APPROVAL SHEET

Title of Thesis: Photosynthetic Plant Stress in Urban Vacant Lots

Name of Candidate: Erin Everton
Master of Science, 2020

Thesis and Abstract Approved: _______________________________
Christopher Swan
Professor
Department of Geography and Environmental Systems

Date Approved: ____________________

Abstract

Title of Document: Photosynthetic Plant Stress in Urban Vacant Lots

Erin Everton

Directed By: Dr. Christopher Swan, Dr. Chris Hawn, Dr. Alan Yeakley, Dorothy Borowy

The trend of increasing vacant land in many cities in the United States has been considered as an avenue to increase urban greenspace, though soils in vacant lots tend to be poorer quality than rural soils of the same region. Using an Fv/Fm chlorophyll fluorometer to measure general stress, this study seeks to determine whether plants grown in this vacant lot soil experience higher stress than those grown in topsoil, as well as whether heightened competition from nonnatives associated with city plant communities induces stress. This study was executed using 32 experimental raised-bed plots on The University of Maryland, Baltimore County's campus, each filled with either topsoil or vacant lot fill soil and each assigned to be weeded, unweeded, or left open for colonization. Only dominant species across all these plots were measured. Species studied are native species *Eupatorium altissimum, Heliopsis helianthoides, Lespedeza capitada, Monarda punctada, Schizachyrium scoparium, Sorghastrum nutans, Tridens flavus* and nonnative species *Digitaria sanguinalis, Melilotus officinalus, Plantago lanceolata, Setaria faberi,* and *Taraxacum officinale.* Data was collected pre-dawn at the end of the growing season, from September 20th through October 18th, 2019. The data was analyzed first in bulk, and then separated into native and nonnative species. No significant difference was found between soil types overall (topsoil mean=0.75, fill

mean=0.761, p=0.15) , as well as for both native (topsoil mean=0.746, fill mean=0.752, p=0.72) and nonnative species (topsoil mean=0.754, fill mean=0.774, p=0.46). Weeding regime was significant overall (weeded mean=0.738, unweeded mean=0.761, open mean=0.763, p=0.016), but this effect vanished once analyzed at the native (weeded mean=0.767, unweeded mean=0.740, p=0.57) versus nonnative (unweeded mean=0.777, open mean=0.757, p=0.56) level. With no significant effect of urban soil or competition, the difficulty of maintaining high diversity of native species within cities is likely due to other factors, such as functional traits related to dispersal and life history strategy. Native species that can tolerate local soil conditions within cities should be considered for plantings and seedings where they are likely unable to disperse.

PHOTOSYNTHETIC PLANT STRESS IN URBAN VACANT LOTS

Erin Marie Everton

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore County in partial fulfillment of the requirements for the degree of
Geography and Environmental Systems
2020

Acknowledgements

I wish to express my deepest gratitude to Dr. Christopher Swan, my advisor and thesis committee chair, who provided me with guidance, not just during my graduate years, but through out my undergraduate career as well. The laboratory skills I developed under his guidance equipped me with the technology and knowledge I needed to perform and interpret this study. He also provided me with advice and encouragement every step of the way.

I also want to thank my thesis committee members, Dr. Chris Hawn and Dr. Alan Yeakley. Their invaluable recommendations and feedback allowed my study to be fully realized. Additionally, this study could not have been completed without the mentorship of Dorothy Borowy. Her dissertation project paved the way for my thesis; I based my experimental design on her past work and greatly benefited from her mentorship.
Lastly, I would like to express my appreciation to the University of Maryland, Baltimore County's Department of Geography and Environmental Systems for providing funding for the soil sample chemical analysis. The data from this analysis provided key insights into the system I studied.

Table of Contents

Introduction

The urban environment creates a different and challenging environment for plants to survive. Urban soils and surface environments are much more heterogeneous than rural soils; they are largely at the mercy of human land usage and districting (Delbecque & Verdoodt, 2016; Vasenev, Stoorvogel, & Vasenev, 2013). Alterations to climate and the ecosystem impact soils as well (Rawlins et al., 2015). Urban areas tend towards higher heavy metal concentrations than rural areas (Johnson & Swan, 2014). In many cities across the globe, population has been decreasing over time; about one sixth of cities around the world are experiencing population decline (Haase, 2008). This leaves many former homes and businesses vacant. Many of these structures are subsequently demolished due to safety concerns, whereafter the lot is backfilled with poor quality soil and typically seeded with grass. Work has begun emerging considering these vacant lots as greenspaces (Anderson & Minor, 2017), though their quality as greenspace has come into question as backfilled vacant lots tend to have lower ability to support plant productivity without amendments (Beniston, Lal, & Mercer, 2016; Herrmann, Shuster, & Garmestani, 2017). Vacant lot fill soil tends to be more compact and contain less nutrients, more heavy metals, and less stratified horizons than undisturbed soils in surrounding areas (Beniston, Lal, & Mercer, 2016; Johnson & Swan, 2014). Plants are mostly limited by the resources they utilize, and these resources tend to be scarcer or poorly distributed in urban areas (Johnson & Swan, 2014).

Competition complicates the relationship of environmental factors to native plant performance. Many non-native herbs establish better in disturbed sites common in urban areas compared with native herbs. The presence of nonnative species is associated with a lower presence of native species (Wallace, Laughlin, & Clarkson, 2017); this is likely to be an evolving issue as species move northward due to climate change (Lososová et al., 2018). Competitive interactions in urban areas could cause natives to perform worse than they would otherwise. However, this is not necessarily true for all native species, with early successional natives commonly colonizing human disturbed areas (Everingham, Hemmings, & Moles, 2019).

Plant stressors almost never occur singularly, as the environment usually has more than one limiting factor. Environmental conditions also change over time. Some stressors can induce other stressors, change the effects of other stressors, or even weaken another co-occurring stressor (Suzuki et al, 2014). Soil element stressors especially affect each other within a plant (Treshow, 1970). Stressors can utilize the same response pathways of plants, which can cause interactive effects (Taiz & Zeiger, 2006). Many stressors can thus evoke similar responses. Nutrient stressors can lower a plant's ability to tolerate other stressors because they cannot synthesize defensive compounds as effectively, and in the case of toxicities, there may be damage to defensive mechanisms (Suzuki et al, 2014). Most of the plant stress studies being conducted are on a single species (often agricultural) with a single stressor of focus. My work is much more generalizable than this by focusing on a community of species under multiple stressors, as the plants are grown in field conditions.

