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ASSESSING PHOTOSYNTHETIC EFFICIENCY IN ORNAMENTAL URBAN SPECIES

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ABSTRACT – Urban green spaces (UGSs) are ecological systems that provide the connection between cities and nature. UGSs offer several ecosystem services (ESs) not only in relation to their aesthetic and recreational values but also for their effects on life's quality, health and wellbeing. These abilities are essential to respond to the challenge of global climate change, but the effectiveness of ESs provided by UGSs depends on their health status. Chlorophyll fluorescence (ChlF) is one of the main methodological approaches to study "urban plant physiology". Here, a review of the studies on the methodological application of ChlF in urban environment is reported and detailed in order to evaluate the potential of this technique in assessing the performance of urban vegetation and its ESs. The reviewed literature (1994-2015) concerning the use of ChlF for the detection of photosynthetic efficiency in UGSs reveals that this technique is (i) increasingly used in researches performed in urban environments; (ii) suitable for several experimental conditions; (iii) suitable for every types of vegetation, allowing the screening of species and genotypes with tolerance to the urban environment; (iv) a powerful tool to asses urban plant stress conditions *in vivo*; (v) based on several methods and instruments usable in urban environments.

KEYWORDS: CHLOROPHYLL FLUORESCENCE, ECOSYSTEM SERVICES, URBAN GREEN SPACES, URBAN PLANT PHYSIOLOGY, URBAN ENVIRONMENT

INTRODUCTION

Urban Green Spaces

An urban area is a living complex mega-organism associated with a host of inputs and outputs, such as heat, energy, materials and others (Decker et al., 2000). To date, 75% of the population in developed countries lives in urban settlements (World Bank, 2013). The rapid growth in urban population [since 1950, from 746 million to 3.9 billion in 2014 (United Nations, 2014)] and the economic development contribute to the high complexity and the dynamism of these environments.

Urban green spaces (UGSs) are ecological systems that provide the connection between cities and nature, where the combinations of different functions are conserved and coexist. UGSs include parks and reserves, sporting fields, greenways and trails, community gardens, street trees, and nature conservation areas, as well as less conventional spaces such as green walls, green alleyways and cemeteries (Wolch et al., 2014). UGSs offer several ecosystem services (ESs) not only in relation to their aesthetic and recreational values, but also for their effects on life's quality, health and wellbeing (Lopucki & Kiersztyn, 2015). These abilities are essential to respond to global climate change which will involve higher temperature, lower air humidity and soil water availability, as well as higher levels of air pollutants (Cotrozzi et al., 2016). However, in order to meet social and psychological needs of citizens, UGSs should be adequately optimal in quantity and quality. According to Haq (2011), UGSs must be uniformly distributed throughout the city area and the total area occupied should be large enough to accommodate the population needs. Actually, UGSs are not enough, occupying only approximately 4% of the earth's

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surface (World Bank, 2013). Moreover, recent studies documented that the urban environment itself affects the longevity and the vitality of UGSs (Ugolini et al., 2012; Savi et al., 2015).
The urban habitat is substantially different from natural community and its ecological features could be potentially limiting for plant life. Here, it is evident that stressors such as emission of greenhouse-gas, change in surface energy balance and high concentration of pollutants can overlap with already existing limiting factors (e.g. soil compaction, period of critical water stress, low air humidity, heat). For this reason, the peculiarities of urban areas may enhance the pressure on (i) plant performance and (ii) vegetation functionality in the form of abiotic [e.g. high air pollution (Pellegrini, 2014) or drought (Cotrozzi et al., 2016)] and biotic [e.g. pest and pathogen attack (Turco et al., 2004)] stress.
Describing the UGSs properties (such as structure, processes and ecophysiological functioning) has now become an essential threat to the global environment in order to enhance and better manage their capacity to provide the specific and/or desired ESs. According to Calfapietra et al. (2013), the quantification and the characterization of regulating services has a pivotal role, especially the mass and energy exchange between vegetation and atmosphere. Indeed, UGSs can (i) sequester and store gaseous air pollutants (such as carbon dioxide, CO_2), (ii) adsorb particulate matter (PM), (iii) dissipate solar energy, and (iv) provide natural cooling mechanisms through evapotranspiration and shade (Roy et al., 2012).
The effectiveness of ESs provided by UGSs depends on their health status. A plant must be in equilibrium with its environment, otherwise, if it is stressed must spend extra energy to survive at the expense of potential benefits for cities. The performance of photosynthesis is considered an excellent physiological parameter to monitor plant health (Clark et al., 2000). Although the field of physiological ecology relies on a large body of well-documented experimental studies, similar rigorous basis are lacking for the urban setting (Ugolini et al., 2012). For this reason, information on the seasonal and circadian trend of ecophysiological parameters and on physiological defense mechanisms involved in UGSs could have greater importance in order to improve their live conditions and, consequently, the efficiency of their ESs.

Urban plant physiology

Analysis and monitoring of plant responses to urban conditions, defined as “urban plant physiology” (Calfapietra et al., 2015), has been identified as a critical component of research programs. It represents an important opportunity to gain immediately an insight in relation to the physiological responses and the mechanisms (type and extent) of plant acclimation/tolerance.
The key physiological parameters that drive the main environmental services of urban plants are: (i) stomatal conductance and assimilation rate, (ii) leaf area index (which is normally related to the health status of plant), and (iii) phenology. They represent the potential foci of experimental studies because any effect of the urban environment on these parameters could deeply influence the ability of plants to mitigate/tolerate environmental changes.
The possibility of studying plant physiology in the urban environment was first suggested two decades ago. Current knowledge is mainly based on two methodological approaches: (i) gas exchange analysis, which allows to identify stress induced alterations of CO_2 assimilation rate, as well as the type of limitation to this process [i.e. stomatal and/or non-stomatal (Pellegrini, 2014)], and (ii) chlorophyll fluorescence (ChlF) measurement, with which it is possible to draw a detailed picture of the impact of stress conditions on the photochemistry of photosystem II (PSII) and, consequently, on the photosynthetic process (Bussotti et al., 2011a). More recently, spectroscopy has been used to evaluate urban plant traits. By utilizing hyperspectral data, it is possible to estimate a variety of plant traits and physiological processes based on foliar optical properties, including their physiological status (Couture et al., 2013).
The frequency distribution of relevant studies published between 1994 and 2015, selected using the online versions of ScienceDirect and Scopus databases, and searching for the terms “gas exchange” or “chlorophyll fluorescence” or “spectroscopy” and “urban”, “plant”, “vegetation”, “stress” and “ornamental”, is shown in Figure 1. Pioneering works on the effects of abiotic stresses on physiological processes of urban trees date back to the early nineties. Grulke & Miller (1994) estimated the impact of current (in those days) and future ozone (O_3) concentrations on gas exchange of *Sequoiadendron giganteum* seedlings (2-year-old) and trees (5- and 20-year-old). The first results based on ChlF measurements were published in the same year. Lanaras et al. (1994) documented that this technique could be used for screening urban pollution in the field and after transfer of plants to the laboratory. From that work onward, an increasing number of studies were carried out on herbs (e.g. Sgardelis et al., 1994), shrubs (e.g. Manes et al., 1999) and especially trees (e.g. Kloeppel & Abrams, 1995; Kjelgren & Montague, 1998; Gratani et al., 2000). In particular, Hamerlynck (2001) integrated both techniques in order to evaluate photosynthetic gas exchange and ChlF responses to irradiance of *Ailanthus altissima* in urban environments. Using ChlF, the Author gave some information on the mechanisms based on the dissipation of

excess of excitation energy that normally occurs in stress conditions. Jung et al. (2005) performed the first hyperspectral remote sensing measurements from the vegetation in a less built-up Hungarian city. However, the first spectroscopy measurements at leaf-level were reported by Gallagher et al. (2008), aiming to evaluate the productivity of *Betula pendula* trees in an abandoned urban brownfield in New Jersey.

