ABSTRACT

Title of dissertation: THE EFFECTS OF FUTURE GLOBAL CHANGE ON ARBUSCULAR MYCORRHIZAL FUNGI AND SOIL CARBON: USING URBANIZATION AS A SURROGATE FOR FUTURE CONDITIONS IN FIELD STUDIES

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Carbon, fixed photosynthetically by plants, cycles through plant, microbial biomass, soil, and atmospheric carbon pools. The effects of global change on this cycling will impact future levels of atmospheric carbon dioxide, but are poorly understood. In urban areas, temperature and carbon dioxide concentrations are often elevated to levels that simulate near-future climate changes. These elevations are not sudden, uniform step increases but are gradual and variable; as such urbanization may provide a means to simulate the effects of near-future climate changes. The dissertation research encompasses two studies utilizing urban macroclimate to study the effects of future climate change.

In the first study, plots containing a common imported soil and seed bank were established at three locations along a 50 km urban-to-rural transect. In these plots, plant community development, temperature, carbon dioxide concentrations, and other factors had been monitored for five years. Subsequently, arbuscular mycorrhizal fungal structures in bulk soil were quantified. These fungi receive carbon directly from plant roots, grow into bulk soil, and can transfer immobile soil minerals to their plant hosts. In

contrast to expectations, fewer fungal structures were found closer to the urban side of the transect.

The second study was an observational study of soil carbon in minimally managed, long-undisturbed soils located at varying distances from urban areas. In sampling sites at 62 golf courses, similar communities of cool-season grasses had been undisturbed for at least 25 years. At each site, total and active soil carbon and many potential explanatory factors were measured and examined with multiple regression analysis. Contrary to expectations, soil carbon was positively correlated with warmer February-only mean daily minimum soil temperatures, suggesting that winter temperatures are more important than mean annual temperature for soil C storage in temperate grassland. Other correlations, including positive correlations with soil cation exchange capacity, soil lead levels, and tropospheric ozone exposure during the peak ozone season, were also detected. Potential mechanisms for the detected relationships are explored.

The results of both experiments demonstrate that commonly-held expectations based on single-factor global change experiments or models are not always borne out in complex natural systems.

THE EFFECTS OF FUTURE GLOBAL CHANGE ON ARBUSCULAR MYCORRHIZAL FUNGI AND SOIL CARBON: USING URBANIZATION AS A SURROGATE FOR FUTURE CONDITIONS IN FIELD STUDIES

by

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TABLE OF CONTENTS

List of Tables:

List of Figures:

Acknowledgements:

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Chapter 1: General introduction and rationale

Soil carbon cycling and arbuscular mycorrhizal fungi:

Soils store more carbon (C) than is contained in the atmosphere and in above-ground biomass (Denman et al., 2007). Soil C is a dynamic quantity: it is determined by the balance between what is added to soil as plant litter, plant root exudates, and analogous microbial and animal materials; and what is removed from soil, when soil organic matter (SOM) is consumed and respired away by soil microbial biomass, along with any losses from erosion or leaching. Without continuous addition of photosynthetically fixed C, soil C stocks will decline. Conversely, if microbial decomposition of SOM is inhibited and/or additions exceed what is decomposed, soil C stocks increase. The rates of both photosynthesis and soil microbial decomposition will be impacted by future global changes in temperature, carbon dioxide concentrations ($[CO_2]$), and other factors, with resulting potential changes in soil C storage.

A significant fraction of photosynthetically fixed C, estimated at between 4 and 20%, passes directly from plants to arbuscular mycorrhizal (AM) fungi (Douds et al., 2000). These fungi associate symbiotically with the roots of most terrestrial plant species, and are obligately biotrophic—their only source of C and energy are living plant roots. The long, thin hyphae (~1 to 12 µm in diameter) of AM fungi can absorb immobile soil nutrients, most notably phosphorus (P), from bulk soil, and transfer them to plant roots; AM plants also withstand drier soils than non-mycorrhizal counterparts (Smith et al., 2010). Therefore, AM fungi often increase the photosynthetic rate, biomass, P content, and/or drought resistance of their host plants. These fungi are belowground sinks for photosynthetically derived C, and confer protection from root

pathogens to their hosts by consuming C and physically occupying infection sites on root surfaces. While the partnerships between plants and AM fungi are not strictly species-specific (unlike some other types of mycorrhizae), the costs and benefits to each partner vary among combinations of plant and fungal species. The relationship can range from mutualistic to neutral or even plant-parasitic (Francis and Read, 1994; Johnson et al., 1997; Sanders, 2004). At one end of this range are some plant taxa whose roots support little or no growth of any AM fungal species; these plants are not dependent on AM fungi for their P nutrition. Notable plant families that are mostly non-mycorrhizal are the *Brassicaceae* (cabbages/mustards) and the *Chenopodiaceae* (including lambsquarters and other weedy annuals) (Newman and Reddell, 1987). On the fungal side of the symbiosis, there are AM fungal species that are considered to be parasitic when growing with some plant species —they decrease the biomass of the host plant, when grown individually in sterilized soil, relative to control plants with no AM fungi. These species of AM fungi might still benefit the host plant in other ways, e.g. drought or pathogen resistance, and so might not be parasitic in more natural growth conditions (but are thought to cause yield decreases in some crops monocultures grown without rotation). Because AM fungi are dependent on their plant hosts for all C requirements, global change factors that affect plant growth or community composition, such as rising carbon dioxide concentration ($[CO_2]$) and warming temperatures, will also affect AM fungi.

In addition to effects on plants, the growth of AM fungal hyphae constitutes a direct transfer of recently fixed, non-structural plant C to soils. The hyphae of AM fungi cover and penetrate soil aggregates, associate closely with soil mineral and organic particles, and promote soil aggregation (Rillig, 2004b). Therefore, the C they contain is

likely to be physically protected from oxidation, decomposition, and mineralization. The walls of AM fungal spores and hyphae are composed of chitin, a polymer found in all fungi and in arthropods, and glomalin, a glycoprotein thought to be specific to AM fungi (Bolliger et al., 2008). These two compounds are thought to be cross-linked in the walls of AM fungal hyphae (Driver et al., 2005), and both are thought to be recalcitrant to degradation in soils. Although chitinase enzymes are found in all fungi, glomalin is believed to be specific to AM fungi and to have a non-repeating, cross-linked structure. Therefore, some of the C transferred to soils by AM fungi will be immediately biochemically resistant to decomposition, as well as immediately physically protected in aggregates (Six et al., 2006). These traits suggest that AM fungal inputs to soil aggregates may have disproportionate impacts on soil carbon sequestration. These inputs are difficult to track experimentally, but are believed to be large: in tropical forest soils, ca. 3% of soil carbon was attributed to glomalin, a glycoprotein produced by AM fungi (Lovelock et al., 2004). In an isotope labeling study under a rapidly growing poplar forest, hyphae of AM and another type of mycorrhizal fungi together accounted for 62% of all new C entering soil organic matter, including inputs from plant litter and roots (Godbold et al., 2006). Because of their inputs to soil C and their effects on primary production and soil aggregation, AM fungi merit attention in global change research.

Global change research—need for new approaches:

If global change factors alter the cycling of photosynthetic C through plants, AM fungi, soils and the atmosphere, large impacts on future greenhouse gas concentrations, as well as on soil fertility and soil structure, may result (Friedlingstein et al., 2006).

Many experiments have attempted to quantify the interactive effects of global change factors on AM fungi and soil C, and many models have been developed to predict the effects on global C cycling. Much of the research on soil C responses to global change, however, is limited in duration, scope, statistical power, and the realism of experimental treatments because of the expense, time, and equipment required. The majority of soil C investigations are short (weeks to months) incubations or intermediate (years to longer than a decade) length field studies. This is problematic, because in intact soils, C changes slowly in response to most global change factors (on the scale of decades to centuries, except in response to land use change, where initial disturbance of untilled soil can cause a large rapid loss of soil C to the atmosphere as CO_2).

As summarized in the most recent IPCC report, the sequestration of C in terrestrial sinks (including biomass and soil C pools) is expected to level off or decrease under future global change conditions (Denman et al., 2007). However, the authors state that there is great uncertainty in this prediction and that many interactions between factors are too complex and/or poorly understood to be adequately modeled or represented by experimental designs. Even single-factor global change experiments have important limitations. The effects of elevated CO_2 or temperature are often investigated as single, sudden step-wise increases, e.g. a doubling of atmospheric CO_2 or a 5°C soil warming. This is very different from the increases occurring globally, which are gradual and vary over time (e.g. daily and seasonally, weekday/weekend differences in gasoline and energy usage, interannually as with El Nino Southern Oscillation cycles, and longer-term variability). Experiments with large step-increases in CO_2 have been shown to overestimate responses (Luo and Reynolds, 1999) because responses are not sustained

over time. In systems with multi-species assemblages, a sudden pulse of C accompanying sudden steep increases in [CO_2] allows opportunistic species to dominate, which can result in overly extreme community changes (Klironomos et al., 2005). These experiments are also expensive, because treatments involving CO_2 must be applied at the whole-plot or block level and require extensive equipment (e.g. sealed growth chambers, open-top chambers, or free-air CO_2 enrichment rings). Therefore the level of replication tends to be low, limiting statistical power to detect responses. Because treatment-induced changes in soil C are expected to be small and slow to accrue against a large background, experiments of short duration and low statistical power often cannot detect soil C changes in the expected range of responses (Smith, 2004). Lastly, large step increases may elicit very different responses than gradual increases do. For example, photosynthetic acclimation to elevated CO_2—an initial jump in photosynthetic rate that is not sustained (Lee et al., 2011)—is evidence for caution in extrapolating results from step to gradual increases in CO_2.

As with elevated CO_2, warming temperatures are not expected to be manifested as sudden, uniform increases. Increasing mean annual temperatures may be manifested as warmer nights, only in certain seasons, as sporadic heat waves, or in any number of other ways. Therefore, results of experiments with uniform temperature increases may not extrapolate to field situations where an increase in mean annual temperature is not uniform over time. In contrast to elevated CO_2 experiments, which can overestimate responses, uniform small-scale warming experiments have been shown to underestimate the responses of plant flowering and leaf-out times, as compared to observations—and results of sudden increase experiments were farther from observations for leaf-out than

for flowering time, and worse for early-season species, which are more sensitive to warming temperature, than later-season species (Wolkovich et al., 2012). The responses of soil C storage and respiration to warming temperatures will be mediated by other global change factors and responses, such as precipitation and soil moisture, net photosynthetic productivity and more; the net effect of all these changes is poorly understood. Therefore, while the results of highly controlled experiments are of interest, new approaches are needed that can incorporate these complex interactions.

