of variation. MDA analysis was analyzed by one-way ANOVA, with volcanic ash treatment as the source of variation. All the means were separated by Fisher's least significant difference (LSD) *post-hoc* test ($P \leq 0.05$). Biometric features, soil parameters, and element concentrations were compared by Student's *t*-test. The software used for the statistical analyses was GraphPad (GraphPad, La Jolla, CA, USA).

3. Results

3.1. Experimental trial 1

3.1.1. Morphological traits of trees

In November, VA trees exhibited larger stem diameters in comparison to Cnt trees, with a 22 % increase at the tree height of 50 cm and a 50 % increase at the tree height of 100 cm. Furthermore, when considering dry weight, root and stem biomasses were notably greater in VA-treated trees than Cnt trees (Table 2). VA trees had a 33 % improvement in root biomass and a 20 % increase in stem biomass, resulting in an overall 27 % increase in total tree biomass compared to Cnt trees. However, the two treatments had no significant differences in dry matter (%).

3.1.2. Physiological analyses and leaf chlorophyll contents

P_n showed the highest values at the mature leaf stage in both treatments, then decreased in both treatments during the leaf senescent stage, with a more pronounced decrease in Cnt trees than in VA trees (–51 in Cnt and –38 % in VA trees compared to mature stage, respectively; Fig. 1a). Statistically significant differences in g_s were only reported at the leaf senescent stage, where g_s decreased in Cnt trees compared to young and mature leaves, and was also lower compared to VA trees (–26 %; Fig. 1b). In both treatments, C_i reaches its lowest values at the mature stage, intermediate values at the young stage, and the highest values at the senescent leaf stage (Fig. 1c). WUE_{int} values were highest at the mature leaf stage and lowest at both young and senescent leaf stages (Fig. 1d). Significant differences were observed in F_v/F_m values among the different leaf stages, with significantly lower values recorded in young leaves compared to mature and senescent leaves (Fig. 1e). However, no differences were observed in VA *vs* Cnt trees throughout the entire vegetative season. Leaf chlorophyll contents peaked at the mature leaf stage in both treatments. Interestingly, no differences were observed between young and senescent leaf stages (Fig. 1f). Differences between treatments were noted at the young and senescent leaf stage, where VA leaves showed lower values compared to Cnt leaves (–19 and –15 % at young and senescent leaf stage, respectively; Fig. 1f).

3.1.3. Leaf element content at the senescent stage

Senescent leaves of VA trees showed −41 % of K and −7 % of Na contents in comparison to Cnt trees (Table 3). Conversely, +19 % of Mg content was found in VA senescent leaves compared to Cnt trees. No harmful accumulation in trace elements or statistical differences between treatments were found for other analyzed elements.

Table 2
Morphological traits (height, diameters and biomass parameters), of trees (*Tilia cordata* L.), measured in November. Trees were grown with 0 (Cnt) or 10 % of volcanic ash (VA). An unpaired *t*-test was used to determine statistical differences between treatments (*: $P \leq 0.05$; **: $P \leq 0.01$).

Parameters		Treatments Cnt	VA
Height	(cm)	100.0 ± 2.4	106.0 ± 5.8
$Ø_{collar}$	(cm)	2.3 ± 0.3	2.1 ± 0.1
$Ø_{50\ cm}$	(cm)	0.9 ± 0.1	1.1 ± 0.0**
$Ø_{100\ cm}$	(cm)	0.4 ± 0.1	0.6 ± 0.0**
Root	FW (g)	134.0 ± 25.3	182.0 ± 23.3*
	DW (g)	59.9 ± 10.0	79.6 ± 10.9*
	DM (%)	44.9 ± 2.7	43.7 ± 2.1
Stem	FW (g)	98.4 ± 21.9	117.0 ± 27.5
	DW (g)	46.7 ± 8.3	56.0 ± 1.7*
	DM (%)	47.8 ± 2.4	48.0 ± 1.5
Branches	FW (g)	4.2 ± 1.2	4.1 ± 1.5
	DW (g)	1.8 ± 0.5	1.7 ± 0.6
	DM (%)	42.3 ± 3.6	41.4 ± 1.2
Total biomass	FW (g)	236.6 ± 40.1	303.1 ± 41.1*
	DW (g)	108.4 ± 16.7	137. 3 ± 19.0*