The large array of instrument types that have become available improved the body of literature discussing urban plant physiology. A quick examination reveals that only in 2015, more than twenty experimental studies based on different methodological approaches (alone and/or combined) were carried out (14, 8 and 2, respectively using gas exchange analyses, ChlF measurements and spectroscopy). Here, a review of the studies on the methodological application of ChlF approach in the urban environment is reported and detailed, in order to evaluate the potential of this technique in assessing the performance of UGSs and their ESs.

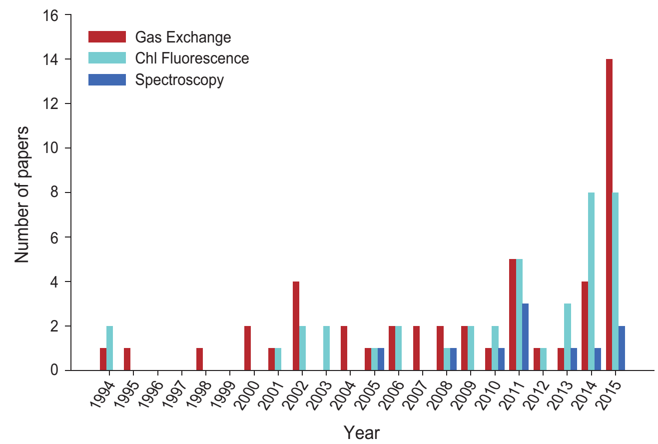

Figure 1. Distribution of relevant studies (total = 76) published between 1994 and 2015, selected using the online versions of ScienceDirect and Scopus, and searching for the terms “gas exchange” or “chlorophyll fluorescence” or “spectroscopy” and “urban”, “plant”, “vegetation”, “stress” and “ornamental”. Papers combining more techniques were counted in each technique class.

CHLOROPHYLL FLUORESCENCE: A POWERFUL TOOL TO EVALUATE URBAN PLANT HEALTH

The reviewed literature (since 1990) concerning the use of ChlF for the detection of photosynthetic efficiency in UGSs is summarized in Table 1. Data presented here are quite heterogeneous due to the property of this technique to be usable in several disciplines, following different approaches. This is confirmed by the high number of scientific journals (focusing on several aspects) where these studies are published, although a large percentage of them (37%) are from Urban Forestry & Urban Greening. For this reason, details of these studies are reported below following the classification used in Table 1, in order to highlight the effectiveness of ChlF in assessing the performance of urban vegetation and its ESs.

A technique increasingly used in the urban environment

Even if it is still scarce, the attention of the scientific community in the evaluation of urban plant performances seems to be increasing, as demonstrated by the big rise of studies published in the last few years focusing on this matter (Figure 1). Simultaneously, works that provide ChlF measurements of urban species are showing the same trend: the number of these papers registered in 2014 and 2015 (8, each) is drastically higher than those published in the previous years (0-3), with the exception of 2011 (5) (see Figure 1 and Table 1). These data reveal the full potential of ChlF in assessing the performance of UGSs and their beneficial services for the environment.

A suitable technique to several experimental conditions

There is no doubt that measurements of ChlF, when carried out with appropriate care, can provide useful information about leaf photosynthetic performances in several experimental conditions (field and environmentally controlled sites). As reported in Figure 2, most of the reviewed experiments (53%) were carried out in controlled environmental conditions (e.g. greenhouses, growth chambers, phytotrons). However, another large percentage of studies was conducted under field conditions: 30% only in urban environment, 5% in urban and periurban sites and 7% in urban, periurban and rural conditions. The residual 5% were studies conducted in both controlled environmental and field conditions (urban). Among the huge number of publications on the matter, key examples which highlight the effectiveness of ChlF technique independently on the experimental conditions is represented by the works of Martinez-Trinidad et al. (2009 and 2010). They measured the potential PSII photochemical efficiency [variable fluorescence (F_v)/maximal fluorescence (F_m), commonly used as indicator of plant stress) in order to evaluate the vitality of *Quercus virginiana* plants both in field (urban forest) and controlled environments. In both experimental conditions, the same measuring protocol was followed and the instruments (HandyPEA, Hansatech, Norfolk, UK) were set equally. At the beginning of both experiments, F_v/F_m values showed a certain degree of homogeneity (near or above 0.8) providing evidence (in addition to visible appearance) that the trees were healthy. These results confirm that the use of standardized and defined protocols gives comparable ChlF measurements across different experimental conditions, according to Bussotti et al. (2011b).

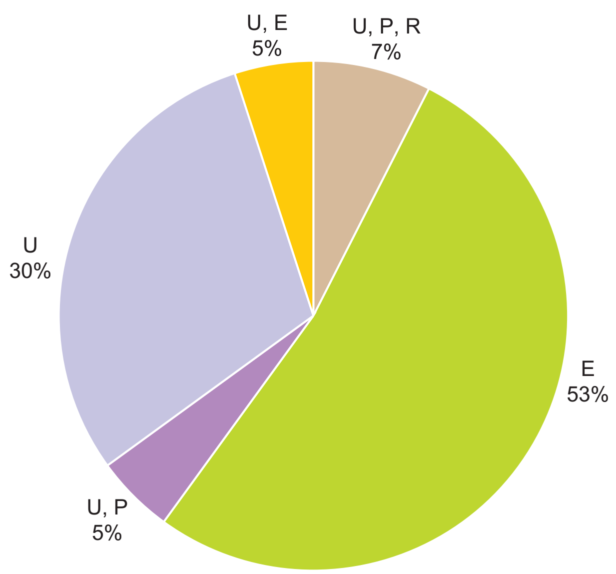

Figure 2. Frequency distribution of the experimental conditions of the studies reviewed (see Figure 1). Abbreviations: E, experimental; P, periurban; R, rural; U, urban.