Cities as global change treatments:

Conditions in urban and surrounding areas can be exploited as a means to simulate future climate change. Recent studies indicate that current conditions in many cities are similar to those expected 50-100 years from now, in terms of temperature and [CO_2] (Pickett et al., 2001; Gregg et al., 2003; George et al., 2007; Grimm et al., 2008). Atmospheric [CO_2] tends to be higher in cities, due to the physical concentration of fossil fuel emissions and the reduced amount of plant cover to draw levels down through photosynthesis. Soil surface temperatures in urbanized areas can rise as much as 0.5 – 2˚C higher than those in rural areas (Pickett et al., 2001) . Increased temperatures in cities result from alteration of land cover and concentrated energy use; they vary with unique qualities of individual cities, landforms, and regions. For example, the city of Phoenix, AZ is sometimes cooler than surrounding rural areas in the daytime in summer, because water, used for irrigation and sprayed to cool outdoor areas, cools the entire city as it evaporates (Baker et al., 2002).

Unlike experimentally imposed uniform treatments, urban increases in [CO_2] and temperature vary temporally with regional weather patterns. However, the parallels to global changes are not perfect. For instance, global warming theory predicts that higher concentrations of greenhouse gases will cause nighttime and winter minimum temperatures to increase more than daytime and summer maximum temperatures, because greenhouse gases trap radiative energy leaving the earth's surface, thus reducing cooling. Recent measurements have corroborated this pattern for both night (Alexander et al., 2006) and winter temperature patterns (Braganza et al., 2003, 2004). While the differences between urban and rural temperatures tend to be larger at evening and/or nighttime than in the day (Montávez et al., 2008, 2008; Pickett et al., 2011), they may tend to be slightly larger during the summer than other seasons (Morris et al., 2001; Oleson, 2012), although mid-latitude U.S.A. cities may be warmed more in winter than in summer (Pickett et al., 2011). Therefore, urban heat islands track global warming patterns but are not perfect reflections of them. The difference from greenhouse warming patterns is due to the difference in causes—causes of urban heat islands include darker paved surfaces and building materials, which absorb and retain more heat, block wind, and reduce evapotranspirative cooling, as well as concentrated waste heat from energy use. Similarly to urban temperature patterns, rising [CO_2] in cities may be expected to track global levels and fluctuate with them, but with some differences in patterns over different time scales, again due to differences in emissions and photosynthesis in cities. This temporal variability in urban temperatures and [CO_2] levels mean that they require repeated or even continuous measurements to be quantified adequately.

Other factors can also vary with degree of urbanization, and these might need to be accounted for when using urbanization as a proxy for global change in research. Cities and areas downwind can have altered rainfall, cloudiness, and fog due to airborne particulates (Pickett et al. 2001). Urban soils often have elevated amounts of heavy metals and salts (Pouyat et al., 2002; Biasioli et al., 2006). Tropospheric (lower atmospheric) concentrations of ozone, and sulfur and nitrogen oxides, as well as terrestrial deposition of elemental sulfur (S) and nitrogen (N), are often elevated in or near cities (Gregg et al. 2003; Grimm et al. 2008). Because elevated ozone can be detrimental to photosynthesis, and because soil acidity, N and/or S levels can impact plant growth, these factors may confound the effect of temperature and [CO_2] differences along urbanization gradients. In addition, plant and animal communities and soil conditions may vary greatly in response to management or disturbance. If these potentially confounding variables can be controlled, or monitored and included as observational variables, then urbanization could serve as a surrogate for global change conditions; this could make large areas of intact soils, exposed to gradually increasing temperatures and [CO_2], available for study.

Justification, Objectives, Design, and Expected Results of Dissertation Research:

To address the limitations of current global change research, I developed a different approach to climate change research by conducting two studies in which urbanization and microsite or intersite differences create a range in temperature and other conditions; this range serves as a surrogate for experimental global change treatments. In the first study, I was fortunate to be able to work in an already-established experiment, which had been monitoring temperature, [CO_2], ozone, and other variables for six years.

In this experiment, a common soil and seedbank had been transplanted into multiple plots at urban, suburban, and rural locations along a single transect (downtown and suburban Baltimore, MD, and rural Buckeystown, MD), and plant communities allowed to develop in place for six years (Ziska et al., 2004; George et al., 2007, 2009). After six years, I made measurements of AM fungi in soils of these plots.

Both rising [CO_2] and warming temperatures are thought to impact AM fungi indirectly, through host plants. Direct effects of rising [CO_2] on AM fungi are not expected, because soil-dwelling organisms already encounter very high ambient levels of [CO_2] in soils. Direct effects of warming on fungal hyphal growth, including AM fungi, are generally expected to be stimulatory as long as physiological thresholds are not crossed. When other factors are not limiting, rising [CO_2] and warming temperatures are commonly expected to increase plant productivity and, subsequently, the abundance of AM fungi and other plant symbionts
(reviewed by Van der Putten et al., 2010; Pritchard, 2011), but this is not found to be a universal response. For example, under prairie plant monocultures and sixteen-species polycultures growing under elevated [CO_2], only one of 11 species of AM fungi increased in abundance, and this only under monocultures (Wolf et al., 2003). In the same study site, the length of AM fungal hyphae in bulk soil increased under elevated [CO_2], but only in some combinations of plant functional group, species richness, and nitrogen enrichment (Antoninka et al., 2011). When 8°C warming was applied to AM fungi growing in root-free compartments, the length of hyphae increased for two out of the three AM fungi included (Heinemeyer and Fitter, 2004). In undisturbed field plots of

annual grasses where the soil surface was heated ca. 1°C by infrared heaters, length of AM fungal hyphae in bulk soil increased by ca. 40% (Rillig et al., 2002).

In the current experiment, AM fungi may be affected by both urbanization and plant community responses to urbanization. This complicates analysis but may capture more of the interactions and feedbacks moderating responses.

I designed the second study, which is observational, specifically to look at soil C stocks along gradients of environmental conditions. I wanted to test the viability of a different approach to the problems of studying soil C under global climate change and, if possible, evaluate current expectations in intact field soils. In order to observe responses of soil C to small changes in temperature, [CO_2], and other factors *in situ,* soils with similar plant communities and plant inputs, and relatively long periods of undisturbed development are needed. Soils on the roughs of golf courses can provide this, and the managers of the study's golf courses keep detailed records, often over all the years since their courses were created. Therefore, working in this setting provides decades-long, managed field sites exposed to differing levels of temperature and [CO_2] that have risen gradually and fluctuate temporally. The records kept by regional golf course managers could also be valuable sources of data and insights (e.g. observations of temperature, leaf-out and frost dates, soil pH and other chemical qualities over time, photosynthesis and other process variability at local scales) that could be used in future studies.

Capitalizing on the abundance of decades-old golf courses in the area, I located 62 study sites across Maryland and Washington, D.C., all with mixtures of cool season grasses, similar management regimes, and at least 25 years since last soil disturbance. Within each of these courses, I selected flat, unshaded sampling locations from which to

remove soil samples and in which to leave a buried temperature probe. All sampling locations were located in the 'roughs', which are the areas that separate areas of active play on the golf courses, and which receive minimal management and little traffic, and have taller mowing heights than fairways, greens, and teeboxes. Total and active C (see Chapter 3) were the focus of this study.

While there is considerable variation in soil texture, mineralogy, landform, and parent material across the study region, there are also important equalizing factors. A large proportion of local soils are likely to have been plowed for agricultural use sometime in the last century (M. Rabenhorst, personal communication); it is likely that most area courses were constructed on active or recently abandoned agricultural land, so that differences in prior land use will be minimal. Furthermore, management practices are likely to be similar at many courses in the region, e.g. the form, frequency, and quantity of fertilizer and lime applications (K. Matthias, personal communication). Therefore, greater similarity in fertility, pH, and calcium levels is likely among golf course soils than might be expected among similar unmanaged soils across the region.

Observational studies in soils can be problematic because of the innate high variability of many soil characteristics. Despite this variability, such studies have been successfully used to examine the effects of temperature and other factors on soil C over large areas, even with uncontrolled variation in other soil qualities (Raich et al., 2006; Kadono et al., 2008; Meier and Leuschner, 2010; Saiz et al., 2012). For example, in tropical forest soils along elevational temperature gradients, organic matter accumulation was shown to decrease, and turnover rates to increase, with mean annual temperature; these patterns were revealed by meta-analysis of several transects in different areas of the

global tropics (Raich et al., 2006). The authors of this work made the assumption that along each tropical elevation gradient, “the five state factors of soil formation” would vary little enough to allow detection of temperature effects—and they were correct. In contrast, surveys of soil C stocks along latitudinal temperature gradients in temperate grasslands have not yielded consistent findings (Jones and Donnelly, 2004).

If soil C in temperate grassland soils does not consistently respond negatively to increasing temperature, that would be important information to incorporate into models of global C cycling, since in the continental U.S.A., grassland, pasture, or rangeland uses comprised 31% of the total area in 2002 (Lubowski, 2006), and nearly 16.4 million hectares are managed as turfgrass (Milesi et al., 2005).

Global models based on the kinetics of photosynthesis and respiration all predict that terrestrial C sinks will decrease in size under future climate warming, but field experiments suggest that additional mechanisms are also in play (Luo, 2007). In irrigated golf fairway soils in Denver and Fort Collins, CO, U.S.A., for example, the CENTURY model of C cycling predicted that soil C stocks would be approximately 6 Mg C ha^{-1} lower in Denver, where daily maximum and minum temperatures are 1.35˚C and 0.95 higher, than in Fort Collins (Bandaranayake et al., 2003). Field measurements don’t conclusively match model predictions, however. In an Oklahoma tallgrass prairie soil, soil surface temperatures were warmed by 2°C for six years; while some increases in soil respiration were observed later in the study, there was no net change in soil C storage (Luo et al., 2009).