Plants make up the base of the ecosystem. If biodiversity is encouraged in the plants of urban areas, greater diversity of animal species can follow; for example, birds that use specific plants as food and/or shelter can establish in areas where these plants are present, despite an urban setting (Boger & Mar, 2018; Zuñiga-Palacios et al., 2020). This can enable urban areas to contribute to conservation, if rare or threatened species establish in greenspaces (Anderson & Minor, 2017). Loss of biodiversity and ecosystem services lowers greenspace quality based on aesthetics, health benefits, recreational benefits, and more (Parris, 2016). Understanding plant performance in urban areas can contribute to planning and management of urban biodiversity. If vacant lot fill soil is inhospitable to planted natives, or competition from nonnatives is forcing natives to underperform, or a combined effect of both, this knowledge can be used to alter management approaches to urban plants and the higher trophic levels that depend on them. The performance of plants in vacant lot soil has implications for the level of intervention required in these soils to prepare them for cultivation, or to set them up for a low-maintenance restoration.

This study seeks to answer the following: will plants experience less stress when grown in topsoil versus vacant lot fill? Does weeding out competitors reduce stress? Does removal of competition have an interactive effect with soil type? And finally, is the overall effect still visible when the plants are broken down into categories by seeded, native species and spontaneous, nonnative species?

Methods

Species

The species represented in this study include 7 native species, *Eupatorium altissimum, Heliopsis helianthoides*, *Lespedeza capitada*, *Monarda punctada, Schizachyrium scoparium, Sorghastrum nutans, Tridens flavus,* and 5 nonnative species, *Digitaria sanguinalis, Melilotus officinalus, Plantago lanceolata, Setaria faberi*, *Taraxacum officinale*. These species were selected based on Braun-Blanquet measurements of their dominance in the community (see Plots subsection), with species of overall across-plot coverage greater than 70 percent being selected for measurement. This was done to reduce noise from the species factor and to ensure healthy, established plants were measured.

Plots

This study was performed on 32 2x2 meter experimental raised-bed plots containing well-established plants grown onsite from seed. These plots are located at The Technology Research Center on University of Maryland, Baltimore County's campus. Two factors, soil type and weeding, were assigned to these plots. The plots contain either topsoil or vacant lot fill soil brought in from Mr. Dirt, a contractor of Baltimore City that performs demolition and backfill. Both soil types are locally sourced. As for competition, the plots have either uncultivated species weeded out, have no weeding, or are uncultivated and left open to spontaneous species colonization. This combination of treatments results in six units: 4 open topsoil plots (number of plants in treatment group=30), 4 open fill plots (n=40), 6 each of weeded (n=36) and unweeded (n=76) fill plots, and 6 each of weeded (n=41) and unweeded (n=67) topsoil plots. This results in a total of 16 plots each in the topsoil (n=138) and vacant lot fill (n=152) groups, 12 plots each in the weeded (n=77) and unweeded (n=143) groups, and 8 plots in the open group (n=70). These plots are visually represented in Figure 1. Uneven sample sizes were the result of differences in establishment and growth of the study species between individual plots. The weeding assignment involved the removal of all species not seeded in that plot and was done twice during the growing season, while the open assignment involved no seeding and was left for recruitment. Each plot was watered throughout the growing season.

All plots with the exception of those left open were split into six groups to receive different mixtures of seeds in order to vary species compositions. Each group contains species found to be dominant, and therefore of interest to this study. Group 1 contains *L. capitada* and *S. scoparium*, Group 2 contains *H. helianthoides*, Group 3 contains *E. altissimum*, *M. punctada*, *S. nutans*,and *T. flavus*, Group 4 contains *M. punctada* and *S. scoparium*, Group 5 contains *H. helianthoides*, *S. scoparium*, and *S. nutans*, and Group 6 contains *E. altissimum*, *L. capitada*, *M. punctada*, *S. nutans*, and *T. flavus*. These mixtures were made for a different study, and the effect of varying community composition is not a part of the scope of this study.

Figure 1: visual representation of the study plots and their location in the field. Green plots contain topsoil (T) while blue plots contain fill soil (F); red plots are left unweeded,

yellow plots are weeded (W), and white plots are left open (O). The numbers 1-6 reflect the seed treatment given to the plot.

Soil samples were taken just before the study period and chemically analyzed by Cornell Soil Health Lab. Results from these tests indicate concentrations of pertinent elements: aluminum (14ppm in topsoil; 16ppm in fill), calcium (2,309ppm in topsoil; 2,064ppm in fill), cadmium (0.23ppm in topsoil; 0.34ppm in fill), copper (0.2ppm in topsoil; 0.31ppm in fill), phosphorus (3.4ppm in topsoil; 3.7ppm in fill), and lead (1.2ppm in topsoil; 0.83ppm in fill). These results are compared with a baseline analysis performed in 2017 as the study organisms were being established, using soil directly from the source instead of from the study plots. Concentrations of the following elements were found: aluminum (25ppm in topsoil; 32ppm in fill), calcium (2,740ppm in topsoil; 2,317ppm in fill), cadmium (0.28ppm in topsoil; 0.67ppm in fill), copper (0.27ppm in topsoil; 0.42ppm in fill), phosphorus (3.27ppm in topsoil; 2.51ppm in fill), and lead (1.54ppm in topsoil; 1.35ppm in fill). Concentrations of potential phytotoxins declined over this time period, especially in the fill soil, potentially indicating a remedial effect with the growth of the study organisms. The baseline represents the original conditions the plants were grown in, while measurements were taken in soil conditions that were more equivalent than initial conditions.

Volumetric soil moisture was measured using the HH2 Moisture Meter with ThetaProbes from Delta-T Devices Ltd. This measurement was taken during the study period two days after a rainfall event to allow soil to drain. Five measurements were taken in each plot, one in each corner and one in the center. Soil moisture values were averaged across all fill and topsoil plots, and both values were found to be normal (20%

and 23% for fill and topsoil, respectively). Because moisture stress is only read at severe levels with the fluorometer used for stress measurement (see Chlorophyll Fluorescence Measurements section), moisture stress is not an issue for the study organisms.

Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence is a protective adaptation in response to light incoming at a rate higher than can be used for photosynthesis. Excess light energy entering chloroplasts can damage photosynthetic mechanisms and reaction centers (Goss & Lepetit, 2015), so plants must find a way to dissipate this excess. There are two processes that deal with this: dissipation as heat, known as nonphotochemical quenching, or chlorophyll fluorescence. Photosystem II can be damaged by a variety of environmental stressors. When photosystem II is damaged, the amount of light energy it can handle for photosynthesis drops, and thus the protective mechanisms of NPQ and chlorophyll fluorescence increase in response (Goss & Lepetit, 2015). Chlorophyll fluorescence reduces damage by reemitting a photon, which can be measured using a fluorometer (Maxwell & Johnson, 2000).