A technique for every type of vegetation, allowing the screening of species and genotypes with tolerance to the urban environment

The examination of the literature reveals that ChlF technique can be used for screening a large number of plant species for their photosynthetic performances. To make the results more comparable, we have grouped the species into three types: (i) trees, (ii) shrubs, and (iii) herbs. The frequency distribution of these types of vegetation is reported in Figure 3. Regardless of experimental conditions, the largest percentage of the results refers to trees (54%), oak species being the most studied, especially *Quercus ilex* and *Q. robur*. Holm oak (*Q. ilex*) is a typical sclerophyllous broadleaved evergreen species largely used as an ornamental in the landscape of parks, gardens and furnishing avenues in many Mediterranean countries (Ugolini et al., 2013). All these studies on *Q. ilex* have been conducted in Italian urban environments (Florence, Naples, Rome and Trieste) by five

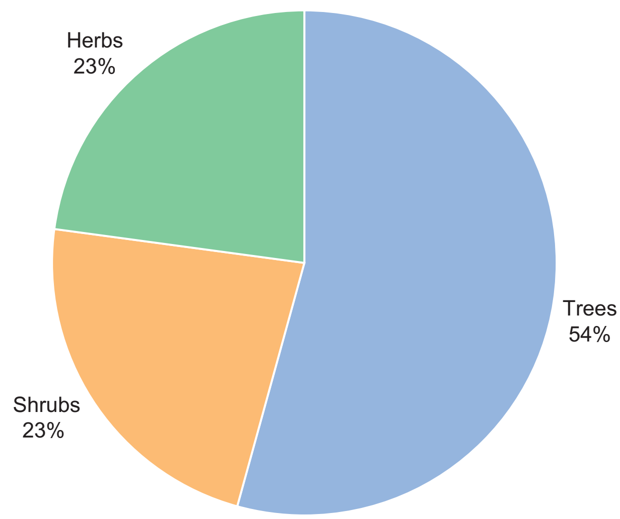

Figure 3. Frequency distribution of the types of vegetation used in the studies reviewed (see Figure 1).

working groups (Alessio et al., 2002; De Nicola et al., 2011; Ugolini et al., 2012; Fusaro et al., 2015; Savi et al., 2015). Unfortunately, not all groups used the same fluorometer's model (four HandyPEA and one PAM-2000, comparisons among instruments are presented later), and the settings were different. However, all of these Authors used the F_v/F_m ratio to evaluate the effects of urban environment on *Q. ilex*. Only Alessio et al. (2002) reported no significant differences among the average values of this ratio measured at Villa Ada (an urban park in the northern part of Rome) along a linear transect perpendicular to via Salaria (a road with very intense car traffic). According to the Authors, this result suggested that air quality in Villa Ada was similar to that outside Rome and that the CO_2 assimilation processes, although being altered, did not indicate a condition of irreversible damage.
English oak (*Q. robur*) is a deciduous tree and is among the five most planted species in urban areas in Northern Europe (Fini et al., 2011). All the studies on this species were conducted in controlled environmental conditions in order to evaluate the effects on plant photosynthetic efficiency of (i) abiotic stress (water shortage), (ii) cultivation practices (e.g. lifting, storage date and girdling) and (iii) biocontrol agents [e.g. Fini et al. (2011) evaluated beneficial effects due to selected mycorrhizae obtained in urban environments]. These works were performed by three groups and also these ChlF measurements were taken with different fluorometers (two HandyPEA and one PAM) set differently. Again, all these studies used the F_v/F_m ratio to evaluate the impact of different agents on *Q. robur* vitality. Fini et al. (2011) documented that the maximal quantum yield of PSII (Φ_{PSII}) was significantly increased by the inoculation with specific mycorrhizal fungi. Percival & Smiley (2015) showed that a

stem girdling > 50% had a detrimental impact on tree vitality (F_v/F_m ratio was lower than control) and that the damage was still evident two years later. Lindqvist & Bornman (2002) reported that the lifting date and the period in cold storage had a strong influence on vitality, as confirmed by the low F_v/F_m values (due to the concomitant increase of minimal fluorescence, F_0) which usually indicates a malfunction in the reaction centers of PSII.

Another large percentage of the works here reviewed were done on shrubs and herbs (23%, each; Figure 3). None shrub species/genus has been studied more than once. By contrast, dandelion (*Taraxacum*) and white clover (*Trifolium repens*) are the genus/species most studied among herbs (thrice and twice, respectively). As reported before, Lanaras et al. (1994) for the first time evidenced how ChlF technique could be used to investigate the stress imposed on dandelion by urban pollution. Later, Sgardelis et al. (1994) documented that dandelion appeared able to survive in the presence of heavy metals contamination in the substrate with no loss in the efficiency of PSII photochemistry. Recently, Vanni et al. (2015) confirmed the low sensitivity to urban pollution of dandelion photosynthetic mechanisms. In particular, the results of ChlF measurements documented a reduction in the fraction of light energy used for photochemistry and a concomitant increase in dissipation mechanisms occurring in plants collected in an urban area. Finally, the Authors affirmed that this mechanism aimed at the photoprotection of the photosynthetic apparatus and for this reason dandelion could be considered an useful biomonitor of urban pollution arising from soil and atmospheric sources.

White clover, *Trifolium repens*, a perennial herb (belonging to the Fabaceae family) cultivated worldwide is also widespread in urban areas, especially in gardens, roadsides and meadows (Cekstere et al., 2015). The two studies on this species here reviewed used F_v/F_m values to evaluate the effects of abiotic stresses in field and controlled environmental conditions. Both working groups used HandyPEA fluorometers, which were set differently from each other. Piraino et al. (2006) documented that F_v/F_m values of *T. repens* cv. Regal plants exposed at 19 monitoring sites distributed homogeneously throughout an Italian province, were close to 0.8 throughout two exposure periods (April-May and September-October). However, only plants exposed in April-May at three sites (where O_3 concentrations often exceeded the limits for plant protection) showed a reduction in efficiency of PSII. Similar conclusions were reported by Francini et al. (2007) in a work performed in controlled environmental conditions aiming to evaluate the effects of O_3 on metabolic activity of NC-S (sensitive) and NC-R (resistant) white clover clones. Recently, Cekstere et al. (2015) investigated the effect of salinity levels and K supply-induced responses and tolerance in the *T. repens* variety Daile in order to evaluate its potential in urban areas. Photosynthesis parameters [F_v/F_m and Performance (P) index] were not negatively affected by increasing NaCl concentrations (0-100 mM, as soil drench) applied to the substrate. The addition of K to the substrate (300 mg l^{-1}, using KNO_3 as soil drench) significantly reduced the F_v/F_m values in elevated salinity conditions, suggesting that the photosynthetic efficiency could be limited by the total concentration of soluble salts. These Authors concluded that the presence of *T. repens* variety Daile was only possible in urban areas with slight or moderate salinity.

The heterogeneity of vegetation types measurable by ChlF merging from this review highlights how this technique will represent a powerful tool to drive local authorities and other practitioners in managing public UGSs and choosing suitable species in current urban greening projects, in order to respond to climate change. Furthermore, one of the most relevant applications of ChlF technique is the screening of genotypes with tolerance to abiotic and biotic stresses, especially in the agronomic and horticultural researches (Gorbe & Calatayud, 2012). However, two studies were also carried out to evaluate this aspect in urban environments. Percival et al. (2006) investigated the response to and the recovery from drought stress in eight *Fraxinus* genotypes. To characterize tree vitality, ChlF values were recorded. During the cessation of watering for two weeks, *F. americana* "Autumn Purple" and *F. velutina* were identified as drought sensitive, with significant reductions in F_v/F_m ratio due to high values of F_0. The concomitant decrease of the light absorption (ABS), trapping (TR_0) and electron transport flux (ET_0) per cross section of PSII indicated that a damage to the leaf photosynthetic system occurred. Considering that during the recovery period ChlF was still lower than the original levels, the Authors concluded that particular care should be taken when planting these three genotypes into urban environments. Recently, Di Baccio et al. (2014) investigated if two poplar clones [I-214 and Eridano, differentially sensitive to O_3, both under acute and chronic exposure (Guidi et al., 1998; Rizzo et al., 2007)] had different sensitivity to cadmium (Cd) pollution. At the constitutive level, significant differences were detected. In particular, ChlF parameters were higher in I-214, suggesting a higher photochemical efficiency and quenching of chlorophyll. These characteristics made I-214 able to counteract the incipient oxidative stress induced by Cd. Indeed, Φ_{PSII} and photochemical quenching (qP) values showed the most pronounced decreases after low and high Cd treatments. In both clones, the impact of Cd was not sufficiently severe to alter the photochemical efficiency of PSII (unchanged F_v/F_m values), suggesting that I-214 and Eridano presented high acclimation to Cd stress and are both suitable to grow in environments contaminated with metals.