Because soil carbon storage and AM fungi are so important, their responses to future climate change should be well-understood. The complex dynamics and multiple

interactions of soil carbon and AM fungi with many biotic and abiotic factors, however, make it difficult to predict their responses based on models or traditional experimental study. The dissertation research was designed to capture this complexity, by using a ambient conditions and microclimate to provide a range of “treatments”.

Chapter 2:

Decreased abundance of arbuscular mycorrhizal fungi in an urban macroclimate

Experimental summary:

Aims: In urban areas, temperature, carbon dioxide concentrations, and plant biomass production are often greater than in rural areas, and both soil and plant community traits may be altered. Increases in temperature, carbon dioxide concentrations, and plant biomass production would be expected to increase the abundance of arbuscular mycorrhizal fungal, but changes in soils and plant community composition may also have impacts. In order to better understand the responses of arbuscular mycorrhizal fungal to this suite of changes, we measured the abundance of these fungi in plots containing a common imported soil and seed bank, established at rural, suburban, and urban locations along a 50 km transect. In these plots, plant community development, temperature, carbon dioxide, and other air and soil variables were monitored for five years.

Methods: Repeated measurements of arbuscular mycorrhizal fungal hyphae in bulk soil, and one-time measurements of spore community composition, glomalin, and phospho-lipid fatty acid content were conducted. After removal of one unusually hot and dry plot, responses were modeled against plant community composition and biomass production, and against small variations in soil characteristics that developed during the years of the study.

Results: In contrast to expectations, we found less arbuscular mycorrhizal hyphae and marker phospho-lipid fatty acid (16:1w5c) in urban plots, and fewer spores in urban and suburban plots. At the plot level, spore and hyphal abundances were negatively correlated with soil temperature, moisture, and acidity, which together explained 86% of

variability. In contrast, no correlations with plant biomass or community composition were found.

Conclusions: If these responses are widespread, then macro-climatic changes associated with urbanization, which simulate near-term global climate changes, may have soil-mediated negative impacts on the abundance of arbuscular mycorrhizal fungal structures in soils.

Introduction:

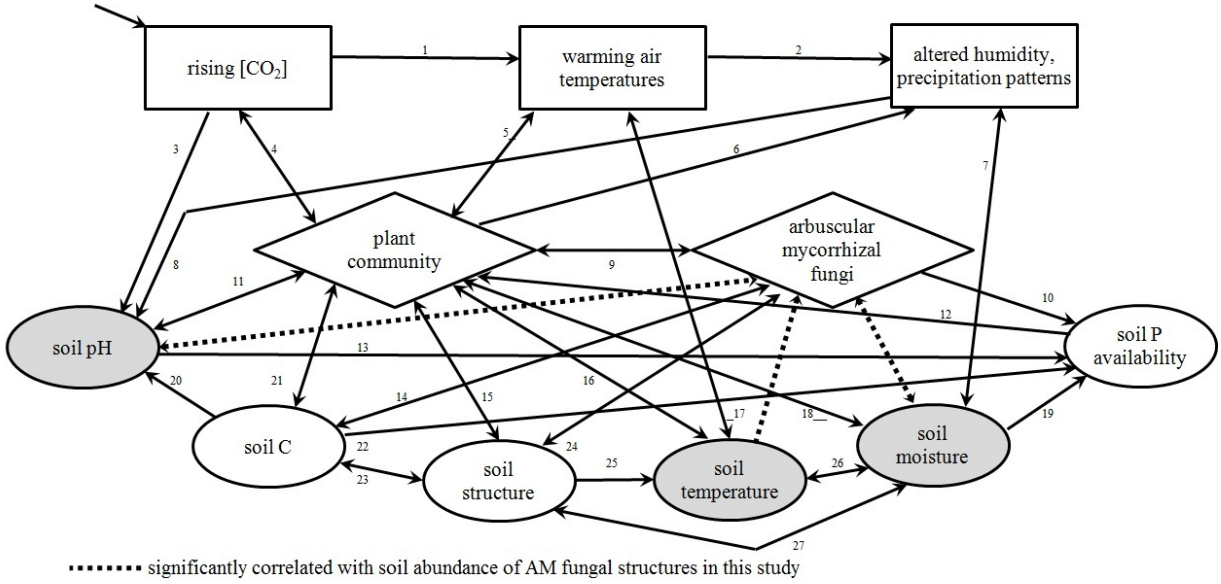
Cities in temperate, mesic zones currently experience warmer temperatures and higher concentrations of atmospheric carbon dioxide [CO_2] than surrounding areas, often matching levels expected globally in 50 to 100 years (Ziska et al., 2003, 2004) These increases are largely due to the urban heat island effect, concentrated fossil fuel combustion, and reduced plant cover, photosynthesis, and evapotranspiration in cities (Pickett et al., 2001; Gregg et al., 2003; George et al., 2007; Grimm et al., 2008). . Differential temperatures and [CO_2] between urban and rural areas can be used to study the local effects of expected climate changes in the field, if other factors that vary with urbanization (e.g. tropospheric ozone levels, deposition of N, S, black carbon, and heavy metals) can be controlled (Ziska et al., 2003; Carreiro and Tripler, 2005). Studies of this nature incorporate some of the complex interactions and feedbacks that occur in intact ecosystems, and can capitalize on many years of gradual, non-linear increases in temperature and [CO_2] (e.g. warmer night and winter temperatures, in contrast to constant steady warming). Often, these aspects of global change are the most difficult to simulate in controlled experimental settings; therefore, urban-to-rural gradient studies may greatly improve our understanding of global change effects.

Many of the changes related to urbanization are likely to impact arbuscular mycorrhizal (AM) fungi. These fungi associate symbiotically with the roots of most terrestrial plants (Brundrett, 2008, 2009) and are obligately biotrophic—their only sources of carbon and energy are living plant roots (Douds et al., 2000). AM fungi can positively impact photosynthetic rate, biomass accumulation, P content, and/or drought resistance of their host plants and variably impact other plant community members

(Johnson, 2010; Smith et al., 2011). Although the majority of plant species form mycorrhizal associations, some early-successional, herbaceous annuals are minimally or non-mycorrhizal; symbiosis with AM fungi has neutral or negative effects on the growth of these plants in experimental conditions, or does not develop at all (Johnson et al., 1997, 2008; Olsson and Tyler, 2004; Brundrett, 2009; Oehl et al., 2011).

Most aspects of urbanization and climate change interact with AM fungi indirectly, via changes in soil qualities and/or the plant communities that supply photosynthate to the fungi (Fig. 1).

Figure 1: Potential impacts and interactions of urbanization and global change factors, soil qualities, and plant and AM fungal communities.



Legend:

1. greenhouse effect
2. increased evaporation and temperature differentials
3. increased carbonic acid dissolution in rain and soil water may alter soil pH
4. stimulation of photosynthesis and drawdown of CO_2
5. altered albedo, emission of GH-associated VOCs
6. transpiration
7. evaporation/precipitation
8. base cation leaching and weathering of soil minerals
9. C and energy to AM fungi/P, H2O, disease protection to AM plants
10. direct access and uptake of immobile P
11 . plant litter qualities impact soil pH/soil pH impacts plants and nutrient availability
12. passive diffusion to plant roots
13. soil P chemical species and solubility change non-linearly with pH
14. soil organic matter stimulates growth of AM hyphae/AM fungal structures contribute to soil C by exudation and turnover
15. plant roots and exudates physically structure soils, plant canopy protects from erosion/soil structure anchors plants, pores allow root respiration
16. shading, evapotranspiration/plant root temperature requirements
17. heat exchange
18. plant water requirements, transpiration/ infiltration or runoff of precipitation
19. dissolution and diffusion of soil P
20. soil organic matter decomposition releases H+
21. plant litter, roots, and exudates contribute to soil C pool/ soil C provides water-and nutrient-holding capacity and CEC
22. soil C can minimize soil P fixation

23. soil C contributes to soil aggregation/soil C inside aggregates protected from microbial degradation and respiration
24. AM fungal hyphae and glomalin contribute to soil aggregation/soil pores permit growth and respiration of fungal structures
25. soil porosity/air permeability contributes to heat exchange
26. evaporative cooling and the high heat capacity of water link soil moisture and temperature
27. soil structure governs permeability and infiltration/ soil water influences soil ….. unknown mechanisms

Rising [CO_2] is thought to impact AM fungi only through host plants, and is generally expected to have positive or neutral effects on AM fungi, based on theoretical models and experiments with doubled [CO_2] (Alberton et al., 2005; Drigo et al., 2008; Pritchard, 2011). Warming temperatures are expected to have complex impacts on plants and on AM fungi, over a variety of timescales. However, a general positive effect of warming temperature has been suggested, based on the expectation of increased plant productivity (Van der Putten et al., 2010). The expected impacts of any single global change factor may not reflect the net effects of multiple interactions and feedbacks (Klironomos et al., 2005; Rustad, 2006; Luo et al., 2008), which are only beginning to be explored (Morgan et al., 2011).

To focus on these complex interactions, we utilized an existing urban-rural transect study (Ziska et al. 2004, George et al., 2009) with a common, imported soil to monitor the net effects of changing [CO_2], temperature, and all other covarying factors on the abundance AM fungi. Plots along this transect were initiated in 2002, and in the first growing season, plant communities were similar at all three transect locations, dominated by weedy annual species. However, total plant biomass production and litter accumulation were greater towards the urban side of the transect (Ziska et al., 2007; George et al., 2009). In subsequent years, plots contained more and larger individuals of woody and perennial species the urban side of the transect; this was attributed to decreased germination success of smaller-seeded plants in plots with greater litter accumulation.

Both increased [CO_2] and temperature, as well as increased biomass of mycorrhizal plants, are expected to positively impact AM fungi (Gamper et al., 2004;

Denef et al., 2007; Drigo et al., 2007; Van der Putten et al., 2010; Pritchard, 2011). Therefore, we expected to find increasing AM fungal spores and extramatrical hyphae (EMH) towards the urban side of the transect. Warming temperatures in and near cities also impact the phenology of plant leaf-out in spring and senescence in the fall. Production of AM fungal structures may track this phenology, because plant allocation of C to AM fungi may be expected to decrease when photosynthesis is decreased. Therefore, we expected to see evidence of differing phenology in repeated measurements of EMH.