Measurements were taken using the Opti-Science Fv/Fm fluorometer. This meter was selected because it provides a normalized test for chlorophyll fluorescence which correlates with photosystem II yield (Schreiber et al., 1995). This measurement was selected due to its sensitivity to soil chemistry and lack of sensitivity to factors outside the interest of this study such as heat, cold, and drought, as well as its efficiency in field measurements.

The normalized ratio Fv/Fm is a simple way to measure chlorophyll fluorescence in the field. Fm is the maximum fluorescence emitted when PSII is completely saturated with light energy; Fv is the difference between this maximum value and the baseline fluorescence being emitted from PSII at the time of measurement (Maxwell & Johnson, 2000). Because maximum fluorescence increases when photosystem II is under stress, the Fv/Fm ratio decreases with increasing stress. Smaller Fv/Fm values indicated a more stressed organism. A value within a range of 0.79-0.84 is considered ideal for plants, with any values below that range indicating stress (Maxwell & Johnson, 2000).

This method is used for describing the total stress a plant is experiencing and cannot be used to diagnose specific causes. Environmental stressors this meter can measure are aluminum (Baker & Rosenqvist, 2004; Joshi & Mohanty, 2004), cadmium (Baker & Rosenqvist, 2004; Joshi & Mohanty, 2004), copper (Baker & Rosenqvist, 2004; Joshi & Mohanty, 2004), lead (Joshi & Mohanty, 2004), and mercury toxicity (Baker & Rosenqvist, 2004; Joshi & Mohanty, 2004), light stress, ozone stress (Calatayud, Pomares, & Barreno, 2006), calcium (Schmitz-Eiberger, Haefs, & Noga, 2002) and phosphorus (Starck et al., 2000) deficiency, and chlorine imbalance (Zhang et al, 2010). It can also measure other stressors such as viruses (Balachandran et al., 1997) and insect larval foot hooks (Hall et al., 2004). The meter will only measure drought (da Silva & Arrabaca, 2004; Zivcak et al, 2008), nitrogen (Baker & Rosenqvist, 2004), sulfur (Baker, 2008), heat (Baker & Rosenqvist, 2004), cold (Ball, Baker, & Bowyer, 1993), and acid stress (Velikova, Ivanov, & Yordanov, 2002) at severe levels. The meter also does not measure nickel and zinc stress (Joshi & Mohanty, 2004), and measures NaCl stress in some plants but not others (Moradi & Ismail, 2007).

Fv/Fm measurements are performed on dark adapted plants to ensure photosystem II is relaxed to the same known state. This is to prevent nonphotochemical quenching

(NPQ) from influencing fluorescence. Nonphotochemical quenching is when incoming light energy is not used for photosynthesis or reemitted by chlorophyll fluorescence, but emitted as heat (Maxwell & Johnson, 2000). These three processes balance the incoming solar radiation; if NPQ is high, it will lead to lower chlorophyll fluorescence if the rate of photosynthesis is held constant (Maxwell & Johnson, 2000). Since NPQ is a response to high light conditions, it relaxes over time in darkness (Lichtenthaler & Babani, 2004). There was likely residual NPQ in the plants in my study from chronic photoinhibition that would need 30-60 hours to fully relax (Lichtenthaler & Babani, 2004), so similar light history is important to keep the measurements consistent with regard to levels of residual NPQ. To ensure similar light history, measurements were collected at night, began at the same time of 3:00AM each night until first light, and were only performed after a sunny to mostly sunny day. Measurements were taken at the end of the growing season on established plants, spanning September 20th through October 18th, 2019. Measurements were completed before first frost. Late season measurements avoid some of the higher light and moisture stress experienced during the summer months. Three leaves from three plants per species were measured in each plot, and the youngest non-diseased mature leaf blades were chosen where applicable. The youngest mature leaf blades are the best to use for determining nutrient stress (Dutkiewicz, Robinson, & Reuter, 1997) Values for the three leaves are averaged to the individual before input to analysis.

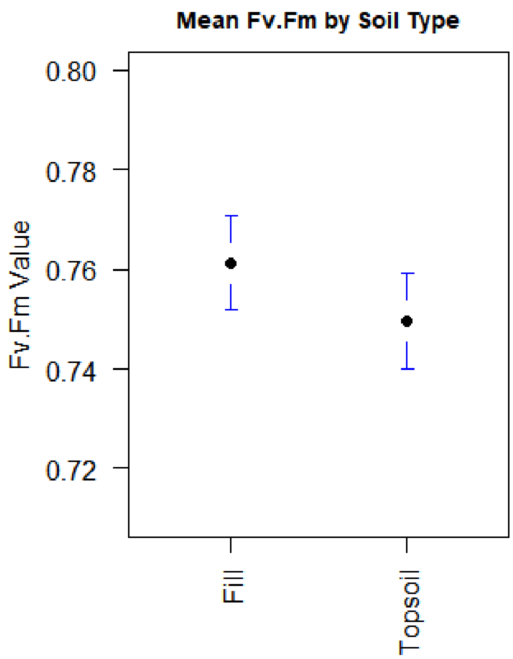
Data Analysis

In order to fit the normality assumptions of ANOVA, the data was power transformed. The boxcox function of the MASS package from Venables and Ripley (2002) was used to find the lambda value for use in the transformation. A linear mixed-effects model was created to test for significance within the soil type and weeding regime factors and to test for interaction, considering species as a random effect, using the nlme package from Pinheiro et al (2020). A two-way, type III ANOVA was run on the linear model to determine if the soil and weeding factors as well as their interaction were significant, followed by a Tukey HSD test to determine which of the weeding levels were significantly different, if any. The Tukey HSD test used was from the R package agricolae from de Mendiburu (2020). The data was then divided into seeded and spontaneous species to determine specific effects for each group. A new mixed effect linear model was made for each sub-dataset and ANOVA was run on both. Eta squared was then calculated to find the effect size of the soil and weeding factors using the R package effectsize from Ben-Shachar, Makowski & Lüdecke (2020). To visualize the data, I used the gplots package from Gregory R. Warnes et al. (2020) to plot the untransformed means of the factors and treatment groups.