A powerful tool to assess urban plant stress conditions *in vivo*

ChlF analysis is a powerful tool to assess plant stress conditions *in vivo* (Adams & Demmig-Adams, 2004). It is well known that a stressed plant reduces metabolism and especially photosynthetic activity. The frequency distribution of the stresses described in the studies here reviewed is reported in Figure 4. Only a little percentage (8%) of these experiments did not focus on the responses of plants to stressors [e.g. Hermans et al. (2003) and Martinez-Trinidad et al. (2010) aimed to show and develop some techniques usable in UGSs]; 13% of the papers specified the stressor generically as "urban environment" (e.g. Rahman et al., 2014); all the others detailed the nature of single stressors. A significant percentage of the experiments (18%) was carried out to investigate the impact of drought [e.g. Percival et al. (2006), Sánchez-Blanco et al. (2008), Fini et al. (2011), Ow et al. (2011), Fini et al. (2014), Jiang et al. (2014)]. Several studies focused on drought because it represents an environmental factor that impairs photosynthetic activity imposing an excess of photosynthetic active radiation (Flexas et al., 2014). In order to highlight the versatility of ChlF as a tool in the screening of genera/species with tolerance to drought, two key examples are reported below. Ow et al. (2011) evaluated the susceptibility of 12 species of flowering plants (commonly planted along streets) to water conditions. They documented that the measurements of F_v/F_m ratios could give not only a quantitative information on how much a plant was stressed, but also a reliable indication of how well the plant was coping with a stressful situation. Five species were identified as susceptible to drought whilst another five were considered to have the ability to resist to water stress and two were identified as drought intermediate species, exhibiting reduced sensitivity to drought conditions where a decline in values of F_v/F_m was observed in the recovery phase. Recently, Swoczyna et al. (2015) used a physiological method (based on the prompt ChlF technique) to evaluate the performance of urban trees (eight species/cultivars planted along streets of medium and heavy traffic in Warsaw) during spring development. Regarding the analysis of ChlF parameters, the taxa could be divided into two groups. The first included species which seemed to be adapted to long periods of water deficit, so their photosynthetic apparatus is usually not severely affected by moderate drought as confirmed by the high values of TR_0/DI_0 (ratio of the energy involved in photosynthetic processes in relation to the lost energy; this indicates that these species were able to overpass previous constrains forcing their photosynthetic apparatus to higher dissipation which occurred). The second group included species that adjust their photosynthetic apparatus activity to low water availability (during drought period) by increasing the dissipation of the excess light energy absorbed [as confirmed by high values of DI_0/CS_0 (dissipated flux per excited cross section at fully opened reaction centers)].

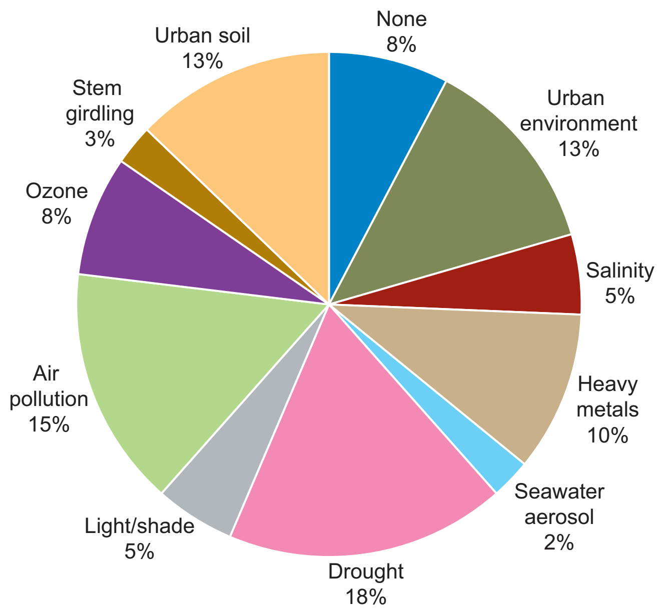

Figure 4. Frequency distribution of the several urban stressors evaluated in the studies reviewed (see Figure 1).

Measurements of ChlF have been performed for decades to determine also the effects of heavy metals (10%, Figure 4). As reported before, Sgardelis et al. (1994) for the first time used this technique to investigate the stress imposed on *Taraxacum* spp. and *Sonchus* spp. by copper (Cu), lead (Pb) and zinc (Zn) present in excess in the substrate. Although the highest metal concentrations measured in the soils were within the range reported to be phytotoxic, a reduction of PSII photochemistry efficiency was not observed, suggesting that these plants were capable of tolerating these conditions. MacFarlane (2003) monitored sub-lethal changes in photosynthesis (via ChlF) of mangrove species in response to Zn stress (125-1000 mg kg^{-1} dry sediment). A significant decline in the F_v/F_m ratio was observed in the presence of 500 mg kg^{-1} Zn and the concomitant reduction of F_m, while F_0 remains constant, suggesting that Zn addition could altered the thylakoid membrane structure, impacting on the electron transport rate and reducing the efficiency of PSII photochemistry. Recently, Dezhban et al. (2015) documented that the slight decline of F_v/F_m ratio observed in *Robinia pseudoacacia* seedlings exposed to high concentrations of Cd (2000 ppm) in the substrate was due to the concomitant increase of F_0, while F_m remained constant. This suggested that the Cd treatment could have an impact (i) directly on the PSII reaction centers (interacting with protein thioyl-, histidyl- and carboxyl-groups) and/or (ii) indirectly reducing the energy transfer from the chlorophyll (Chl) *a* antennae attached with the PSII light-harvesting complex to the reaction centers.