Materials/Methods:

Establishment, monitoring and sampling of field sites

A single urban-to-rural transect between Baltimore and Buckeystown, MD was established in 2002 (Ziska et al., 2004). Four plots were initially established within 25 m^2 areas at each of three sites along the transect: downtown Baltimore (39.27°N, 76.60°W, elev 6.8m) , suburban Baltimore (39.30°N, 76.68°W, elev 98.9 m), and rural Buckeystown, MD (39.30°N, 77.43°W, elev 109.8 m). After establishment, one rural plot was damaged by groundhog activity and one suburban plot damaged by stream flooding; therefore the experimental n of 10 plots is unevenly distributed among transect locations (three plots at rural and suburban sites and four plots at the urban site).

Topsoil and subsoil from a single location at the Beltsville Agricultural Research Center (Beltsville, MD) were separately homogenized and placed into all plots. The plots were 2 x 2 m in area and 1.1 m deep. Plastic barriers between the imported and surrounding soils were placed to a depth of 20 cm. The imported soil was a Codorus silt loam containing excessive soil test levels of N, P, and K at the start of the experiment due to prior fertilization, and with pH of 5.5 (calcium chloride method). Plant communities were allowed to develop from seed and to grow naturally from 2002 to 2006; temporal dynamics of the plant communities during these years have been described (Ziska et al., 2004, 2007; George et al., 2007, 2009). Plant community composition was assessed as the aboveground biomass produced each year by each plant species. During growing seasons, plots received supplemental water based on weekly, site-level estimations of evapotranspiration; this reduced but did not eliminate differences in soil moisture among plots.

Measurements of soil temperature, moisture, and respiration in each plot were made weekly through the end of the last complete growing season of the experiment. To match collection dates for AM fungi (see below), averages over all measurement dates from May 2 through Nov 7, 2006, when measurements ended, were used as explanatory variables in this study. Because on-site measurements were discontinued after the end of the 2006 growing season, daily mean air temperatures over the entire soil sampling period (July 2006 – May 2007) were obtained from the National Climatic Data Service (http://cdo.ncdc.noaa.gov/cgi-bin/cdo/cdostnsearch.pl) from monitoring stations adjacent to the urban (MD Science Center in Baltimore City, MD, U.S., 39.26°N, 76.60°W, elev 6.1 m) and near the rural (Frederick 2 NNE, Frederick, MD, U.S., 39.43°N, 77.38°W, elev 85.4 m) transect locations (Fig. 3b).

In each plot, soil samples were taken as composites of three individual cores, each to a depth of 10 cm, which were removed from arbitrary positions in the plots without severing live plant stems. Samples for spore extraction and trap culture initiation (see below) were collected with a 2-cm diameter soil probe on Feb. 9th, 2006. Samples for hyphal extraction were collected with a 1-cm diameter cork borer on the following dates: July 26th, August 2nd and 24th, September 7th, 12th, 21st, and 26th, October 5th, 18th, and 31st, November 15th and 29th, and December 11th and 27th, 2006; January 23rd, March 14th and 26th, and April 24th, 2007. Snow accumulated intermittently at all transect locations throughout February and early March, 2007, with deeper and longer-lasting accumulations at the rural site; no sites were sampled at times when any site had snow

cover. All soil samples were placed in a cooler with ice packs immediately after collection, and were stored at 4˚C upon return to the laboratory. Before processing, samples were thawed and homogenized by manual shaking and disruption of clods for 2 min. The weight change of subsamples after oven-drying at 105˚C was used to estimate the dry weight of soil used for each measurement. Soil pH in each plot was measured in soil subsamples from March 26th and April 24th, 2007; pH in three replicate 1:1 soil-water slurries was measured using a Beckman 34 pH meter **(**Beckman Coulter, Inc., Brea, CA, U.S.) and the average over all measurements from each plot is presented.

Quantification and identification of AM fungal spores from soil

Colonization of plot soils by AM fungi from the surrounding soil would confound the detection of site-level effects relating to urbanization and plant community development. Therefore we compared AM fungal spore communities inside and outside each plot. Three cores were taken from the center of each plot, an additional three from ~10 cm inside the most distal corner of each plot, and another three from the native soil ~10 cm outside of the plot, adjacent to the sampled corner. This sampling scheme was carried out to check for invasion of plot soil by AM fungal morpho-species from the native soil. The replicate cores were combined, and homogenized as described above, subsamples were removed for initiating trap cultures (see below), and the remainder stored frozen until spores were extracted.

Spores of AM fungi were extracted from approximately 30 grams (fresh weight) of soil from all field samples and from approximately one fourth of pot soil from each trap

culture. Trap cultures were grown, and spores were extracted from field and trap culture soil following a published protocol (Johnson et al., 1999) using sieves ranging from 38 to 250 µM in pore size. Supernatants from all steps were drained through a 250 µM sieve, and retained material was combined and examined under a dissecting microscope (Nikon SMZ1500, Nikon Instruments Inc., Melville, NY, U.S.); all spores found attached to or contained within soil mineral or organic particles were removed with fine jeweler's forceps. AM fungal spores were identified to morpho-species based on visual descriptions made by Schenck and Perez (1990) and published online by the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm).

Extraction and quantification of extramatrical hyphae

EMH were extracted from soil samples following a published protocol (Rillig et al., 1999) with the following changes: no stain was used; after thorough drying filters were mounted on glass slides using Permount™ solution (Fisher Chemical, Fairlawn, NJ) and allowed to set for at least seven days; and hyphae were quantified at 400x magnification using differential interference contrast (Zeiss Axioplan II compound microscope). Hyphae were categorized in five classes: i-iii) hyaline, clear - to - yellow, irregularly septate, thin (< 2.5 µM), medium (2.5 – 5 µM), and thick (> 5 µM) diameter hyphae; iv) hyaline, irregularly septate, orange -to-light brown hyphae of all diameters; and v) matte, pale tan-to- grayish brown, regularly septate hyphae of all diameters (Figure 2a – 2e) . The first four categories are presumed to be AM fungal hyphae based on published descriptions (Nicolson, 1959; Sylvia, 1990, 1992). The fifth category is presumed to

represent hyphae of zygomycetous or ascomycetous non-AM fungi. No hyphae with clamp connections, which would indicate basidiomycetes, were observed.

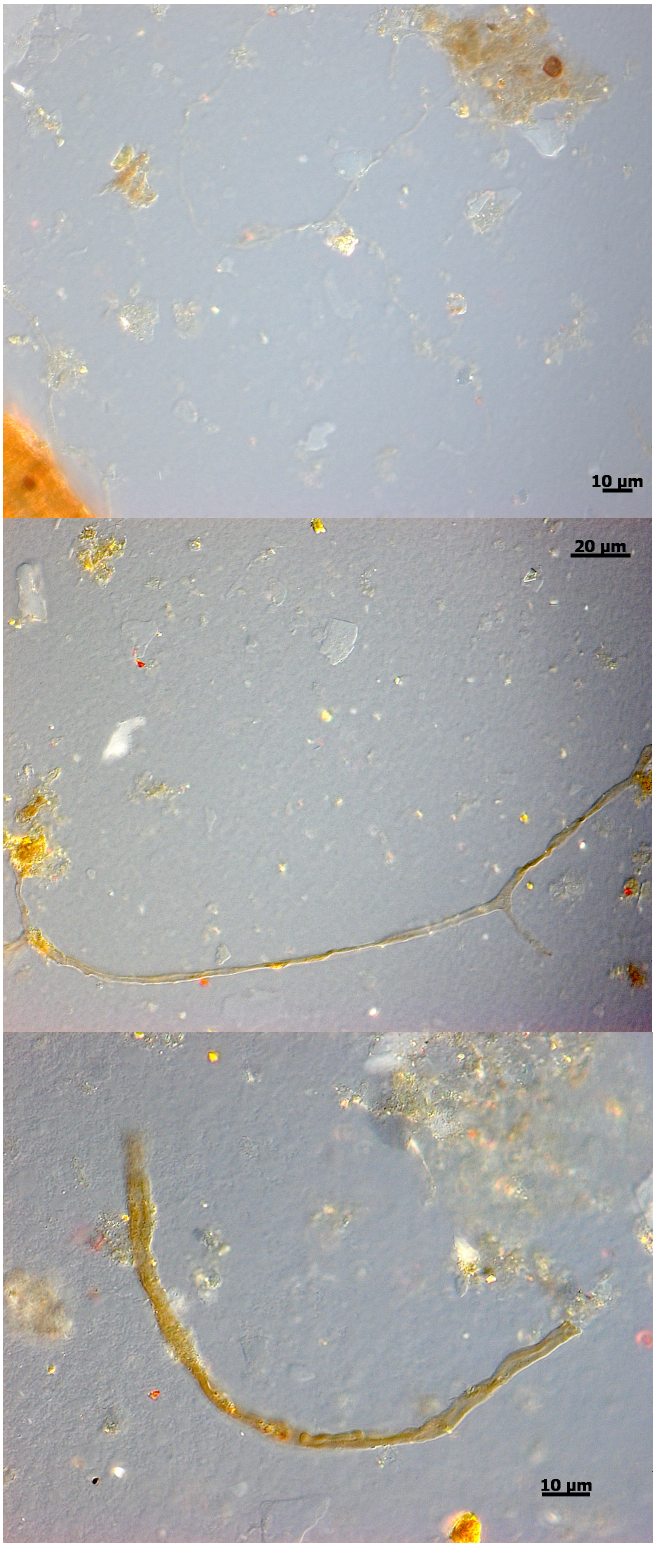


Figure 2 a – 2 c: examples of thin, medium, and thick AM fungal hyphae size classes