3.2. Experimental trial 2: drought stress

3.2.1. Soil water loss and midday leaf water potential

At T0, water-saturated pots of both Cnt- and VA-S treatments showed no statistically different weights (*data not shown*). From T1 to T4, the percentage of soil water decreased in both -S treatments (Fig. 2a). Nevertheless, VA-S soils exhibited elevated percentages of soil water loss compared to Cnt-S soils.

At T1, the Ψ_{MD} decreased only in the Cnt-S treatment when compared to its respective controls, but no statistical differences were observed between stressed treatments (Fig. 2b). At T2, T3, and T4, ΨMD values continued to decline in both -S treatments compared to their respective controls, reaching values of approximately –2.5 MPa at T4.

3.2.2. Physiological analyses and leaf chlorophyll contents

At T1, the P_n values declined in Cnt-S and VA-S in comparison to their corresponding controls. However, smaller reductions were observed for VA-S trees, with decreases of –40 % and –26 % for Cnt-S and VA-S, respectively (Fig. 3a). At T2, P_n values in both -S trees were further reduced compared to their respective controls (–81 % in Cnt-S and –72 % in VA-S) showing no differences between the -S treatments. The net CO_2 assimilation rate in both treatments under stress conditions reached its minimum values at T3 and T4 (approximately a 96 % reduction on average compared to the controls). The g_s parameter exhibited a comparable pattern to Pn, with the sole distinction between the -S treatments noted at T1. During this sampling time, VA-S trees demonstrated a lesser reduction in g_s compared to Cnt-S (a reduction of 24 % and 60 % in comparison to their respective controls, respectively; Fig. 3b). C_i values experienced a decrease solely in Cnt-S at T1 (a reduction of 14 % compared to Cnt trees; Fig. 3c). At T2, C_i values decreased in VA-S (–7 % compared to VA trees), while in VA-S, C_i values started to increase. At both T3 and T4, C_i values were highest in both -S treatments compared to their respective controls (~+14 % in -S treatments compared to controls). The WUE_{int} parameter increased at T1 only in Cnt-S compared to Cnt trees by 54 % (Fig. 3d), while VA-S trees increased the WUE_{int} at T2 (+26 % compared to VA trees). At T3 and T4, both -S treatments showed very low WUE_{int} values compared to respective controls (~–43 % in -S treatments compared to controls). At T1, F_v/F_m data were lower in Cnt-S with respect to their controls (–9 % in -S treatments compared to controls), but values were not statistically different from VA-S trees (Fig. 3e). The major variations in F_v/F_m were observed in -S compared to respective control trees starting from T3 (9 days of drought stress), with a reduction of –11.9 and –16.0 % in Cnt-S and VA-S trees, respectively (Fig. 3e). After 12 days of treatment (T4), the decrease in F_v/F_m reached –59.9 and –53.5 % in Cnt-S and VA-S trees, respectively. However, no variation in F_v/F_m occurred between Cnt-S and VA-S trees during the entire duration of the experiment. Chlorophyll content started to constantly decrease in both -S treatments from T1 (–12 and –16 % for Cnt-S and VA-S compared to their relative controls, respectively; Fig. 3f) reaching the lowest contents at both T3 and T4 (~–33 % in -S treatments compared to controls).

3.2.3. Lipid peroxidation level

At T4 the levels of lipid peroxidation were higher in both Cnt-S and VA-S compared to their respective controls. However, lipid peroxidation levels were higher in Cnt-S trees than in VA-S trees, showing an increase of +86 % and +56 % for Cnt-S and VA-S, respectively, compared to their relative controls (Fig. 4).