A considerable fraction of the studies monitored (about 15%) showed that ChlF is also a good indicator of stress due to air pollution. In addition to the already mentioned studies by Lanaras et al. (1994) and Piraino et al. (2006), Matsushima et al. (2009) evaluated the photosyntethic efficacy and the resistance of street trees to vehicular exhausts by cold neutron radiography with D_2O tracer and pulse-modulation ChlF imaging. With these techniques, information on the responses of water usage and photosynthetic activity of plants exposed to simulate toxic fumes (2 ppm sulphur dioxide in air, however an exceptionally elevated level) were obtained in a controlled environment. F_v/F_m immediately dropped after supplying the simulated auto-exhaust fumes and the Authors concluded that the photosynthetic activity of *Hibiscus* leaves was negatively affected. More recently, Kardel et al. (2013) compared leaf saturation isothermal remanent magnetization (SIRM) with leaf area, leaf dry weight, specific leaf area, stomatal density, relative Chl content and ChlF parameters (F_v/F_m and PI) for three urban tree types in Ghent to evaluate their responses to particulate and gaseous pollution. The results of the regression analysis for F_v/F_m revealed just significant relationship between leaf SIRM of one species (*Carpinus betulus*) and F_v/F_m, but no significant relationship was observed between PI and SIRM for all the tree types. Authors concluded that ChlF parameters could not significantly distinguish among the urban tree types and suggested the use of SIRM for this purpose. The effects of air pollution on urban plants were also investigated by Van Wittenberghe et al. (2013 and 2014, described later).

Other studies (8%) focused on air pollution in terms of O_3, which is the most important air contaminant that affects wide areas down-wind of urban and industrialized regions (Pellegrini, 2014). Several reports have focused on O_3 because it represents an environmental factor that impairs photosystem I, decreasing the ability of the final acceptors of electrons (ferredoxine and NADP+) and RuBP to manage the flux of electrons effectively (Bussotti et al., 2011b). All the experiments with O_3 were carried out in controlled environment conditions in order to investigate the impact of the pollutant (in relation to the duration of the experiment and O_3 concentrations) on trees (Pellegrini et al., 2011, 2013; Pellegrini, 2014). In these works, Authors used the same (i) sampling methods, (ii) equipment, (iii) settings (iv) measuring protocol, and (iv) type of stress (120 ppb O_3, 5 h d^{-1} for 45 consecutive days). In both *Liriodendron tulipifera* and *Tilia americana*, Authors concluded that the primary target of oxidative stress induced by O_3 was the PSII photochemistry. In fact, a decline of F_v/F_m ratio (due to an increase of F_0) and a concomitant reduction of Φ_{PSII} values were reported, suggesting that a photodamage and a concomitant photoinhibition occurred. Significant differences in terms of onset and extent of the photosynthetic alterations were observed among species. In *L. tulipifera*, ChlF parameters showed a marked reduction starting from eight days from the beginning of the exposure (FBE, Pellegrini et al., 2011). By contrast, the effect of O_3 treatment in *T. americana* was evident starting to 28 days FBE (Pellegrini et al., 2013; Pellegrini, 2014). It is easy to conclude that ChlF technique is an useful tool in the screening of tree species with different sensitivity to O_3.

Recently, Tan & Ismail (2014) evaluated the effects of shade on some urban shrubs using ChlF [as well as the already mentioned papers by Hamerlynck (2001) and Huang et al. (2011)]. They observed changes in photosynthetic efficiency (F_v/F_m and Φ_{PSII}) at reduced PAR levels in *Ficus microcarpa* 'Golden', *Hymenocallis speciosa*, *Wrightia religiosa* and *Duranta erecta* 'Variegata'.

Only a minority (5%, Figure 4) of the experiments here reviewed evaluated the effects of salinity: Cekstere et al. (2015) and Thomas et al. (2013). The latter paper investigated the ability of biochar (pyrolized biomass, at two dosages: 5 and 50 t ha^{-1}) to mitigate salt-induced stress, simulating road salt additions in a factorial glasshouse experiment involving the broadleaved herbaceous plants *Abutilon theophrasti* and *Prunella vulgaris*. Biochar did not affect ChlF (F_v/F_m) in either species. There were also no detectable differences between salt addition and no-salt treatments under high (50 t ha^{-1}) biochar levels for *Abutilon* (the only species exposed to salt).

Ferrante et al. (2011) investigated the effect of seawater aerosol on the leaves of six plant species potentially useful for ornamental purposes in coastal areas. ChlF parameters (such as F_v/F_m and PI) were the most affected by the treatment and allowed a screening for sensitivity to salt.

Finally, a significant number of studies (13%, Figure 4) have shown that ChlF is also a good indicator of soil stress conditions, such as compaction and pollution. In addition to the already mentioned works of Savi et al. (2015) and Vanni et al. (2015), Philip & Azlin (2005) examined the effects of soil compaction on a range of ChlF parameters (F_0, F_m and F_v/F_m) in foliar tissues of *Lagerstroemia speciosa*, a widely planted Malaysian street tree. Soil compaction above 180 MPa affected tree form and reduced ChlF. The Authors concluded that ChlF offers a rapid screening technique for assessing soil compaction tolerance. Song et al. (2014 and 2015) evaluated several environmental factors and ecophysiological characteristics of *Ginkgo biloba* planted on two totally impervious surfaces, and one non-impervious surface (grass land). Compared to urban grass land, F_v/F_m and Φ_{PSII} decreased significantly on impervious surfaces.

Most of the experiments presented in this subchapter were conducted in controlled environmental conditions, where

(i) duration (from instantaneous to long-term responses), (ii) intensity (from moderate to severe), and (iii) effect (from acute to chronic) of stress were established. However, the literature revealed the potential application of the ChlF technique to assess several urban plant stress conditions *in vivo* both in the laboratory and directly in field sites.

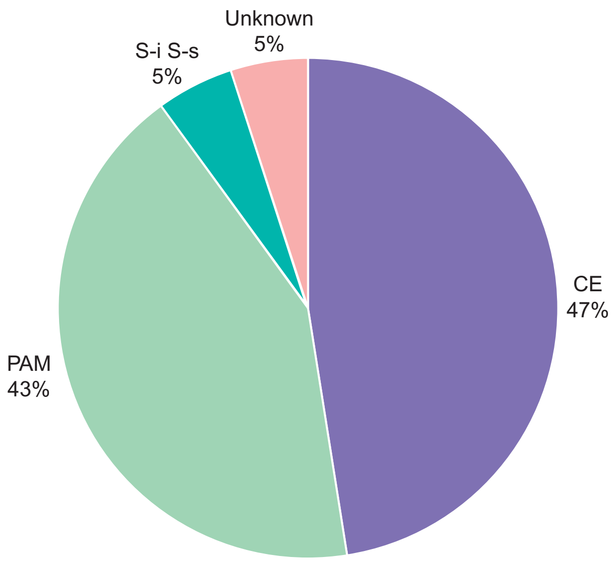

Figure 5. Frequency distribution of the different types of methods used in the reviewed studies (see Figure 1). Abbreviations: CE = Continuum Excitation; PAM = Pulse Amplitude Modulated; S-i S-s = Solar-induced Steady-state.

A technique with several methods and instruments usable in urban environments

Certainly, the strong increase in the last decades in the use of ChlF to evaluate the photosynthetic efficiency of UGSs is partly due to the relative simplicity and availability of the instrumentations, devices and facilities needed. The frequency distribution of the types of methods and instruments merging from the reviewed papers is reported in Figure 5. Only two of them (5%) did not specify the methods and instruments used. Most of the results refer to the Continuum Excitation [(CE), or direct or prompt] or Pulse Amplitude Modulated (PAM) ChlF methods (47% and 43%, respectively). A CE fluorometer is designed to measure the Kautsky Induction, or Fast Chlorophyll Induction (Adams & Demmig-Adams, 2004). Unfortunately, such a system must be shielded from ambient light during use, otherwise the red/far-red component of day-light is super-imposed in the fluorescence signal. Differently, a PAM fluorometer uses sophisticated electronics to separate ChlF from ambient light. The system achieves this using a rapid pulsing excitation light in order to induce a corresponding pulsed fluorescence emission. The PAM fluorometer uses a highly sensitive photodiode to detect and record the pulsed fluorescence signal and to ignore any non-pulsed signal. Therefore, the relative yield of fluorescence can now be measured in the presence of background illumination and, most significantly, in the presence of full sunlight in the field (Maxwell & Johnson, 2000).