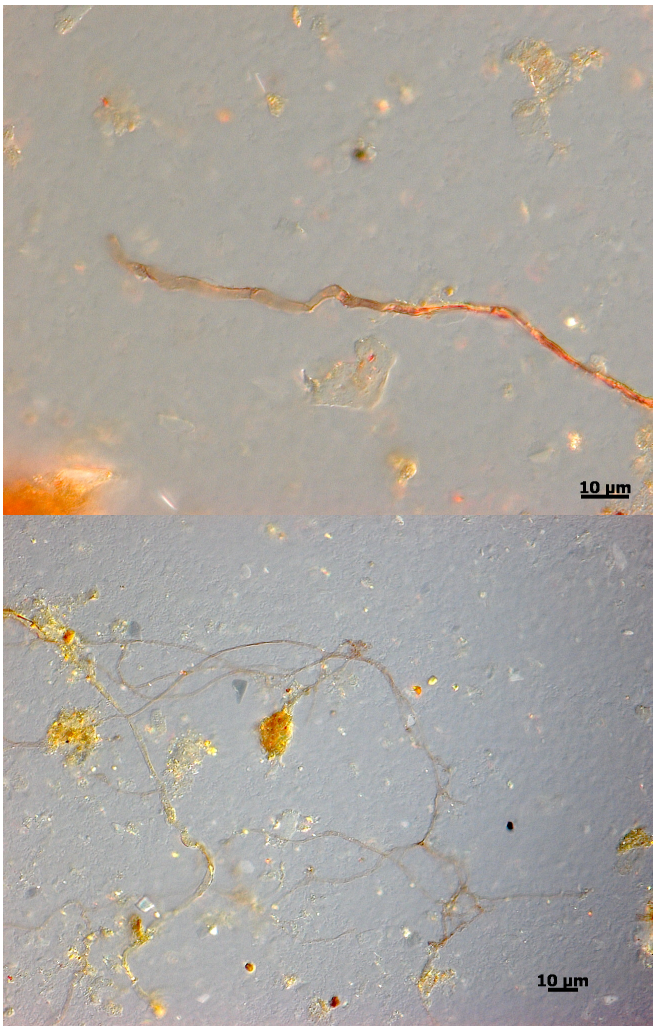


Figure 2 d: example of an ‘orange’ AM fungal hypha, and e: brown septate hyphae (at center-right of photo)

Analysis of Phospholipid Fatty Acids

Phospholipid fatty acids were extracted from soil samples collected on Dec. 17, 2007 as described above. Chemical extraction and purification of lipids from 5 g of lyophilized soil, and separation by gas chromatography, was conducted as described by Buyer et al. (2010). Because single fatty acids do not necessarily reflect the abundance of single members of the soil community, abundances of groups of fatty acids were analysed (Buyer et al., 2010; Frostegård et al., 2011). Groups included: arbuscular mycorrhizal fungi (16:1 ω5cis); actinomycetes (10-methyl fatty acids); gram positive bacteria (all branched iso and anteiso fatty acids); gram negative bacteria (all monounsaturated fatty acids), total fungi (18:2 ω 6 cis); and protozoa (20:3 and 20:4).

Quantification of glomalin

Glomalin-related soil protein (GSRP) (Rillig, 2004a) was extracted from 2-g subsamples separated from soil samples collected on December 17, 2007 with alkaline sodium pyrophosphate (Wright et al., 2006). Bradford-reactive soil protein (BRSP) was used to quantify total protein in the extract solution, and immunoreactive protein was measured using procedures described by Wright et al. (2006). The detection limit for measuring total protein is 8.33ng/ul and for immunoreactive protein is 0.1 ng/ul.

Statistical methods

In this study, the treatments had low and unbalanced n (three plots each in rural and suburban locations; four plots in the urban location) and some uncontrolled factors. We used traditional analysis of variance to evaluate simple site-level differences in univariate

responses. We then used non-parametric permutation (resampling) tests to evaluate multivariate plot-level factors, plant community and PLFA responses. Tukey's HSD was used for *post hoc* comparisons. We did not employ any correction for multiple tests of significance.

All statistical analyses were carried out using R (version 2.12.2, R Development Core Team, 2011). The R package vegan (version 1.17-9, Oksanen et al., 2011) was used to assess Bray-Curtis dissimilarities among plant communities, AM fungal spore communities, and the above-described groups of PLFAs from each of the ten plots, using the function "metaMDS". The function "adonis" was used to look for community differences among transect locations. This function assigns variability in dissimilarity matrices to levels of a given variable or function; the likelihood of this variability is then evaluated by permutation. When significant community differences were detected among the transect locations, the function "indval" from the R package labdsv (Roberts, 2010) provided indicator species analysis as described by Dufrene and Legendre (1997). The function "envfit" from the vegan package was used to compare community composition dissimilarity matrices to multivariate environmental measurements or to other community matrices (e.g. AM fungal spore communities to plant or soil PLFA communities), and to indicate which individual components were significantly correlated with dissimilarity among community composition. Test results with $0.05 < P \leq 0.10$ are presented as suggestive of differences or trends.

Table 1. Mean abundances (spores-g-1 soil) ± one standard deviation, of visually identified AM fungal spore morpho-species in rural, suburban, and urban plots. Significant indicator species are in bold.

Site (no. of plots):	Rural (n = 3)	Suburban (n = 3)	Urban (n = 4)
Acaulospora spinosa	0.01 ± 0.01	0	0.01 ± 0.01
Archeospora leptoticha	0	0.06 ± 0.1	0.02 ± 0.03
Ar.trappei	0.8 ± 0.26	1.18 ± 0.84	3.12 ± 3.25
Entrophospora infrequens	0.01 ± 0.01	0	0.01 ± 0.01
Gigospora margarita	0.04 ± 0.02	0.07 ± 0.07	0.05 ± 0.09
Glomus clarum	0.4 ± 0.08	0.33 ± 0.08	0.37 ± 0.32
Gl. etunicatum	0.38 ± 0.25	0.47 ± 0.21	0.89 ± 1.17
Gl. fasciculatum	0	0.09 ± 0.15	0
Gl. geosporum	0.01 ± 0.02	0.01 ± 0.01	0
Gl. intraradices	**13.18 ± 1.44**	**6.08 ± 2.8**	**5.23 ± 3.15**
Gl. mosseae	0.12 ± 0.12	0.23 ± 0.18	0.33 ± 0.31
Gl. sinuosum	0.56 ± 0.48	0.21 ± 0.23	0.12 ± 0.24
Gl. sp., unknown *Sclerocystis*-type	0.02 ± 0.03	0	0
Paraglomus occultum	**1.2 ± 1.08**	**0.17 ± 0.18**	**0.1 ± 0.21**
Scutellospora calospora	0.23 ± 0.1	0.29 ± 0.26	0.01 ± 0.01
S. gregaria	0.01 ± 0.01	0	0.07 ± 0.13
S. pellucida	0.02 ± 0.01	0.05 ± 0.07	0.01 ± 0.01

Results:

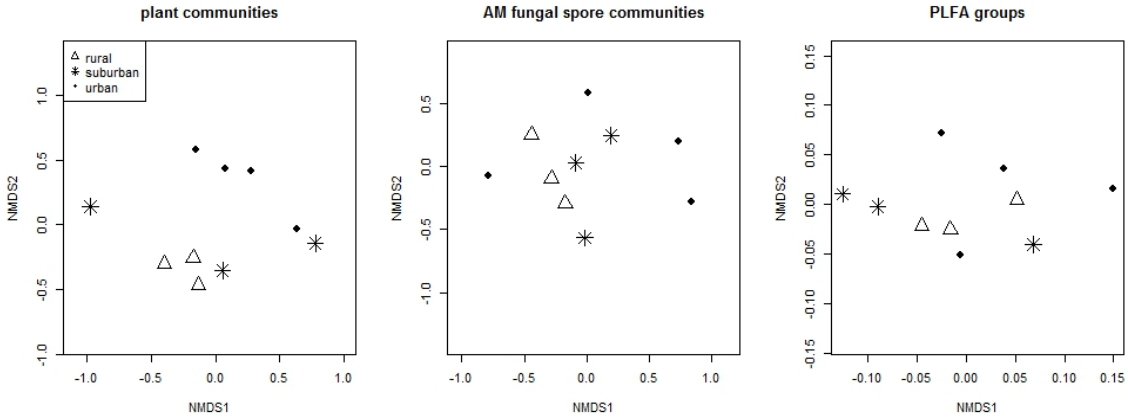
Environmental and plant community variation

Mean plot soil temperatures over the summer and fall of 2006 did not vary significantly among transect locations; means over these dates were 19.2 ± 1.5˚C in rural, 18.5 ± 0.2˚C in suburban, and 18.8 ± 0.3˚C in urban plot soils (P = 0.61). In contrast, over the five full years of the experiment mean soil temperature was significantly higher in urban than in suburban and rural plots (George et al., 2007). In 2006, [CO_2] averaged 402 µmol mol^{-1} at the rural site, which was significantly lower than the averages at the suburban and urban sites, which were 449 and 448 µmol mol^{-1} respectively. Over the five full years of experimental measurements, average [CO_2] levels were significantly different among all three sites; levels were 409 at the rural, 435 at the suburban, and 482 µmol mol^{-1} at the urban site (George et al., 2009). Despite supplemental watering of the transect plots, comparison of mean soil gravimetric moisture over the summer and fall of 2006 is suggestive of differences among transect locations (P = 0.065). *Post hoc* comparison (Tukey's HSD) indicates that rural plot soils (mean soil moisture of 7.43 ± 2.73%) were on average dryer than suburban plots (mean 11.27 ± 1.06%), and urban plots were not different from either (mean 10.34 ± 1.09%). As measured in the fall of 2006, soil pH (1:1 soil to water) ranged from 6.30 to 6.72 among individual plots, but did not vary significantly among transect locations (P = 0.37).

Comparison of 2006 plant community composition was suggestive of differences among transect locations (P = 0.053, Fig. 3a), although plant species richness and Shannon-Weiner index of diversity did not vary detectably (P = 0.193 and 0.760, respectively).

The plant indicator species driving community differences were lambsquarters (*Chenopodium album*), which was most abundant and frequent in rural plots (P = 0.029), and curly dock (*Rumex crispus*, P = 0.033), Jimson weed (*Datura stramonium*, P = 0.051), oak (*Quercus* sp., P = 0.030), and red maple (*Acer rubrum*, P = 0.005), which were all most abundant and frequent in urban plots. Neither total nor mycorrhizal plant biomass varied significantly among transect locations in 2006 (P = 0.189 and 0.195, respectively) although cumulative aboveground biomass over all five years of the experiment was significantly greater at the urban site than at the suburban and rural sites (George et al., 2009).

Figure 3 Multidimensional scaling ordinations of (a) plant community composition, (b) spore community composition, and (c) PLFA group abundances.