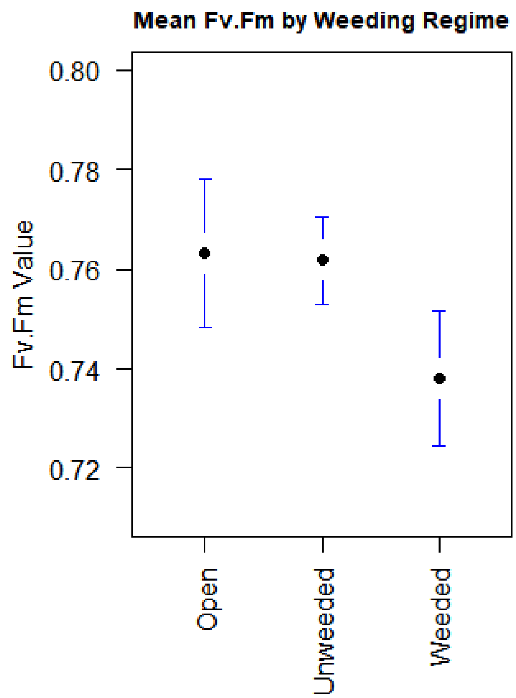
Results

The only significant difference in treatments found was in the weeding treatment: weeded plots were more stressed than unweeded and open plots. The soil factor and the interaction between soil and weeding were not significant. The weeding factor became insignificant once the data was split into native and nonnative categories, and the soil factor remained insignificant for both sub-datasets. All treatments showed signs of some stress, as all treatment means fell below the optimum Fv/Fm range of 0.79-0.84.

Pre-transformed, plants in all topsoil plots had a mean Fv/Fm value of 0.750 while plants in all fill plots had a mean Fv/Fm value of 0.761. Plants in all weeded plots had a mean Fv/Fm value of 0.738, all unweeded plots had a mean value of 0.761, and all open plots had a mean value of 0.763 (Figure 2). Plants in weeded topsoil plots had a mean Fv/Fm value of 0.743, unweeded topsoil plots had a mean Fv/Fm value of 0.757, and open topsoil plots had a mean Fv/Fm value of 0.743. Plants in weeded fill plots had a mean Fv/Fm value of 0.732, unweeded fill plots had a mean Fv/Fm value of 0.767, and open fill plots had a mean Fv/Fm value of 0.778 (Figure 3). The grand mean of all Fv/Fm values was 0.756.

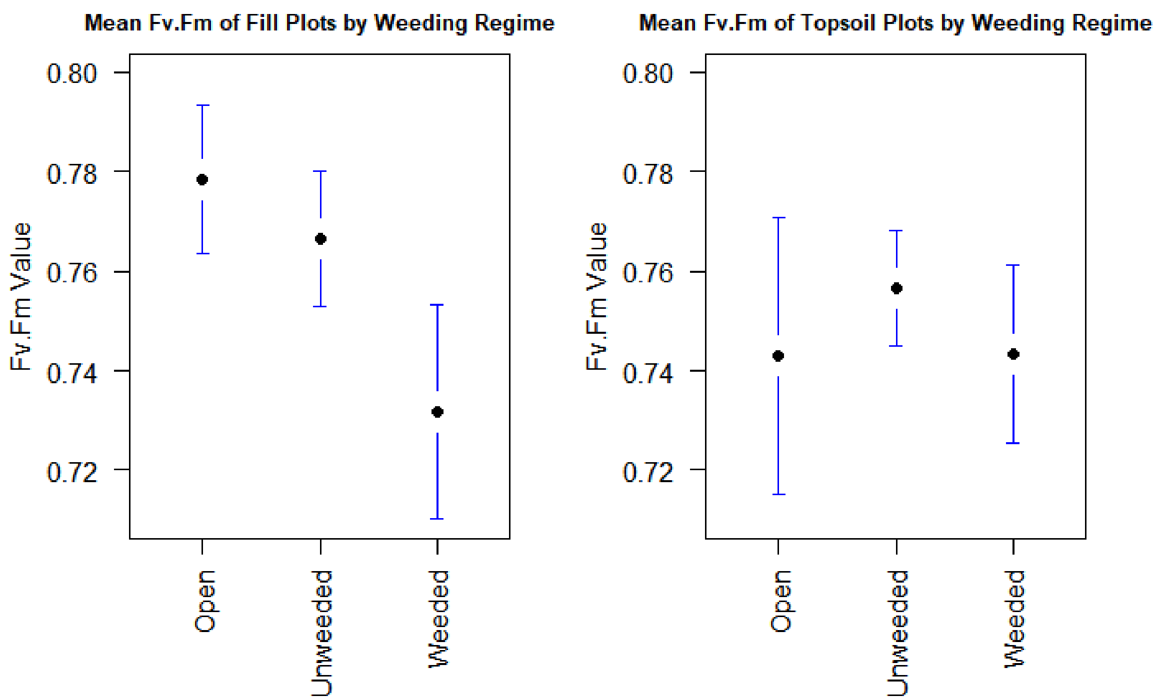


(a)



(b)

Figure 2: Pre-transformed means of Fv/Fm values measured for (a) the soil type factor and (b) the weeding regime factor. Lower values indicate higher stress. Bars displayed are 95% confidence intervals.



(a) (b)

Figure 3: Pre-transformed means of Fv/Fm values measured for the treatments from combining the two factors, where (a) is weeding levels for fill plots and (b) is weeding levels for all topsoil plots. Lower values indicate higher stress. Bars displayed are 95% confidence intervals.

ANOVA on the mixed effects model, once transformed to fit assumptions of normality, indicated that while the differences between all topsoil and fill plots are not statistically significant ($p=0.15$), at least one of the weeding groups significantly differed from the others ($p=0.016$) (Figure 4). The Tukey-HSD test revealed that the weeded plots were significantly different from both the unweeded and open plots, which were not significantly different from each other. There was no significant interaction between weeding regime and soil type ($p=0.21$) (Figure 5). The partial eta-squared effect size of the weeding regime was 0.03, while for soil type it was 0.01.

	numDF <int>	denDF <dbl>	F-value <chr>	p-value <chr>
(Intercept)	1	269	184.59661	<.0001
Soil	1	269	2.06656	0.1517
Weeding	2	269	4.17550	0.0164
Soil:Weeding	2	269	1.55193	0.2137

Figure 4: ANOVA output table for the mixed effects linear model. Weeding regime was significant, while soil type and the interaction were not.