The CE fluorescence has been used in both field and controlled environment studies (9, each; one work used this method in both experimental conditions), because it combines the maneuverability of the instruments with the rapidity with which each measurement is taken and the information obtainable. Suitable instruments are produced by (i) Hansatech (in 43% of reviewed studies HandyPEA fluorometers were used), (ii) ADC BioScientific, Hoddesdon, UK [Sánchez-Blanco et al. (2008) measured F_0, F_m and F_v values using an OS-3 OptiScience fluorometer in geranium seedlings subjected to moderate and severe deficit irrigations for 2 months], (iii) BioMonitor S.C.I. AB, Umeå, Sweden (Sgardelis et al., 1994).

The modulated fluorescence technique which proposes a large number of parameters aimed at describing the functionality of PSII (e.g. actual yields and efficiency of PSII), the redox state of Q_A and the heat-dissipation (e.g. fluorescence quenching parameters). Also these methods have been applied in both field and controlled conditions (6 and 11, respectively). Suitable instruments are produced by (i) Walz, Effeltrich, Germany: PAM series [PAM-2000 (used in about 28% of studies), PAM and MINI PAM utilized by Lindqvist & Bornman (2002) and Thomas et al. (2013)]; (ii) Hansatech, Norfolk, UK (FMS2): used in about 10% of studies in field [Hamerlynck (2001); Tan & Ismail (2014)] and in controlled conditions (Ferrante et al., 2011; Huang et al., 2011); and (iii) Li-COR, Lincoln, NE, USA (Song et al., 2014 and 2015).

Furthermore, Van Wittenberghe et al. (2013 and 2014) used a third method providing solar-induced steady-state fluorescence measurements (F_s). This technique provided a direct diagnosis of the functional status of vegetation photosynthesis allowing to detect plant stress in urban environments. Upward and downward hyperspectral fluorescence yield and indices based on the two F_s peaks (at 687 and 741 nm) were calculated on trees of common species in Valencia (Spain) using a spectroradiometer (FieldSpec 3 Hi-Res, ASD, Analytical Spectral Devices, Boulder, CO, USA).

Table 1. Measurements of chlorophyll fluorescence to assess the responses of plants to the urban environment. Abbreviations used in the table: ChlF = Chlorophyll fluorescence; CE = Continuum Excitation; E = Experimental; Ins = Instrument; P = Periurban; PEA = Photosynthetic Efficacy Analyser; PAM = Pulse Amplitude Modulated; PPFD = Photosynthetic Photon Flux Density; R = Rural; S-i S-s = Solar-induced Steady-state; U = Urban.

Authors	Journal	Year	Site	Species	Stress	Aim	Method (Ins)	Parameters
Alessio et al.	Atmos Environ	2002	U, P, R	*Quercus ilex, Pinus pinea*	Urban environment	Use of 14C, sampled in leaves of evergreen species, as a natural geochemical marker to estimate the contribution of artificial sources to the complex of atmospheric gases	CE (Handy PEA, Hansatech)	F_0, F_m, F_v, F_v/F_m, M_o, ET_0/RC, RC/CS, ABS/RC, D_0, K_P, K_N, K_F, K_P/K_N
Cekstere et al.	Urban For Urban Gree	2015	E	*Trifolium repens*	Salinity	Examine the effect of different salinity levels and K supply-induced responses and tolerance	CE (Handy PEA, Hansatech)	F_v/F_m, PI_{TOT}
De Nicola et al.	Water Air Soil Pollut	2011	U, P	*Quercus ilex*	Urban environment	Assess the impact of urban environmental stresses on photosynthetic efficiency	PAM (PAM-2000, Walz)	F_v/F_m, Φ_{PSII}, Φ_{PSII}/Φ_{CO2}
Dezhban et al.	J For Res	2015	E	*Robinia pseudoacacia*	Heavy metals	Evaluate changes in photosynthetic responses and chlorophyll and proline contents of seedlings exposed to high concentrations of Cd and Pb	PAM (PAM-2000, Walz)	F_0, F_m, F_v/F_m
Di Baccio et al.	J Plant Physiol	2014	E	*Populus × canadensis, I-214; Populus deltoides × maximowiczii, Eridano*	Heavy metals	Verify if the two hybrid poplars have different sensitivity to Cd pollution	PAM (PAM-2000, Walz)	Fv/Fm, ΦPSII, qP, NPQ
Ferrante et al.	Sci Hortic	2011	E	*Acacia cultriformis, Callistemon citrinus, Carissa edulis microphylla, Gaura lindheimeri, Jasminum sambac, Westringia fruticosa*	Seawater aerosol	Evaluate the physiological responses of six plant species (characterized by different levels of resistance to salt stress) to seawater nebulisation treatments	CE (Handy PEA, Hansatech,), PAM (FMS2, Hansatech)	F_v/F_m, TR_0/CS, RC/CS_m, ET_0/CS, DI_0/CS, PI_{TOT}, Φ_{PSII}, qP, NPQ, ETR
Fini et al.	Mycorrhiza	2011	E	*Acer campestre, Tilia cordata, Quercus robur*	Drought	Evaluate the effects of selected mycorrhiza obtained in the urban environment on growth, leaf gas exchange, and drought tolerance	CE (Handy PEA, Hansatech)	F_v/F_m, OJIP test
Fini et al.	Urban For Urban Gree	2014	E	*Fraxinus ornus*	Drought	Test the hypothesis that the irradiance at which plants grow in the nursery may impact on their responses to a subsequent drought stress	PAM (PAM-2000, Walz)	$F_v/_{Fm}$, Φ_{PSII}, NPQ, 1-qP
Fusaro et al.	Urban For Urban Gree	2015	U, P	*Quercus ilex*	Urban environment	Quantify how the environmental differences and stress factors affect the functionality of plants and consequently their capacity to provide ecosystem services	CE (Handy PEA, Hansatech)	F_v/F_m, ET_0/TR_0, DI_0/RC, PI_{TOT}
Hamerlynck	Photosynthetica	2001	U	*Ailanthus altissima*	Light/shade	Examine the leaf-level anatomical and photosynthetic responses to PPFD variability under field conditions	PAM (FMS2, Hansatech)	F_0, F_m, F_v/F_m, Φ_{PSII}, 1-qP, NPQ