AM fungal responses

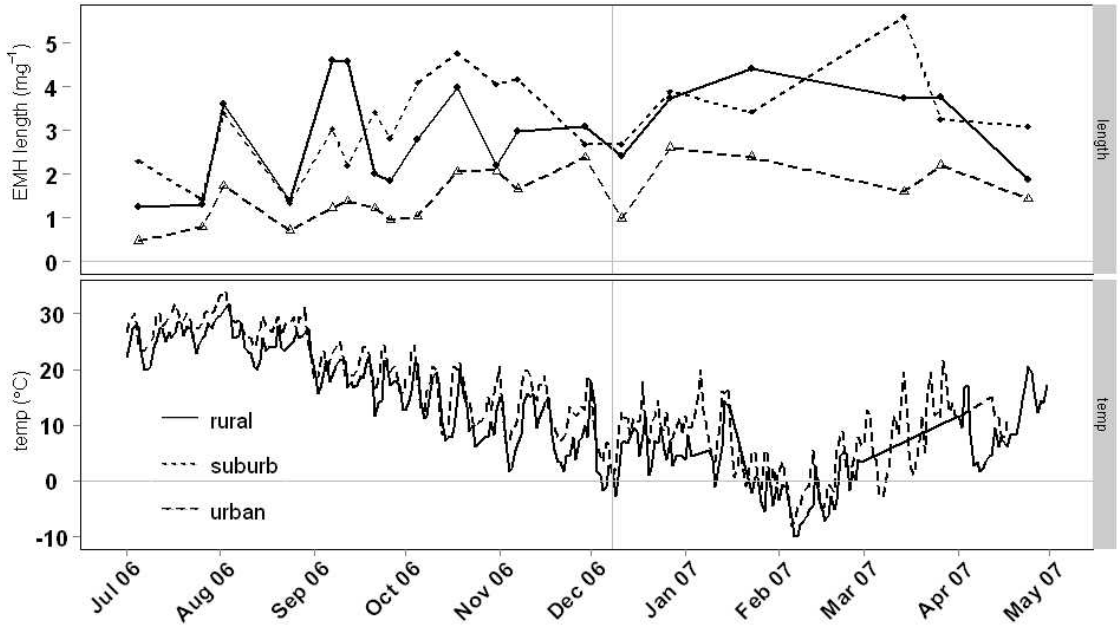
No AM fungal species unique to local soils outside of plots were detected in plot soils (not shown). Comparison of AM fungal spore communities may suggest differences among transect locations (P = 0.089, Table 1, Fig. 3b). Richness of AM fungal spore morpho-species trended lower in urban than in rural plots (P = 0.063); the Shannon-

Weiner index of diversity did not vary detectably among locations (P = 0.243). Community differences among transect locations were attributed to spores resembling *Glomus intraradices* (P = 0.026) and *Paraglomus occultum* (P = 0.043). Both indicator morpho-species were more abundant, and *P. occultum* was present more frequently, in rural plots than in urban and suburban plots.

Community composition of AM fungal spores was not detectably correlated with any of the following plot-level measurements: mean soil temperature, moisture, pH, or aboveground biomass of mycorrhizal, non-mycorrhizal, woody, herbaceous, and non-native plants. Procrustes rotation analysis of plant and AM fungal spore community ordinations did not reveal correlation between the two (P = 0.253).

The repeated measurements of AM fungal EMH length in plot soils are shown in Fig. 4a, with concurrent daily mean air temperatures from nearby weather stations aligned in Fig. 4b. The length of AM fungal hyphae in soil, when averaged over all collection dates, differed significantly among transect locations (P = 0.010). Mean AM fungal EMH length over all dates was 3.86 ± 0.60 m hyphae - g^{-1} soil in the urban plots; *post hoc* comparison (Tukey's HSD) showed this to be significantly less than in suburban and rural plots, which had means of 6.19 ± 0.44 m - g^{-1} soil (P = 0.017) and 6.91 ± 1.42 m – g^{-1} soil (P = 0.020), respectively. The majority (99 ± 0.7%) of AM fungal hyphae present in all plots were thin-walled with diameters in the ≤ 2.5 µm or 2.5 – 5.0 µm classes. Hyphal length did not decline for any appreciable winter period. Glomalin concentrations were below the detection limits in all plot soils.

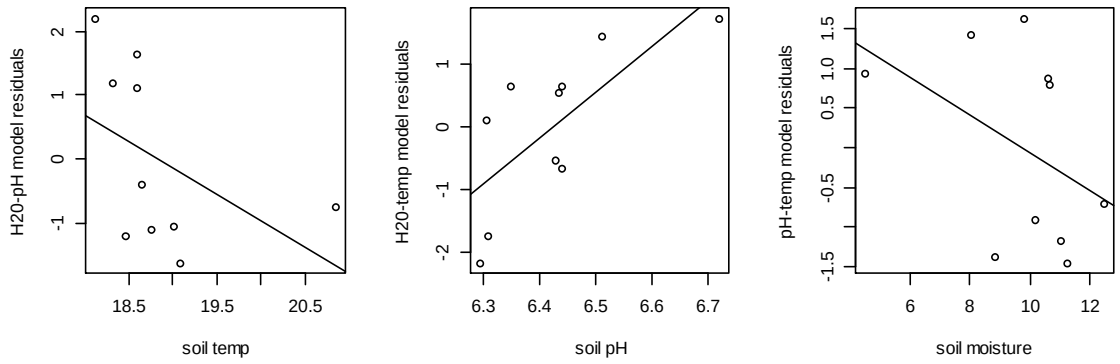
Figure 4 Mean AM fungal extra-matrical hyphal length at urban (dashed), suburban (dotted), and rural (solid line) sites (a), and daily mean air temperatures from National Climatic Data Service (http://cdo.ncdc.noaa.gov/cgi-bin/cdo/cdostnsearch.pl) monitoring stations adjacent to the urban (dashed line, MD Science Center in Baltimore City, 39.26°N, 76.60°W, elev 6.1 m) and near the rural (solid line, Frederick 2 NNE, 39.43°N, 77.38°W, elev 85.4 m) transect locations (b).



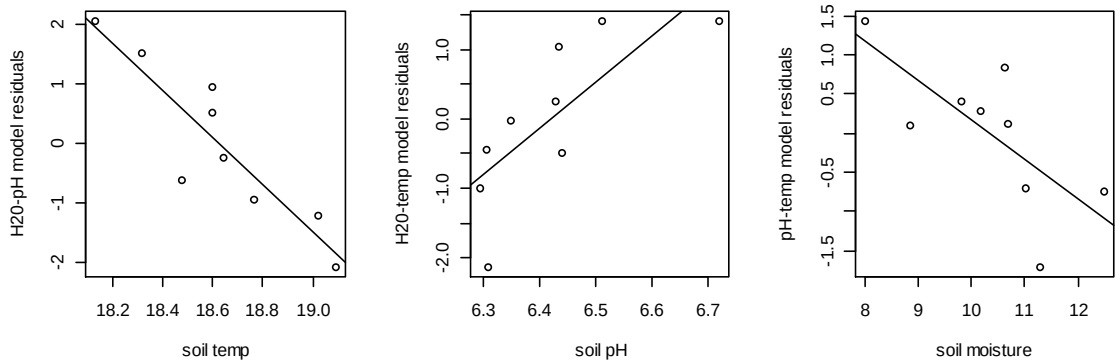
To better understand the responses of AM fungal EMH length to plant and soil factors, we conducted exploratory multiple regression analyses to model the abundance of *Glomus intradices* and *Paraglomus occultum* spores and of mean EMH length in each plot against the plot-level measurements of total, mycorrhizal, woody, and/or non-mycorrhizal plant aboveground biomasses, soil pH, temperature, and moisture. Site-level location along the transect (urban, suburban, or rural) was also included. No plant group biomass explained the abundance of either the AM fungal indicator species or or of mean EMH length. In contrast, all three soil variables were retained in the final statistical models of these responses. When the residuals of this regression were plotted, one plot at the rural site was revealed to be an outlier, with much higher temperature and lower soil moisture (Fig. 5a). When this outlier was removed, the overall fit improved (adjusted R^2 increases from 72 to 86% for EMH; Fig. 5b). If any soil factor was removed, no correlations could be modeled—soil temperature, moisture, and pH were all needed. With all three soil factors in the model, site-level location along the transect (urban, suburban, or rural) no longer had explanatory value. Principle components analysis of soil temperature, moisture, and pH in the plots retained three axes of variability and did not suggest dominant or redundant roles for any of these factors (not shown).These three soil factors were similarly correlated with the abundance of *P. occultum* spores but the abundance of G. intradices spores was weakly correlated with only soil moisture (not shown)

Figure 5. Plot-level modeling of mean EMH against soil temperature, moisture, and pH, in a) all plots, and b) after omitting one outlier

A



B



Phospholipid Fatty Acids

No differences in soil community composition, as represented by grouped phospholipid fatty acids, were found among urban, suburban, and rural transect locations (Fig. 3c, $P = 0.316$). No correlation was detected between soil PFLA groups and AM fungal spore community composition ($P = 0.450$). Univariate analysis of only 16:1ω5c, frequently used as a marker for many but not all AM fungal species, was suggestive of decreased abundance in the urban plots ($P = 0.074$); this urban decrease matches the pattern of AM fungal EMH length (lower in urban plots) but not that of AM fungal spore abundance (lower in both urban and suburban plots).

Discussion:

We observed decreased soil abundance of AM fungal EMH in urban plots, and of AM fungal spores in suburban and urban plots, along a single rural-to-urban transect. Three soil factors— moisture, temperature, and pH—were significant predictors of the soil abundance of AM fungal EMH and of spores of one of the two species that varied across the transect. In contrast, plant community factors were not correlated with the abundance of any AM fungal structures in bulk soil. Together, soil temperature, moisture, and pH accounted for c. 70% of the variability in the abundance of AM fungal EMH outside of plant roots, but without all three factors, no variability in AM fungal abundance could be explained.

Soil abiotic characteristics have complex relationships with each other, with plant and soil communities, and with atmospheric and climatic changes, over a variety of temporal and spatial scales (Fig. 1). In mesic, temperate field settings, soil temperature and moisture may inversely covary to some degree, but not so much that either could represent the variability of the other in models, and each impacts and responds to many other factors. Similarly, soil pH strongly influences and interacts with many other soil characteristics, including the chemical speciation and solubility of P. This complexity explains the need for all three explanatory soil variables, and highlights that even small changes in these variables (relative to many experimental treatments) impacted the abundance AM fungal structures in our plots.