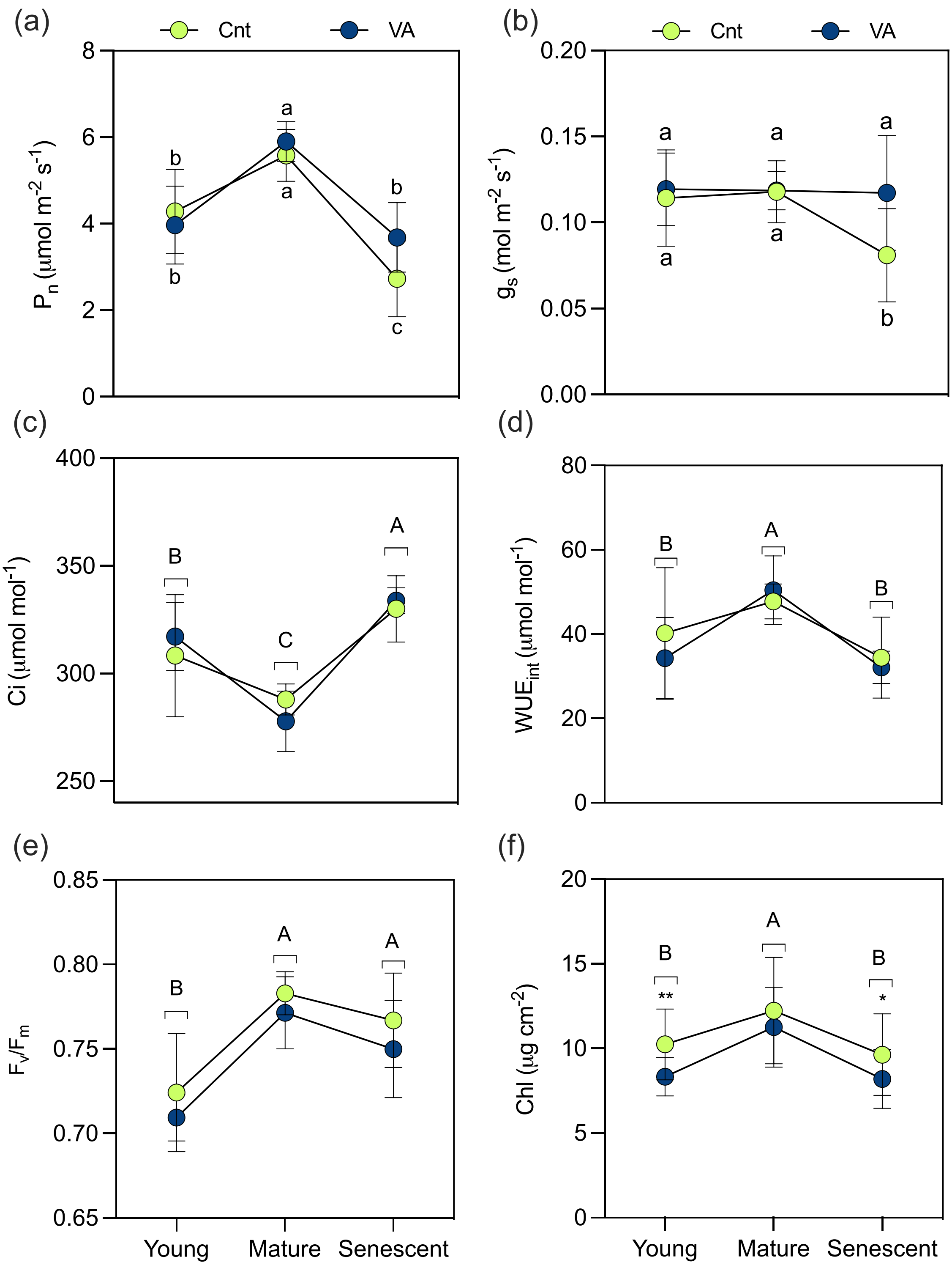

Fig. 1. Gas exchanges, the maximum quantum yield of photosystem II (PSII), and chlorophyll concentration measured in leaves of controls (Cnt) and 10 % volcanic ash amended (VA) trees (*Tilia cordata*) at young, mature and senescent leaf stage. The parameters analyzed were: net CO_2 assimilation (P_n; a), stomatal conductance (g_s; b), intercellular CO_2 concentration (C_i; c), intrinsic water user efficiency (WUE_{int}; d), PSII maximum photochemical efficiency (F_v/F_m; e), and leaf chlorophyll content (Chl; f). Means ± SD were subjected to two-way ANOVA with volcanic ash treatment and sampling time as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$). When the interaction F ratio is not significant, capital letters indicate significant differences among means over leaf stages, while asterisks (*: $P \leq 0.05$; **: $P \leq 0.01$) indicate differences between treatments.

Table 3