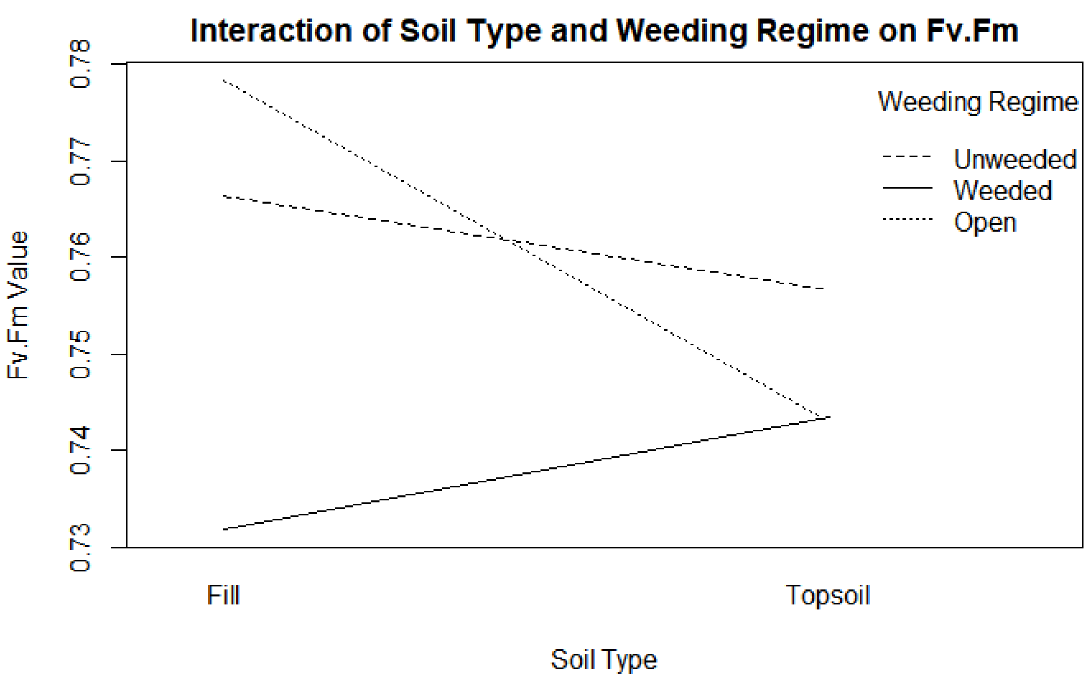
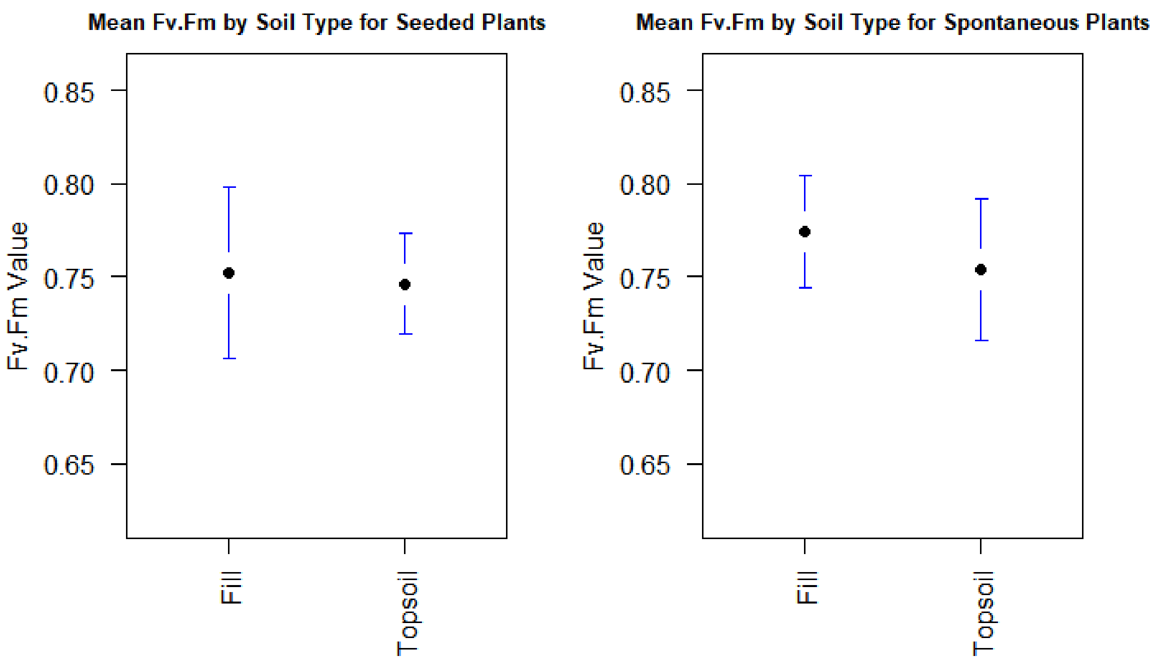


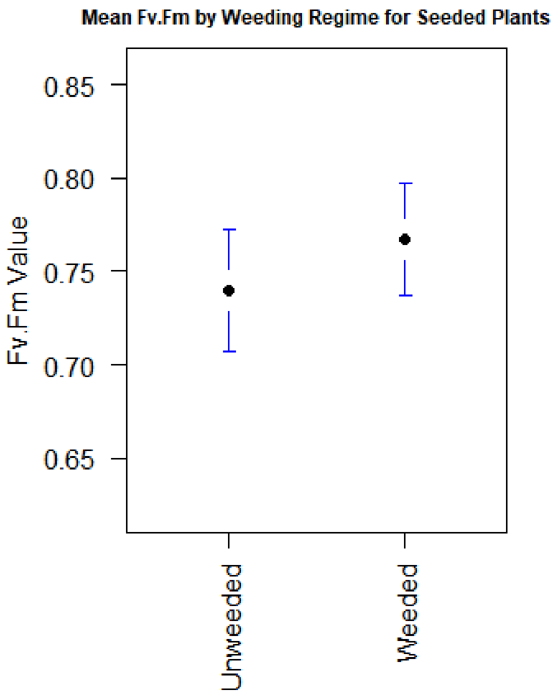
Figure 5: Interaction plot for the soil type and weeding regime factors, using pre-transformed Fv/Fm values. While the lines are not parallel, the ANOVA found no significant interaction.

Following this analysis, the study organisms were broken into groups based on whether their species was intentionally seeded or was spontaneous. Pre-transformed, seeded plants had an average Fv/Fm value of 0.746 in topsoil and 0.752 in fill soil; spontaneous plants had an average Fv/Fm values of 0.754 in topsoil and 0.774 in fill soil (Figure 6). Seeded plants had an average Fv/Fm value of 0.767 in weeded plots and 0.740 in unweeded plots; spontaneous plants had an average Fv/Fm value of 0.757 in open plots and 0.777 in unweeded plots (Figure 7). The grand mean of seeded plants was 0.749, while the grand mean of spontaneous plants was 0.764.