following →

Authors	Journal	Year	Site	Species	Stress	Aim	Method (Ins)	Parameters
Hermans et al.	J Plant Physiol	2003	U	*Platanus acerifolia*		Demonstrate the complementary utility of chlorophyll fast fluorescence OJIP transient (from 50 μs to 1 s) measurements in the aerial study of rows of trees	CE (Handy PEA, Hansatech)	OJIP test, PI_{TOT}
Jiang et al.	App Mech Mater	2014	E	*Abelia grandiglora* 'Francis Mason'	Drought	Test the effect of water stress on the physiology of plants to ascertain the optimum irrigation	*unknown*	F_v/F_m, Φ_{PSII}, qP, ETR
Kardel et al.	Environ Pollut	2013	U, P, R	*Carpinus betulus, Tilia* sp.	Air pollution	Compare leaf saturation isothermal remanent magnetisation with anatomical, morphological and physiological leaf triats for assessing urban habitat quality	CE (Handy PEA, Hansatech)	F_v/F_m, PI_{TOT}
Lanaras et al.	Sci Total Environ	1994	U,E	*Taraxacum* spp.	Air pollution	Investigate the stress imposed on the plants by urban pollution	*unknown*	F_0, F_m, F_v, F_v/F_m
Lindqvist & Bornman	Scientia Horticulturae	2002	E	*Betula pendula, Quercus robur*		Assess the effect of lifting date and storage time on partial photosynthesis measured by chlorophyll fluorescence	PAM (PAM, Walz)	F_0, F_m, F_v/F_m, Φ_{PSII}
MacFarlane	Bull Environ Contam Toxicol	2003	E	*Avicennia marina*	Heavy metals	Investigate the effects of Zn exposure on chlorophyll a fluorescence under laboratory conditions	PAM (PAM-2000, Walz)	F_0, F_m, F_v/F_m
Martinez-Trinidad et al.	Urban For Urban Gree	2009	E	*Quercus virginiana*		Evaluate the effects of exogenous applications of glucose and starch on the growth and vitality	CE (Handy PEA, Hansatech)	F_v/F_m
Martinez-Trinidad et al.	Urban For Urban Gree	2010	U	*Quercus virginiana*		Evaluate different techniques to determine vitality of trees	CE (Handy PEA, Hansatech)	F_v/F_m
Matsushima et al.	Nucl Instrum Meth A	2009	E	*Hibiscus* sp.	Air pollution	Evaluate photosynthetic efficacy and auto-exhaust-fume resistance of street trees by cold neutron radiography with D2O tracer and chlorophyll fluorescence imaging	PAM	F_v/F_m
Ow et al.	Urban For Urban Gree	2011	E	*Exocecaria cochinensis, Russelia equisetiformis, Lantana montevedensis, Phyllantus cochinchinensis, Evolvulus pilosus, Talinum triangulare, Coreopsis grandiflora, Chlorophytum cosmosum, Elephantopus scaber, Periscaria hydropiper, Stenotaphrum secundatum*	Drought	Evaluate fluctuation in the Fv/Fm ratio as a result of drought and periods coupled with adequate water supplied via irrigation to identify species to be drought tolerant or susceptible to water stress	CE (Handy PEA, Hansatech)	F_v/F_m
Pellegrini et al.	Acta Hortic	2010	E	*Tilia americana*	Ozone	Evaluate the effects of chronic exposure to ozone on photosynthetic performance	PAM (PAM-2000, Walz)	F_0, F_m, F_v/F_m, Φ_{PSII}, qP, qNP

Pellegrini et al.	Environ Exp Bot	2011	E	*Liriodendron tulipifera*	Ozone	Clarify if ozone limits the physiological performance	PAM (PAM-2000, Walz)	F_v/F_m, Φ_{PSII}, Φ_{exc}, $(1/F_0)$-$(1/F_m)$, 1-qP, qL, %P, %D, %X
Pellegrini	Urban For Urban Gree	2014	E	*Tilia americana*	Ozone	Characterize the behavior of saplings exposed to ozone in a controlled environment in terms of ecophysiological and biochemical responses	PAM (PAM-2000, Walz)	F_0, F_m, F_v/F_m, Φ_{PSII}, Φ_{NPQ}, Φ_{NO}
Percival et al.	Urban For Urban Gree	2006	E	*Fraxinus americana, F. excelsior 'Aurea Pendula', F. excelsior 'Jaspidea', F. americana 'Autumn Purple', F. excelsior, F. ornus, F. velutina, F. angustifolia 'Raywood', F. nigra*	Drought	Identify wether differences in drought tolerance exist within the genus and thereby provide information as to their usefulness for planting in urban landscapes	CE (Handy PEA, Hansatech)	F_0, F_v/F_m, ABS/CS, TRo/CS, ETo/CS, DIo/CS
Percival & Smiley	Urban For Urban Gree	2015	E	*Quercus robur, Betula pendula*	Stem girdling	Investigate the effect of stem girdling performed either during the winter or spring	CE (Handy PEA, Hansatech)	F_v/F_m
Philip & Azlin	Urban For Urban Gree	2005	E	*Lagerstroemia speciosa*	Soil compaction	Test the effects of soil compaction in two Malaysian urban areas on the growth and development	CE (Handy PEA, Hansatech)	F_0, F_m, F_v/F_m
Piraino et al.	Sci Total Environ	2006	U, P, R	*Trifolium repens*	Air pollution	Assess the genotoxic potential of air pollutants througout the Novara Province (North Italy)	CE (Handy PEA, Hansatech)	F_v/F_m
Rahman et al.	Urban For Urban Gree	2014	E	*Pyrus calleryana*	Urban environment	Quantify the sensitivity of the growth and cooling potential to urbanization and climate	PAM (PAM-2000, Walz)	F_v/F_m
Sánchez-Blanco et al.	J Plant Physiol	2008	E	*Pelargonium* × *hortorum*	Drought	Evaluate the response of potted geraniums to different irrigation levels	CE (OS-3 OptiScience)	F_v/F_m
Savi et al.	New Phytol	2015	U	*Quercus ilex*	Urban impervious surfaces	Investigate the water relations and xylem hydraulic safety/efficiency of trees growing at urban sites with different percentages of surrounding impervious pavements	CE (Handy PEA, Hansatech)	F_v/F_m
Sgardelis et al.	Sci Total Environ	1994	U/E	*Sonchus* spp., *Taraxacum* spp.	Heavy metals	Compare chlorophyll fluorescence and some heavy metal concentrations along an urban pollution gradient	CE (BioMonitor S.C.I. AB)	F_0, F_m, F_v/F_m
Song et al.	Acta Ecologica Sinica	2014	U	*Ginkgo biloba*	Urban impervious surfaces	Study the effects of impervious surfaces on habitat and ecophysiology	PAM (Li-6400-40 leaf chamber fluorometer, LiCOR)	F_v/F_m, Φ_{PSII}
Song et al.	Urban For Urban Gree	2015	U	*Ginkgo biloba*	Urban impervious surfaces	Measure the effects of impervious surfaces on photosynthesis and fluorescence	PAM (Li-6400-40 leaf chamber fluorometer, LiCOR)	F_v/F_m, Φ_{PSII}, NPQ