Ca, K, Mg, Na, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn, Hg, As and P concentrations in leaves of senescent *Tilia* leaves grown with 0 (Cnt) or 10 % of volcanic ash (VA). The showed values represent the mean ± SD of three samples. An unpaired *t*-test was used to determine statistical differences between treatments (*: $P \leq 0.05$; **: $P \leq 0.01$). Abbreviation: lq, limit of quantification.

Elements	Cnt	VA	
P (mg kg^{-1})	112.28 ± 14.21	138.69 ± 13.41	*Total*
Ca (g kg^{-1})	17.48 ± 1.15	17.55 ± 0.29	
K (g kg^{-1})	5.53 ± 1.20	3.25 ± 0.27*	
Mg (g kg^{-1})	3.70 ± 0.01	4.41 ± 0.17**	
Na (g kg^{-1})	0.56 ± 0.01	0.52 ± 0.00**	
Cd (mg kg^{-1})	<lq	<lq	
Cr (mg kg^{-1})	<lq	<lq	
Cu (mg kg^{-1})	3.02 ± 0.48	3.03 ± 0.46	
Fe (mg kg^{-1})	101.04 ± 18.38	124.18 ±19.97	
Mn (mg kg^{-1})	19.23 ± 3.80	15.14 ± 3.08	
Ni (mg kg^{-1})	<lq	<lq	
Pb (mg kg^{-1})	<lq	<lq	
V (mg kg^{-1})	<lq	<lq	
Zn (mg kg^{-1})	4.95 ± 2.19	3.03 ± 1.70	
Hg (mg kg^{-1})	<lq	<lq	
As (mg kg^{-1})	<lq	<lq	