In contrast to the small ranges in soil factors among experimental plots, plant community composition varied widely among transect locations and individual plots (Fig. 3a). While oak stems of various size were present in most plots, all of the urban

plots were dominated by one or two species of fast-growing woody perennials, including the AM hosts mulberry (*Morus* sp.), tree-of-heaven (*Ailanthus altissima*), and red maple, and the dual AM/ectomycorrhizal hosts poplar (*Populus* sp.) and oak. Two of the three suburban plots were dominated by the viney or shrubby perennials porcelainberry (*Ampelopsis brevipedunculata*) and multiflora rose (*Rosa multiflora*), both AM hosts. In contrast, rural plots were dominated by annuals, mostly asters (*Asteraceae* sp.) and ragweed (*Ambrosia artemisifolia*), which are AM hosts, and lambsquarters, a non-mycorrhizal member of the Amaranthaceae.

AM fungi can only receive C compounds from living plants, but this is not necessarily the most limiting aspect of their existence. Because plant roots direct sugars to parts of the root system that are sources of P and other nutrients, any AM fungus colonizing a root should obtain photosynthate if it is able to supply such nutrients to plant roots. This means that AM fungal EMH may be more influenced by the soil environment from which these nutrients are obtained than by the plant community per se (Helgason and Fitter, 2009). In situations where plant nutrient supply is not limiting, any AM fungi present in plant roots must obtain plant C either by fulfilling a different plant need (e.g. water availability or protection from root pathogens) or by simply scavenging C from plant roots. In these situations, plant community composition may not have any bearing on the abundance of AM fungal spores and EMH.

A number of recent studies have demonstrated that AM fungal community composition and/or abundance are better correlated with edaphic conditions than with plant community factors (Oehl et al., 2003; Ji et al., 2010; Santos-González et al., 2011). Several experiments correlated changes in AM fungal community composition with soil

pH and weakly or not at all with plant community composition (Dumbrell et al., 2009; Lekberg et al., 2011; Maček et al., 2011), and in one study, a one-unit drop in soil pH (from c. 6.0 to c. 5.0) was associated with decreased production of AM fungal EMH (Van Aarle et al., 2002). Large changes may push locally adapted species outside of their optimum ranges of soil temperature, moisture, or pH, but in our study, the ranges in these variables seem too small for this to have occurred.

Increasing soil temperatures and moisture levels may affect both the metabolism of soil decomposers and the supply of photosynthate from plant hosts. Almost all hyphae observed in this study were thin-walled and of thin diameter; these are likely to have turnover times of less than a week (Staddon et al., 2003; Olsson and Johnson, 2005). Therefore, temperature-related increases in decomposition rates may be speeding the decomposition of these thin hyphae in our plot soils over time scales relevant to our repeated measurements. Increasing soil moisture may ameliorate plant water stress and increase the diffusion of dissolved nutrients towards roots; both would serve to decrease the amount of photosynthate the plants send belowground to roots and AM fungi. These effects would be in line with the observed negative correlations of soil temperature and moisture with AM fungal structures.

The availability of soil P to plant roots is known to vary with small changes in pH (Shen et al., 2011). The range in soil pHs that developed among plot soil over the five experimental years was 6.30 - 6.72; changes across this range are unlikely to eliminate any locally-adapted strains of AM fungi, especially considering that pH can decrease much more drastically when moving from bulk to rhizosphere soil. In this pH range, the adsorption of P to soil particle surfaces (clay minerals, aluminum and iron oxides, and

soil organic matter) may limit plant P availability more than overall soil P content (Devau et al., 2009). In one study, the availability of inorganic P rose and the adsorption to montmorillonite decreased as soil pH was lowered across this pH range (Devau et al., 2011). If our plot soils exhibit similar P dynamics, then the negative correlation of AM fungal EMH with soil acidity (i.e. positive correlation with soil pH) may be due to increased P availability to plant roots.

The single experimental transect used in this study may encompass a number of confounding influences; and additional work will be needed to confirm the generality of these results in the context of urbanization. There are very few studies using urbanization as an experimental variable, but the ones that exist corroborate our findings. Bainard et al. (2011) found decreased AM fungal colonization of urban tree roots compared with rural conspecifics, and in a study of nematodes, Pavao-Zuckerman and Coleman (2007) found evidence of decreased fungal dominance of food webs in urban soils.

We found negative impacts of increasing soil temperature, moisture, and acidity on the abundance of AM fungal structures. These factors are all likely to increase under future levels of global change as well in urbanizing areas. Because greenhouse gas emissions have not decreased, global mean air and soil temperatures will continue to rise. Precipitation patterns are difficult to predict, but independent of precipitation changes, many soils will become wetter as [CO_2] continues to increase, due to decreased stomatal conductance (Ainsworth and Rogers, 2007, 2007). While human activities can raise soil pH dramatically on local scales (due to liming, proximity to cement, etc.), rain and soil water will contain more dissolved carbonic acid as [CO_2] rises (although warmer

temperatures will also reduce the solubility of CO_2); over long periods this may increase leaching of soil cations, and may even decrease soil pH (Oh and Richter Jr., 2004; Oh et al., 2007; Cheng et al., 2010) . These impacts are separate from (but will compound the problem of) soil acidification related to N and S pollution (Richter, 2007). To date, experimental investigations and modeling have highlighted the indirect effects of climate change on AM fungi, working via altered plant performance, with neutral or slight positive effects of rising [CO_2] and temperature on AM fungi (Van der Putten et al., 2010; Pritchard, 2011) . Our results suggest instead that the soil impacts of factors common to urbanization and climate change will negatively impact AM fungi, and we encourage more investigation focusing on these relationships.

Chapter 3:

Positive correlation of soil carbon with warming winter temperatures, ozone, and soil lead content in intact turfgrass soils

Experimental Summary:

Aims: The factors that regulate soil carbon pools and fluxes are of great interest, particularly to better predict the responses of soil carbon to multiple global changes. However, many experimental designs are too short and not sufficiently complex to adequately represent responses in intact soils. Here, soils with long exposures to varying environments were used to study these responses.

Methods: In the temperate mesic region of Maryland and Washington, D.C., U.S.A., 63 golf courses were located where soils had similar plant communities and low levels of management, and had been undisturbed for at least 25 years. At sampling sites within 'roughs' at each course, I measured hourly soil temperature over a period of seven months, ozone exposure during 'peak ozone season', soil chemical and textural qualities, and management/site variables. Complete explanatory data was available for 39 of the courses. Using these measurements, I investigated the relationships of total and active soil carbon with potential factors of influence. I specifically tested two hypotheses: i) total soil carbon is impacted by warming mean temperature and ii) labile soil carbon, operationally defined as 'active' carbon, is less sensitive to temperature increases than more recalcitrant pools.

Results: Contrary to hypothesis i), total soil carbon was not correlated with warming mean temperature, but was positively correlated with increasing mean February daily-minimum temperature. There were no significant relationships between active soil

carbon and any temperature measurements. Along with the response of total carbon, this finding does not lend support to hypothesis ii). In addition to the positive correlation with mean February daily-minimum temp, we also observed positive relationships of total soil C with soil CEC, soil lead content, and relative ozone exposure, and smaller negative relationships with fertilization and soil clay content (which is the opposite of what would be expected). Active C was strongly positively correlated with total C, positively correlated with soil pH and relative ozone exposure, and negatively correlated with course age (since establishment) and fertilization. No other factors yielded significant relationships with active or total soil carbon.

Conclusions: Our findings suggest that in temperate, mesic grassland soils, soil C decreases with the frequency, duration, and/or severity of winter cold temperatures (freezes and/or thaws); there are many mechanisms that could drive this relationship. While warming is likely to have negative impacts on terrestrial carbon storage in some areas, e.g. permafrost, our results suggest that is not the case in temperate turfgrass systems with mild winters, where warming of a few degrees may have small positive impacts on total soil carbon content of soils. These results also show observational studies across urban-rural gradients can provide insight into carbon cycling under future climate change conditions, and should be used to complement experimental and modeling approaches.

Introduction:

Soils store more carbon (C) than is contained in the atmosphere and in above-ground biomass (Denman et al., 2007). High levels of soil C are associated with improved soil fertility and structure, and maximizing the size and lifetime of soil C pools is also desirable in order to reduce the size of the atmospheric pool of carbon dioxide (CO_2). However, the impacts of global change factors on soil C stocks and transformations are still poorly understood (Conant et al., 2011). The large amount of C stored in soils is not stagnant; a significant portion of the soil C pool, ca. 5%, is added and removed every year by the processes of photosynthesis, plant and soil respiration, and burning. Anthropogenic combustion of fossil fuels and land use change add additional large quantities of CO_2 to the atmosphere (Denman et al., 2007). Even small changes in soil C storage and/or the processes that control it may result in very large changes in pool size over many years. Detecting changes in soil C is difficult, however, because the pools and fluxes are large relative to the change that may be measured experimentally—even very long experiments only begin to approach the decades-to-centuries time scale over which many soil C storage changes are measurable (Trumbore, 2006).

A main tenet of global change expectations is the positive feedback of soil C to global warming. Because decomposition of soil C is a microbially mediated process, and because microbial/enzymatic processes tend to increase with temperature when substrate is not limiting, increased soil respiration with warmer temperatures has been predicted based on kinetic theory and demonstrated globally (Bond-Lamberty and Thomson, 2010) but whether the rate increase is due to direct temperature effects, or due to plant growth

increases with warmer temperatures, is not clear. To reiterate, the actual responses of soil respiration to temperature in real soils/ecosystems, independent indirect plant-mediated responses, is not known with certainty, nor is it known what the net increases in plant inputs to soils (e.g. through increased photosynthesis under rising CO_2 and warmer and longer growing seasons) might be under future climate change conditions (Davidson et al., 2006). The goal of this study is to address these unknowns.