following →

Authors	Journal	Year	Site	Species	Stress	Aim	Method (Ins)	Parameters
Swoczyna et al.	Urban For Urban Gree	2015	U	*Tilia cordata 'Greenspire', Tilia × europaea 'Pallida', Acer campestre, Quercus rubra, Gleditsia traicanthos, Pyrus calleryana, Platanus × hispanica 'Acerifolia'*	Drought	Follow several chlorophyll a fluorescence parameters to get an insight into eight tree species photosynthetic machinery performance affected by drought stress during spring development	CE (Handy PEA, Hansatech)	ABS/CS_0, TR_0/CS_0, DI_0/CS_0, ET_0/CSv, ET_0/TRv, RC/CS_0, TR_0/DI_0
Tan & Ismail	Urban For Urban Gree	2014	U	*Axonopus compressus, Bougainvillea 'Angus', Calliandra haematocephala, Cordia subcordata, Cordyline fruticosa 'Firebrand', Duranta erecta 'Variegata', Excoecaria cochinensis 'Firestorm', Ficus microcarpa 'Golden', Hymenocallis speciosa, Ixora 'Sunkist', Ochna kirkii, Phyllantus mytrifolius, Piper sarmentosum, Schefflera arboricola, Sphagneticola trilobata, Wrightia religiosa*	Shade	Characterize the level and distribution of PAR, as well as the spectral quality of solar radiation in these green spaces through simulations and direct measurements; evaluate the in situ plants responses under varying levels of PAR encountered on site	PAM (FMS2, Hansatech)	F_v/F_m, Φ_{PSII}
Thomas et al.	J Environ Manage	2013	E	*Abutilon theophrasti, Prunella vulgaris*	Salinity	Investigate the ability of biochar to mitigate salt-induced stress, simulating road salt additions in a factorial glasshouse experiment	PAM (MINI PAM, Walz)	F_v/F_m
Ugolini et al.	Urban For Urban Gree	2012	U	*Quercus ilex*	Urban environment	Analyze the water relations and the performance of photosynthesis of exemplars growing in different conidtions of urban green areas	CE (Handy PEA, Hansatech)	TR_0/ABS, ET_0/TR_0, RE0/ABS, RE_0/ET_0, PI_{ABS}, PI_{TOT}, ΔV and ΔW curves
Vanni et al.	Water Air Soil Pollut	2015	U	*Taraxacum officinale*	Soil pollution	Evaluate physiological and biochemical characteristics in plants growing naturally in an urban environment	PAM (PAM-2000, Walz)	F_v/F_m, Φ_{PSII}, qP, qN, ETR
Van Wittenberghe et al.	Environ pollut	2013	U	*Celtis australis, Morus alba, Platanus × acerifolia, Phoenix canariensis*	Air pollution	Investigate the effect of ambient air pollution in an urban environment on high spectral resolution solar-induced steady-state ChlF	S-i S-s (Field-Spec3 Hi-Res, ASD Inc., coupled with the FluoWat leaf clip)	Fluorescence Yield indices
Van Wittenberghe et al.	Sci Total Environ	2014	U	*Celtis australis, Morus alba, Platanus × acerifolia, Phoenix canariensis*	Air pollution	Investigate both the importance of within-canopy and between-canopy variation for several fluorescence yield and pigments parameters	S-i S-s (Field-Spec3 Hi-Res, ASD Inc., coupled with the FluoWat leaf clip)	Fluorescence Yield indices

Parameter abbreviations and brief explanations: %D, fractions of light absorbed by PSII antenna that are thermally-dissipated; %P, fractions of light absorbed by PSII antenna that are used in phytochemistry; %X, fractions of light absorbed by PSII antenna not used in photochemistry not dissipated in the antenna; Φ_{CO_2}, quantum efficiency for CO_2 assimilation; Φ_{exc}, efficiency of excitation energy transfer to open PSII traps; Φ_{NPQ}, quantum yield of regulated energy dissipation in PSII; Φ_{NO}, quantum yield of non-regulated energy dissipation in PSII; Φ_{PSII}, maximal photochemical efficiency in light adapted leaves; (1-qP), excitation energy on PSII; $(1/F_0)$-$(1/F_m)$, indicator of PSII functionality; ΔV and ΔW curves, $\Delta V = (V_{treated} - V_{control})$ and $\Delta W = (W_{treated} - W_{control})$, where V is the variable relative fluorescence at any time *t* between F_0 and F_M [$V_t = (F_t - F_0)/(F_M - F_0)$] and W is the relative variable fluorescence at any time *t* between F_0 and F_J [$W_t = (F_t - F_0)/(F_J - F_0)$]; ABS, photon absorption; ABS/CS_0, total light energy flux absorbed per excited cross section at fully opened reaction centres; CS, cross section; D_0, density of the reaction centres per unit of leaf area; DI_0, energy dissipation; DI_0/RC, effective energy dissipation in an active reaction centre; ET_0, maximum electronic transport; ET_0/TR_0, probability that a trapped electron goes forward the primary acceptors of the electron transport chain; ETR, electron transport rate; F_0, minimal fluorescence yield in dark-adapted leaves; F_m, maximum fluorescence yield in dark-adapted leaves; F_v, variable fluorescence yield; F_v/F_m, potential PSII photochemical efficiency; K_F, energy de-excitation velocity per fluorescence emission; K_N, non-photochemical de-excitation rate; K_P, photochemical de-excitation rate; M_0, relative velocity of closure of the reaction centres; NPQ, non-photochemical quenching ; PI_{ABS}, potential performance index (PI_{TOT}) for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors; PI_{TOT}, potential performance index; qL, coefficient of photochemical quenching; qN, non-photochemical quenching; qP, photochemical quenching; RC, number of reaction centres; RC/CS_m, density of reaction centres at P stage; RE_0/ABS, quantum yield of electron transport from Q_A^- to the PSI end electron acceptors; TR_0, energy flux for trapping; TR_v/ABS, maximum quantum yield of primary photochemistry.

CONCLUSIONS

The recent literature concerning the use of ChlF for the detection of photosynthetic efficiency in UGSs highlights the effectiveness of this methodological approach in assessing the performance of urban vegetation and its ecosystem services. More in detail, this review reveals that ChlF: (i) is increasingly used in researches performed in urban environments; (ii) can provide useful information about leaf photosynthetic performance in different experimental conditions (such as controlled environmental and field sites), when applied with appropriate care; (iii) is an excellent technique in the screening of several species and genotypes with tolerance to the urban environment; (iv) is a powerful tool to assess several urban plant stress conditions *in vivo* both in laboratory and directly in field sites; and (v) has several methods to be adopted (the most performed are the CE or PAM) and F_v/F_m is the most used parameter.

Although ChlF is an useful and promising technique in the urban environment, it has some weaknesses that should be overcome. For example, it is important to take into account that the different types of equipment, of measuring protocols and parameters make the comparison among experiments really difficult. It would be advisable to standardize and define common protocols, so that the experiments can be actually reproducible. In addition, in realistic conditions, the plant is subjected simultaneously to several kinds of stresses. Although the technique has been proved as a good stress detector, it is not easy to find out through ChlF which is the stress actually affecting the plant. Maybe, an in-depth analysis of the parameters affected and their distribution within the leaves would shed some light on this aspect.

In conclusion, future directions for research in this field could/should provide the establishment of a national/international network of ChlF/urban environment specialists able to deal with the weaknesses and critical points mentioned above and to drive local authorities and other practitioners in managing public UGSs and choosing suitable species, in order to respond to the challenge of climate change.

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