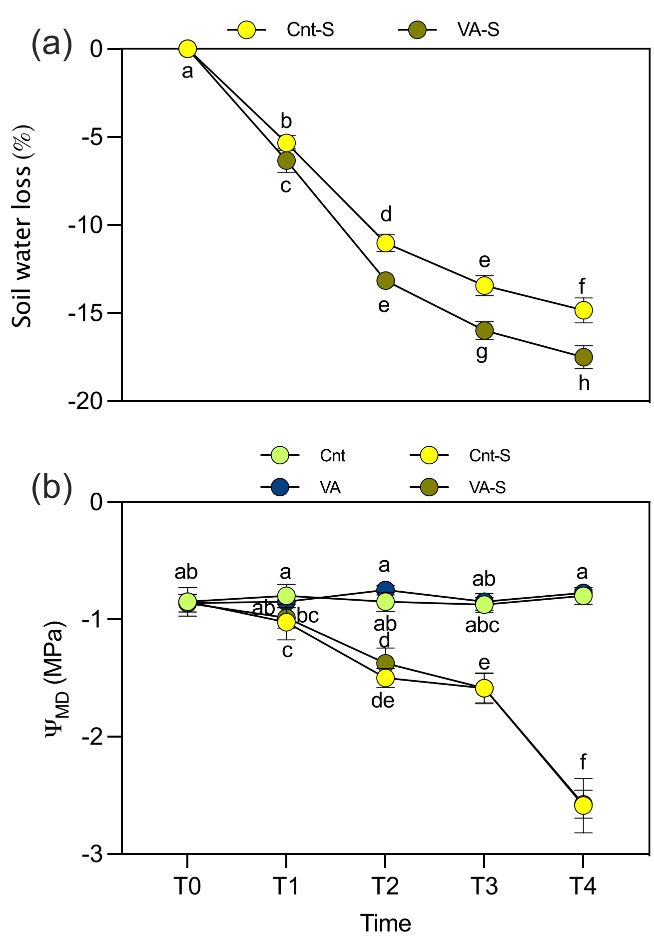

Fig. 2. Soil water loss (a) and leaf midday water potential (Ψ_{MD}; b) analyzed in controls (Cnt), drought-stressed controls (Cnt-S), 10 % volcanic ash amended (VA) and drought-stressed 10 % volcanic ash amended (VA-S) trees (*Tilia cordata*), analysed at T0, and every 3 days of drought stress (T1, T2, T3 and T4). Means (± SD; n = 3 and n = 4 for soil water loss and $\Psi_{MD,\ respectively}$) underwent a two-way ANOVA with volcanic ash treatment and sampling time as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$).

4. Discussion

4.1. Experimental trial 1

4.1.1. Volcanic ash as a possible inorganic conditioner for urban soils

Volcanic eruptions are powerful events capable of releasing into the atmosphere vast amounts of ash, which eventually settles over large areas. The dispersion of VA often challenges affected areas, particularly in terms of cleanup and disposal. To the best of our current knowledge, the utilization of VA as an inorganic soil conditioner in urban environments remained an unexplored avenue.

A primary concern associated with the use of volcanic material in urban environments is the potential presence of trace elements, which could exacerbate the contamination level in an already potentially polluted environment, such as urban soil. Indeed, volcanic ash may contain harmful amounts of trace elements, including Zn, Cu, Cd, Cr, V, Hg, Pb, and As (Bardelli et al., 2020; Barone et al., 2021). In our experiment, the utilized volcanic ash did not contain harmful levels of trace elements and consequently did not pollute amended VA soil. The concentrations of these elements (Table 1) remained below the threshold values set by the Finnish Ministry of Environment (MEF, 2007), which closely aligns with the mean values observed in various European national systems (Tóth et al., 2016). Our findings are in line with a work conducted by De La Rosa et al. (2023), in which soil amended with 5 % VA consistently exhibited trace element levels below the maximum permitted concentrations set by European regulations for soil amendments. Furthermore, in our experiment, the concentrations of trace elements dissolved in water were below the detection limit of the instrument, indicating a very low risk for these elements being released into the urban environment.

A second concern encompasses the potential uptake and translocation of trace elements to the leaves, potentially creating a pollution hazard in urban environments, especially for deciduous trees. Previous studies have indicated that trace elements can be transferred from the contaminated soil to the various tree organs (Liang et al., 2017; Olowoyo et al., 2010; Patel et al., 2022). In our experiment, no increase in harmful trace elements content in *Tilia* leaves was observed; however, further research is required since the translocation of these elements depends not only on the trace element content in soil, but also on the specific plant species (De La Rosa et al., 2023; Patel et al., 2022; Rajput et al., 2021).

Despite the understanding of the environmental risks, a fundamental question remains: how do plants respond to volcanic ash amendments? To date, no studies have documented the impact of such amendment on the photosynthesis processes of tree species. Two works were conducted on *Picea* species by comparing natural volcanic soil to forest brown soil (Ishii et al., 2007, 2003). In these two studies, volcanic soil, being nutrient-poor, led to a decrease in tree growth and CO_2 assimilation in both analyzed species. However, in our study, the addition of volcanic ash did not significantly affect nutrient content. Furthermore, physiological responses (eg., P_n, g_s, C_i, WUE_{int}, F_v/F_m and Chl) for most of the growing season were similar between VA-treated trees and their controls, indicating the absence of apparent plant stress due to VA amendment.