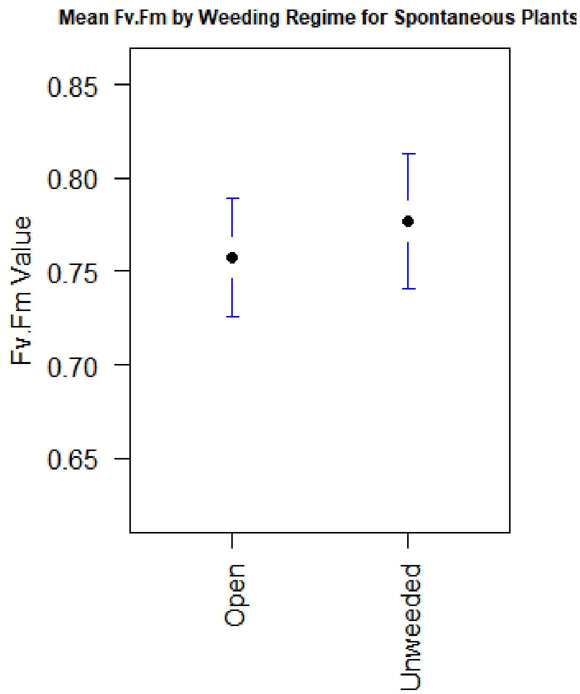


(a) (b)

Figure 6: Pre-transformed means of Fv/Fm values measured for both (a) seeded plants (b) and spontaneous plants for the soil factor. Lower values indicate higher stress. Bars displayed are 95% confidence intervals.



(a)



(b)

Figure 7: Pre-transformed means of Fv/Fm values measured for both (a) seeded plants (b) and spontaneous plants for the weeding regime factor. Lower values indicate higher stress. Bars displayed are 95% confidence intervals.

A mixed effects ANOVA was run on each group of species. The analysis indicated no statistically significant differences in any factor for both seeded and spontaneous species (Figure 8). The interaction between weeding regime and soil type was also not significant for either seeded or spontaneous species (Figure 8). The partial eta-squared effect size for seeded species was 0.01 for soil and 0.03 for weeding while for spontaneous species it was 0.02 for soil and 0.02 for weeding.

	numDF <int>	denDF <dbl>	F-value <chr>	p-value <chr>
(Intercept)	1	15	122.15294	<.0001
Soil	1	15	0.13368	0.7197
Weeding	1	15	0.33657	0.5704
Soil:Weeding	1	15	0.00398	0.9505

	numDF <int>	denDF <dbl>	F-value <chr>	p-value <chr>
(Intercept)	1	11	175.90230	<.0001
Soil	1	11	0.58705	0.4597
Weeding	1	11	0.36785	0.5565
Soil:Weeding	1	11	0.10059	0.7571

Figure 8: ANOVA tables for seeded species (top) and spontaneous species (bottom). None of the factors or their interaction were significant for either group.

Discussion

Due to the initial, baseline poor quality of vacant lot fill, I expected that plants would perform lower in the fill plots than in the topsoil plots. Similarly, I expected the plants in unweeded plots to perform lower due to increased competition. None of the treatments yielded average results within the optimal Fv/Fm range, indicating a mild level of stress throughout the study. This is likely due to the general field conditions, such as full sun exposure, limiting nutrients, less water than optimal, and potential other factors. Full sun exposure is likely a large contributor to overall lower values due to its increase of chronic NPQ (Lichtenthaler & Babani, 2004). Because all of the values fall below the optimal range, differences between the values are of more interest than the question of whether they lie within the optimal range or not.

In general, plants in weeded plots tended to have lower Fv/Fm values on average. This could be due to some type of disturbance of the weeding process, such as root damage to the study species or removal of beneficial species such as nitrogen fixers. However, it could also be due to the difference in species composition between weeded, unweeded, and open plots. The latter two contain weedy species that may weather soil stressors more effectively than the native species in the weeded plots. However, examining the means of natives versus nonnatives indicated that there was not a large difference in Fv/Fm between native species (average 0.75) and nonnative species (average 0.76). Because the effect size of the weeding regime is so low, it is likely that the variation between species is partially responsible for the differences seen in weeding regime, especially due to the close relation of weeding regime and species present. Fv/Fm does vary across species (Maxwell & Johnson, 2000), so the difference seen in weeding regimes could also simply be due to natural variation of PSII functioning between the study species present in different regimes. Additionally, while the difference between weeding regimes was statistically significant, it is not necessarily true that a difference of this magnitude has any biological difference on survival and function. More inquiry into species' PSII performance due to weeding regimes is required to better understand this relationship.

Breaking the species into groups of seeded and spontaneous species yielded no significant differences in any factor for these groups. The effect of weeding is completely lost once the data is examined at this level, likely because the weeding effect did simply stand in for the species effect, and this species effect decreased when the species included in analysis were limited. The effect size for weeding also remained close to the same once the species were split up, another indicator that species caused much of the variation seen in the data, as weeding did not strongly impact either native or spontaneous species.

The lack of significant difference between the topsoil and fill soil plots at all levels is of particular interest in the context of the shift in soil conditions from the baseline to the study period. There was a much higher difference in heavy metal concentrations in the baseline soil, with the baseline fill soil containing 28% more aluminum, 139% more cadmium, and 56% more copper than baseline topsoil on average, though baseline topsoil did have 12% more lead than baseline fill soil on average. While both soils saw a decrease in metal concentrations across the three year period between the baseline measurement and the current measurements, metal concentrations in the fill soil dropped 12% more than in topsoil on average. This narrowing of difference between soil conditions suggests a remedial effect, which should be explored in future research, but also could explain the current lack of difference between stress in the two soil types, given that the soil metals did not cause permanent damage earlier in the study organisms' lifespan.

If vacant lot fill does depress plants' performance, especially through early-life damage to plants before the soil is biologically amended, I would have expected a clear signal of this across the community, or at least in the native, non-spontaneous species. The lack of difference between plant stress on vacant lot fill and topsoil points towards the ability of plants to survive and function in urban vacant land soils similarly to plants grown in human-amended or natural soils. If plants can grow and function with minimal soil preparation in vacant lots, less time and capital can be invested in amending their soils, and native plants can be selected for plantings (Anderson & Minor, 2017). Due to the lack of difference between soil types as well as the remedial effect seen from baseline soils, native plants may also have the ability to both tolerate and remediate fill soil's conditions, though further inquiry should be made into this issue. Locations such as vacant lots that experience little to no intervention in plant growth and soil health have the possibility to contribute to urban greening; this greening can be onsite or by means of providing additional patches for dispersal into city cores (Rupprecht et al., 2015). This less-is-more approach to vacant lot management could promote plant well-being through time. In turn, increased presence of native plants in urban areas can help contribute to ecosystem services and promote urban biodiversity (Boger & Mar, 2018; Zuñiga-Palacios et al., 2020). The lack of significant difference between both soil types and weeding regime for native species provides evidence that perhaps lower quality soil and higher presence of nonnative species are not as critical of a reason for a drop off in native biodiversity with increasing urbanization. This is supported by growing evidence that traits such as mode of dispersal and life history strategy have significant impact on biodiversity in the urban environment (Aronson et al., 2016; Johnson & Swan, 2014; Planchuelo, Kowarik, & von der Lippe, 2020). Native species that do have traits that allow for continued survival in urban areas, such as rapid growth early in life and self-pollination (Johnson & Swan, 2014), may do just as well as nonnative species in urban ecosystems. Natives that perform well in urban soil, but don't disperse in ways best fit for the urban environment, may require planting, but could survive and thrive once the dispersal hurdle is overcome.

Because this study did not take place in situ on a vacant lot within city boundaries, it reflects the impact of soil type and weeding regime alone and does not necessarily reflect the full mosaic of environmental conditions in the field such as soil compaction, presence of wastes, the urban heat island effect, differences in water availability, and others. Further study that can compare in situ treatments will be needed to further understand plant performance in urban environments. Additionally, more species-specific studies will be needed in determining whether the lack of effect of soil type is true for a broader variety of native species, including woody species. Additionally, threshold studies for determining what Fv/Fm values indicate a species is not going to survive or limits its function would be useful for understanding the biological importance of Fv/Fm as a stress index. Lastly, the remedial effect over time of the study organisms seen here merits further inquiry into the ability of natives to perform well in and amend vacant lot backfill across a time period.

Conclusion

Neither soil type nor weeding created a significant difference in plant stress. This was counter to expectation, as vacant lot fill soil is considered poor quality, a factor in the urban environment being inhospitable to native plants. Given no significant effect of vacant lot fill soil or competition from nonnatives on the performance of native species, the challenges to native plant diversity within cities is likely due to spatial traits, such as dispersal, or temporal traits, such as life history strategy. Planting and seeding projects on vacant land aimed at increasing native plant species diversity should consider natives that may not be present in the city due to dispersal and early life limitations.

References

Anderson, E. C., & Minor, E. S. (2017). Vacant lots: An underexplored resource for ecological and social benefits in cities. *Urban Forestry & Urban Greening*, *21*, 146–152. https://doi.org/10.1016/j.ufug.2016.11.015

Aronson, M. F. J., Nilon, C. H., Lepczyk, C. A., Parker, T. S., Warren, P. S., Cilliers, S. S., Goddard, M. A., Hahs, A. K., Herzog, C., Katti, M., Sorte, F. A. L., Williams, N. S. G., & Zipperer, W. (2016). Hierarchical filters determine community assembly of urban species pools. *Ecology*, *97*(11), 2952–2963. https://doi.org/10.1002/ecy.1535

Baker, N. R. (2008). Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. *Annual Review of Plant Biology*, *59*(1), 89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Baker, N. R., & Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. *Journal of Experimental Botany*, *55*(403), 1607–1621. JSTOR.

Balachandran, S., Hurry, V. M., Kelley, S. E., Osmond, C. B., Robinson, S. A., Rohozinski, J., Seaton, G. G. R., & Sims, D. A. (1997). Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. *Physiologia Plantarum.*, *100*(2), 203–213.

Ball, M. C., Baker, N. R., & Bowyer, J. R. (1993). The role of photoinhibition during tree seedling establishment at low temperatures. *ENVIRONMENTAL PLANT BIOLOGY*, 365–376.

Ben-Shachar, Makowski & Lüdecke (2020). Compute and interpret indices of effect size. CRAN. Available from https://github.com/easystats/effectsize.

Beniston, J. W., Lal, R., & Mercer, K. L. (2016). Assessing and Managing Soil Quality for Urban Agriculture in a Degraded Vacant Lot Soil. *Land Degradation & Development*, *27*(4), 996–1006. https://doi.org/10.1002/ldr.2342

Boger, A. M., & Marr, D. L. (2018). Effect of Native and Non-Native Plantings in Urban Parking Lot Islands on Diversity and Abundance of Birds, Arthropods, and Flower Visitors. *Proceedings of the Indiana Academy of Science*, *127*(2), 115–123.

Brown, E. M., Roswell, A. S., College, K., Schade, K. M., & Smith, M. T. (2018). *Vacant Properties in Baltimore City: Practical Solutions to City Revitalization* (p. 3).

Calatayud, A., Pomares, F., & Barreno, E. (2006). Interactions between nitrogen fertilization and ozone in watermelon cultivar Reina de Corazones in open-top chambers. Effects on chlorophyll a fluorescence, lipid peroxidation, and yield. *Photosynthetica.*, *44*(1), 93–101.

da Silva, J. M., & Arrabaca, M. C. (2004). Photosynthesis in the water-stressed C4 grass Setaria sphacelata is mainly limited by stomata with both rapidly and slowly imposed water deficits. *Physiologia Plantarum.*, *121*(3), 409–420.

de Mendiburu, F. (2020). agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-3. https://CRAN.R-project.org/package=agricolae

Delbecque, N., & Verdoodt, A. (2016). Spatial Patterns of Heavy Metal Contamination by Urbanization. Journal of Environmental Quality, 45(1), 9–17. https://doi.org/10.2134/jeq2014.11.0508

Dutkiewicz, C., Robinson, J. B., & Reuter, D. J. (1997). Guidelines for Collecting, Handling and Analyzing Plant Material. In *Plant Analysis: An Interpretation Manual : An Interpretation Manual: Vol.* (2nd ed, pp. 53-70). CSIRO PUBLISHING.

Everingham, S. E., Hemmings, F., & Moles, A. T. (2019). Inverted invasions: Native plants can frequently colonise urban and highly disturbed habitats. *Austral Ecology*, *4*, 702.