Typically, during the leaf senescent stage, both stomatal and non-stomatal limitations contribute to a reduced photosynthetic capacity in leaves (Grassi and Magnani, 2005). At this stage, the higher values in net CO_2 assimilation found in VA-treated leaves were attributed to an improved stomatal conductance, as no differences were found in apparent CO_2 carboxylation efficiency values between treatments (Fig. S2). This suggests few hydromechanical limitations during this leaf ontogenetic stage. Such physiological adaptations, indicating enhanced stomatal functionality, can be associated with broader changes within the plant, especially when considering shifts in plant biomass. The root system is crucial for stabilizing plants and optimizing water and nutrient absorption (Rogers and Benfey, 2015), and the significantly greater root

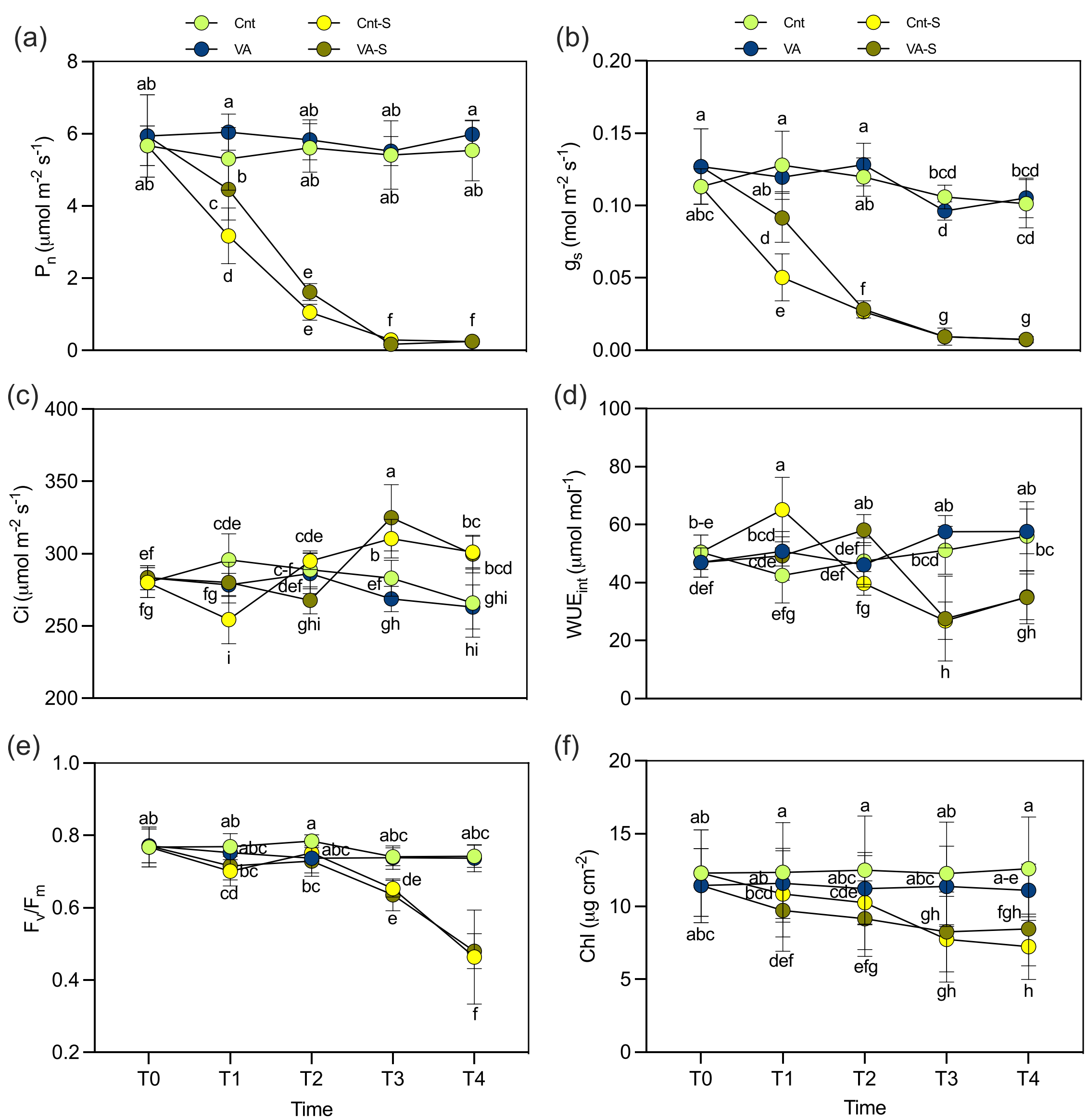

Fig. 3. Leaf gas exchanges, maximum quantum yield of photosystem II (PSII), and and chlorophyll concentration measured in leaves of controls (Cnt), drought-stressed controls (Cnt-S), 10 % volcanic ash amended (VA) and drought-stressed 10 % volcanic ash amended (VA-S) trees (*Tilia cordata*), measured at T0, and every 3 days of drought stress (T1, T2, T3 and T4). The parameters analyzed were: net CO_2 assimilation:(P_n; a), stomatal conductance (g_s; b), intercellular CO_2 concentration (C_i; c), intrinsic water user efficiency (WUE_{int}; d), PSII maximum photochemical efficiency (F_v/F_m; e), and leaf chlorophyll content (Chl; f). Means were subjected to two-way ANOVA with volcanic ash treatment and sampling time as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$).

biomass found in VA-treated trees contributes to improved plant water balance, as also reported in the following experimental trial 2.

4.2. Experimental trial 2

4.2.1. VA amendment improved the tolerance of lime trees to drought stress

One of the pressing environmental challenges faced by plants in the urban environment is drought stress (Haase and Hellwig, 2022; Lüttge and Buckeridge, 2023). Drought stress refers to the adverse effects on plant metabolism that impact growth and development (e.g., chlorophyll contents, CO_2 assimilation, plant hydraulic balance), resulting from insufficient water availability (Lo Piccolo et al., 2022; Moore, 2012). The severity of these effects is heightened in urban environments due to factors such as poor soil conditions and elevated temperatures. In fact, newly planted trees are particularly susceptible to perishing due to these extreme conditions (Haase and Hellwig, 2022). To the best of our knowledge, no studies have been conducted to examine the impact of VA amendment on plants experiencing drought stress. Therefore, this report is the first of its kind, investigating the effectiveness of this potential soil conditioner. In our experimental trial, at T1 (early drought), both VA- and control-S trees responded to drought conditions, with the -S trees exhibiting lower Ψ_{MD} values compared to the relative controls. Simultaneously, diminished stomatal conductance restricted the rates of net CO_2 assimilation in both -S treatments, albeit to a lesser extent in VA-S trees. This is a typical first plant response to drought stress, in which decreasing stomatal conductance, leads to an elevated diffusion resistance for CO_2, ultimately reducing the CO_2 assimilation (Fang and Xiong, 2015). Notably, the Cnt-S trees demonstrated improved WUE_{int} at T1, suggesting that they were experiencing greater stress than the VA-S trees. They adapted by temporarily reducing transpiration to save water and counteract the stress amplitude (Zhang et al., 2015). The same mechanism was adopted by VA-S trees three days later (T2), highlighting a delayed physiological adjustment and a heightened tolerance to drought stress. Indeed, the VA-S trees were more effective at utilizing the