Goss, R., & Lepetit, B. (2015). Biodiversity of NPQ. *Journal of Plant Physiology*, *172*, 13–32. https://doi.org/10.1016/j.jplph.2014.03.004

Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber, Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson,

Steffen Moeller, Marc Schwartz and Bill Venables (2020). gplots: Various R Programming Tools for Plotting Data. R package version 3.0.3.

https://CRAN.R-project.org/package=gplots

Haase, D. (2008). Urban Ecology of Shrinking Cities: An Unrecognized Opportunity? *Nature and Culture*, *3*(1), 1–8. https://doi.org/10.3167/nc.2008.030101

Hall, D. E., MacGregor, K. B., Nijsse, J., & Bown, A. W. (2004). Footsteps from Insect Larvae Damage Leaf Surfaces and Initiate Rapid Responses. *European*

Journal of Plant Pathology, *110*(4), 441–447. https://doi.org/10.1023/B:EJPP.0000021072.89968.de

Herrmann, D. L., Shuster, W. D., & Garmestani, A. S. (2017). Vacant urban lot soils and their potential to support ecosystem services. *Plant and Soil*, *413*(1), 45–57. https://doi.org/10.1007/s11104-016-2874-5

Johnson, A. L., & Swan, C. M. (2014). Drivers of Vegetation Species Diversity and Composition in Urban Ecosystems. In R. A. McCleery, C. E. Moorman, & M. N. Peterson (Eds.), *Urban Wildlife* (pp. 75–90). Springer US. https://doi.org/10.1007/978-1-4899-7500-3_5

Joshi, M. K., & Mohanty, P. (2004). Chlorophyll a Fluorescence as a Probe of Heavy Metal Ion Toxicity in Plants. *Advances in Photosynthesis and Respiration.*, *19*, 637–661.

Lichtenthaler, H. K., & Babani, F. (2004). Light Adaptation and Senescence of the Photosynthetic Apparatus. Changes in Pigment Composition, Chlorophyll Fluorescence Parameters and Photosynthetic Activity. In G. C. Papageorgiou & Govindjee (Eds.), *Chlorophyll a Fluorescence: A Signature of Photosynthesis* (pp. 713–736). Springer Netherlands. https://doi.org/10.1007/978-1-4020-3218-9_28

Lososová, Z., Tichý, L., Divíšek, J., Čeplová, N., Danihelka, J., Dřevojan, P., Fajmon, K., Kalníková, V., Kalusová, V., Novák, P., Řehořek, V., Wirth, T., & Chytrý, M. (2018). Projecting potential future shifts in species composition of European urban plant communities. *Diversity and Distributions*, *24*(5/6), 765.

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—A practical guide. *Journal of Experimental Botany*, *51*(345), 659–668. JSTOR.

Moradi, F. & Ismail, A.M. (2007). Responses of Photosynthesis, Chlorophyll Fluorescence and ROS-Scavenging Systems to Salt Stress During Seedling and Reproductive Stages in Rice. *Annals of Botany*, *99*(6), 1161.

Parris, K. M. (2016). *Ecology of urban environments* (UMBC Library Stacks QH541.5.C6 P37 2016). John Wiley & Sons Ltd.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2020). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-148, <URL: https://CRAN.R-project.org/package=nlme>.

Planchuelo, G., Kowarik, I., & von der Lippe, M. (2020). Endangered Plants in Novel Urban Ecosystems Are Filtered by Strategy Type and Dispersal Syndrome, Not by Spatial Dependence on Natural Remnants. *Frontiers in Ecology and Evolution*, *8*. https://doi.org/10.3389/fevo.2020.00018

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rawlins, B. G., Harris, J., Price, S., Bartlett, M., & Davidson, D. (2015). A review of climate change impacts on urban soil functions with examples and policy insights from England, UK. Soil Use & Management, 31, 46.

Rupprecht, C. D. D., Byrne, J. A., Garden, J. G., & Hero, J.-M. (2015). Informal urban green space: A trilingual systematic review of its role for biodiversity and trends in the literature. *Urban Forestry & Urban Greening*, *14*(4), 883–908. https://doi.org/10.1016/j.ufug.2015.08.009

Schmitz-Eiberger, M. A., Haefs, R., & Noga, G. J. (2002). Enhancing biological efficacy and rainfastness of foliar applied calcium chloride solutions by addition of rapeseedoil surfactants. *ZEITSCHRIFT FUR PFLANZENERNAHRUNG UND BODENKUNDE*, *5*, 634.

Schreiber, U., Hormann, H., Neubauer, C., & Klughammer, C. (1995). Assessment of Photosystem II Photochemical Quantum Yield by Chlorophyll Fluorescence Quenching Analysis. *Australian Journal of Plant Physiology*, *22*(2), 209–220.

Starck, Z., Niemyska, B., Bogdan, J., & Tawalbeh, R. N. A. (2000). Response of tomato plants to chilling stress in association with nutrient or phosphorus starvation. *Plant and Soil*, *226*(1), 99–106. JSTOR.

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist*, *203*(1), 32–43. https://doi.org/10.1111/nph.12797

Taiz, L., & Zeiger, E. (2006). *Plant Physiology* (4th ed.). Sinauer Associates, Inc.

Treshow, M. (1970). *Environment & plant response* (UMBC Library Stacks QK754 .T7). New York : McGraw-Hill, 1970.

Vasenev, V. I., Stoorvogel, J. J., & Vasenev, I. I. (2013). Urban soil organic carbon and its spatial heterogeneity in comparison with natural and agricultural areas in the Moscow region. CATENA, 107, 96–102. https://doi.org/10.1016/j.catena.2013.02.009

Velikova, V., Ivanov, A. & Yordanov, I. (2002). CHANGES IN LIPID COMPOSITION OF Phaseolus vulgaris LEAVES AFTER SIMULATING ACID RAIN TREATMENT. Retrieved from ResearchGate on 21 July 2020. www.researchgate.net/publication/237292264_CHANGES_IN_LIPID_COMPOSITION_OF_Phaseolus_vulgaris_LEAVES_AFTER_SIMULATING_ACID_RAIN_TREATMENT

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Wallace, K. J., Laughlin, D. C., & Clarkson, B. D. (2017). Exotic weeds and fluctuating microclimate can constrain native plant regeneration in urban forest restoration. *Ecological Applications*, *27*(4), 1268–1279. https://doi.org/10.1002/eap.1520

Zhang, Z., Wang, L., Huang, S., Xing, L., & Wang, L.. (2010). EFFECT OF CL-STRESS ON PHOTOSYNTHETIC, CHLOROPHYLL FLUORESCENCE CHARACTERS AND CHLOROPLAST ULTRA-STRUCTURE OF WATERMELON SEEDLINGS. *Acta Horticulturae*, *871*, 377–384. https://doi.org/10.17660/ActaHortic.2010.871.51

Zivcak, M., Brestic, M., Olsovska, K., & Slamka, P. (2008). Performance index as a sensitive indicator of water stress in Triticum aestivum L. *PLANT SOIL AND ENVIRONMENT.*, *54*(4), 133–139.

Zuñiga-Palacios, J., Zuria, I., Moreno, C. E., Almazán-Núñez, R. C., & González-Ledesma, M. (2020). Can small vacant lots become important reservoirs for birds in urban areas? A case study for a Latin American city. *Urban Forestry & Urban Greening*, *47*, 126551. https://doi.org/10.1016/j.ufug.2019.126551