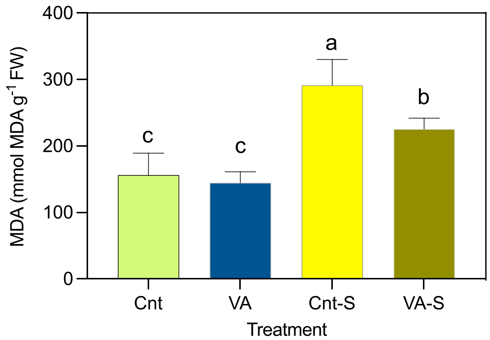

Fig. 4. Malondialdehyde (MDA) content in controls (Cnt), drought-stressed controls (Cnt-S), 10 % volcanic ash amended (VA) and drought-stressed 10 % volcanic ash amended (VA-S) trees (*Tilia cordata*), measured at 12 days (T4) of drought stress. Means were subjected to one-way ANOVA with volcanic ash treatment as the source of variation. Means with different letters are significantly different after Fisher's LSD post-hoc test ($P \leq 0.05$).

available soil water than Cnt-S trees, as suggested by the higher water loss from the VA-S pots compared to the Cnt-S pots. This was likely due to a more developed root system that covered a larger surface area (Rogers and Benfey, 2015). It is conceivable that the VA amendment facilitated root system growth by improving soil physical characteristics such as soil porosity, thereby decreasing soil resistance to root penetration. Existing literature supports the idea that volcanic soils typically have low bulk densities, attributed to the development of highly porous soil structure (due to the presence of VA), providing an optimal environment for root growth (Nanko et al., 2014; Nanzyo et al., 1993).

Under prolonged drought stress conditions (T3 and T4), additional factors besides stomatal limitations to CO_2 assimilation (e.g., photochemical and biochemical factors) become the primary limitations to the photosynthetic performance (Flexas et al., 2004; Lo Piccolo et al., 2022). In our experiment, starting from T3, the alterations in PSII photochemical efficiency, coupled with biochemical limitations, as highlighted by increased C_i levels in both -S treatments (Li et al., 2017), contributed to the reduced rates of P_n in both -S treatments. This could further endanger the photosynthetic process, leading to an escalation of oxidative stress reactions (Flexas et al., 2004; Hussain et al., 2019; Lo Piccolo et al., 2022). Accumulation of excessive Reactive Oxygen Species (ROS) can result in cellular damage, affecting components such as membrane lipids, proteins, and the nucleus, potentially culminating in cell death (Hussain et al., 2019; Reddy et al., 2004). As a result, the increase in MDA contents in drought-stressed trees, encompassing both Cnt-S and VA-S groups, was expected. However, our findings confirm that prolonged water stress primarily disrupts the redox balance in the leaves of Cnt-S trees. This renders plants more susceptible to photooxidative damage, which could, in turn, result in a slower plant recovery following possible irrigation (Kirova et al., 2021; Lo Piccolo et al., 2022). In an urban environment, and especially in the global climate change era, even few additional days of tolerance to drought can determine the success or failure of newly planted trees.

5. Conclusion

In the present study, a two-step experiment was conducted to elucidate the impacts of VA soil amendments on the concentration of potentially toxic trace elements in soil and in trees, thus affecting their physiology. Although a small sample size was used for the soil analyses, our findings reveal no significant harmful increase in trace element concentrations in the soil or in the leaves of trees grown in VA amended soil. The inclusion of VA appears to have positively influenced the development of the root system, possibly by reducing resistance to root penetration, although this hypothesis warrants further investigation. The enhanced root system growth of trees grown in VA amended soil improved water uptake, mitigating the drought stress effects by approximately three days compared to control trees. Taken together, these findings support the use of VA in urban soils as a sustainable alternative to the "conventional" landfill disposal. Simultaneously, it enhances soil properties for newly planted trees.

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Declaration of 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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ufug.2024.128458.

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