In addition to the uncertainty in net outcome of global changes on total C, differing temperature sensitivity of soil C pools of different quality (e.g. chemical structure, N content, degree of physical protection) has been suggested but not clearly demonstrated (von Lützow and Kögel-Knabner, 2009). The total pool of soil C is often thought of and measured as a group of fractions or pools that differ in their lability/recalcitrance—having varying chemical complexity, degradability, turnover rate, and/or residence time in soils (Kleber, 2010). Current experiments use operationally defined methods to quantify conceptual soil C pools based on their age, residence time, or recalcitrance, but methods and concepts of pools do not match (Six et al., 2002), nor are there clear definitions of what constitutes a soil C pool, or if discreet pools even exist (as opposed to just a range of soil C qualities) (Bruun et al., 2010). If the decomposition rate of more recalcitrant pools increases more with rising temperature than labile pools as some work suggests, then very large positive feedbacks to [CO_2] and climate warming may result.

Many of the experiments done to investigate the responses of soil C to warming temperature are inucubation studies, in which whole soil or isolated soil C fractions, sometimes with added substrates of known lability, are incubated at different

temperatures. These studies track decomposition by quantifying the production of CO_2 from incubated soils, or the loss of soil C from the substrate (e.g. by mass or concentration), and/or changes in isotope ratios or other chemical qualities in remaining soil substrate after a known incubation period. Most incubation studies find that the decomposition rate of more recalcitrant soil C increases more with warming temperature than that of more labile soil C, as predicted by reaction kinetics (reviewed by Conant et al., 2011). This is based on the concept that more complex soil organic molecules require higher activation energies to initiate chemical decomposition; in warmer temperatures, these activation energy requirements will be met more easily (von Lützow and Kögel-Knabner, 2009).

This concept had become widely accepted but may now be losing favor, because pools defined by kinetics (e.g. temperature sensitivity) cannot be firmly linked to known qualities of soil C fractions such as mean residence time in soil (Dungait et al., 2012). Recently, more focus has been placed on the idea that the qualities of soil organic carbon quality are not as important for turnover/respiration as is the ability of microbial biomass to access it, which depends on having non-dormant cells with adequate oxygen, moisture, physical and temporal proximity to substrate, and appropriate soil pH and other conditions (Dungait et al., 2012). Because so many of these variables depend on soil structure, air and water permeability, and other soil characteristics, all of which are disrupted upon disturbance, responses in tilled versus untilled soils, and in recently disturbed versus long-undisturbed soils, may be quite dissimilar. This is a problem because almost all research takes place in disturbed or recently disturbed soils.

Incubation experiments obviously use disrupted soils, but even field experiments allowed to run for long periods usually take place in soil recovering from disturbance. Experimental areas are often plowed or otherwise disrupted to yield more homogenous study conditions. For example, the BioCON experiment, part of the Long-Term Ecological Research site at Cedar Creek, Minnesota, in which six Free Air CO_2 Enrichment (FACE) rings have been running since 1997, was constructed on subsoil and/or parent material after topsoil was entirely removed by bulldozer (because the seedbank in topsoil precluded establishment of target plant communities). All of the measurements and publications that have originated from that study site so far have taken place in the context of soil development and sequestration of C in newly forming topsoil; extrapolating these findings to undisturbed soils is, in my opinion, problematic. However, it is easy to see why this is done. The Duke FACE site, in which mixed forest communities are grown, was established in 1983 after clear-cutting and burning of the existing forest. Researchers here detected significant changes in soil acidification under elevated CO_2 (Oh and Richter Jr., 2004) a large part of which was later attributed to underlying soil variability (Oh et al., 2007). I think that, while these large rare experiments are quite important, they must be augmented with more experimental sites and higher replication in undisturbed soils.

There is a need for measurements of soil C dynamics in intact soils that have experienced decades of climate change, but no existing long-term field experiments with climate change treatments have run that long. Additionally, many of the limitations of current soil C/climate change research—high variability, small results against large background, complex interactions, multiple statistical comparisons from each rare

experiment—will still be problematic, even as the few existing experiments are allowed to run longer. Therefore, a different approach is needed to complement existing traditional experiments. Instead of controlling soil variability and measuring short term respiration or changes in soil C with high precision, I will allow soils to vary freely, include a large number of experimental sites, and measure extant soil C in soils that have been in place for decades. The goal is to capture the net effect of small differences in conditions.

Differences between urban and rural areas, and among individual locations in general, in terms of temperature, [CO_2], ozone, and other factors, have been suggested as a means to study these complex interactions *in toto* (Ziska et al., 2003; Carreiro and Tripler, 2005). This approach may allow us to capture soils that are near steady state, and the long times of development mean that changes in soil C storage should not be as small or variable against a large background—giving us an actual chance at detection. The aspects which would need to be controlled to make meaningful comparisons—plant communities and land use/management—can be found in abundance in managed turfgrass areas.

A compilation of soil measurements from 16 golf courses of varying age in Colorado and Wyoming suggested that in fairways and putting greens, soil organic matter content reached equilibrium within 25 - 35 years after course establishment (Qian and Follett, 2002). There are many courses older than this in the mid-Atlantic area. Golf courses maintain large areas to separate areas of play, called “roughs”, which are managed to have relatively homogeneous plant communities but otherwise have minimal

management regimes compared to fairways and greens. Furthermore, the staffs at most courses keep detailed records of soil inputs and disturbances over many years.

Although the concept of differential SOM quality is being deemphasized in the literature, there is still information that can be gained from measuring different parts of the soil C pool. The most labile C in soils might represent recent additions which are not (yet) associated with soil chemical bonding sites or mineral surfaces (Jones and Donnelly, 2004; Jones et al., 2009). Differential responses of labile C and total C pools might give additional insight into possible mechanisms of change, and there is still a question of differential temperature sensitivity between labile and recalcitrant fractions of soil C. I will use operationally defined active C—the amount that is oxidized by dilute potassium permanganate in 12 minutes (Weil et al., 2003) as measure of labile C (Six et al., 2002; Weil et al., 2003).

I located 62 courses established more than 25 years ago and willing to participate in this type of study, in locations ranging from downtown Baltimore and Washington D.C. to rural areas north and west throughout the state of Maryland (Fig. 6).Using measurements in these 62 golf courses, I will evaluate two hypotheses: i) total soil C will decrease with small increases in mean temperature, and ii) active soil C is less sensitive to warmer temperatures than total soil C; therefore the proportion of active : total C will increase with temperature.

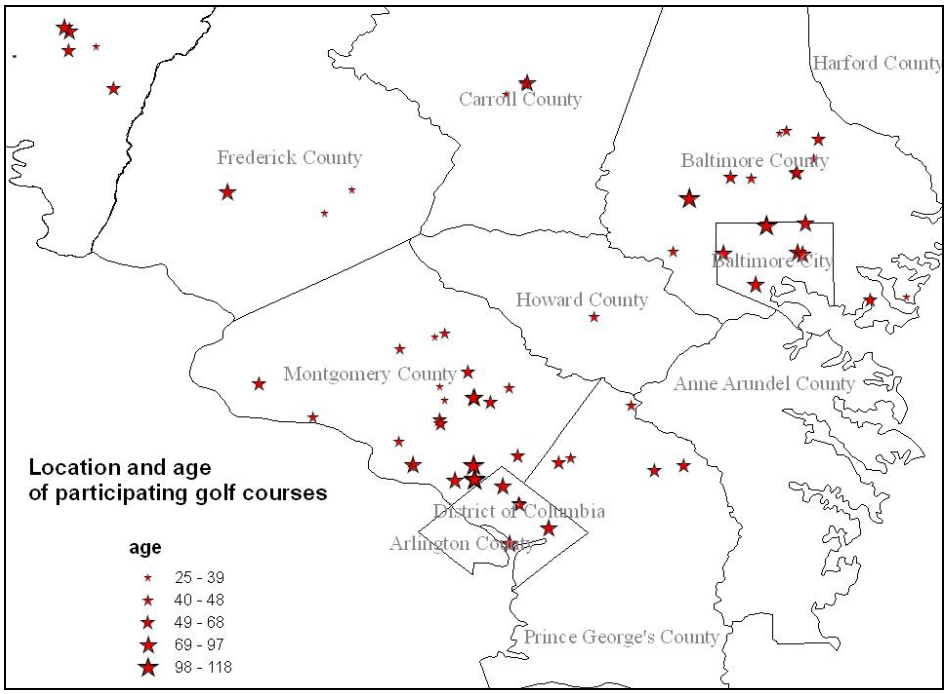


Figure 6. Detail of Maryland and Washington, D.C. region, showing study sites.

Materials and Methods:

Sampling locations and collection:

Golf courses were located and asked to participate at the beginning of 2009. Participating courses were visited and soil samples were collected between Feb. 12 and Apr. 2, 2009. This timing was based on the assumption that soil C would change most slowly during colder times of the year when the rates of both photosynthesis and soil respiration are lowest, to minimize bias introduced by different sampling dates. At this visit, course managers were interviewed regarding management practices, such as aeration, fertilization (applied or not), use of pesticide/herbicide, target (planted/desired) plant community composition, and asked to recommend likely sampling locations in the roughs (flat, unshaded, with good plant cover and minimal weediness). Because chemical inputs can be a sensitive issue, no details regarding fertilization or use of pesticides and herbicides were requested beyond the frequency of fertilizer application (zero, one, two, or three applications per year) which was then reduced to a binary applied/not applied variable. Only courses with a target mixture of cool-season (C3 photosythetic pathway) grasses were included in the study. Species grown include tall fescue (*Schedonorus phoenix*), annual and Kentucky bluegrasses (*Poa annua* and *Poa pratensis*), perennial rye (*Lolium perenne*), and creeping bentgrass (*Agrostis palustris)*. Areas wherewarm-season (C4) grasses encroached as weeds (e.g. zoysiagrass, bermudagrass) were avoided; this was possible because the warm-season grasses were dormant at the time of sampling, and so were very obviously senescent and tan in color, in contrast to the deep green maintained throughout winter and spring by the C3 grass species.

When an appropriate sampling location was found on a course, eight intact soil cores were collected within a uniform 1 x 1.5-m area (two rows, 1 m apart, of four cores taken every 50 cm; with 10 cm of leeway in case rocks or roots obstruct a core). A GPS reading was recorded at the midpoint of the rectangular sampling area, slope was recorded using an inclinometer, aspect was noted if applicable, and a written description of the area was made. Slopes at all sampling locations were 5% or less, except at one course where the flattest location that could be found had a slope of 8%. Sampling was not made within 3m of any tree trunks. Soil cores were collected using a JMC intact-core probe and disposable plastic collection tube inserts that were 30.5cm in length and 2cm in diameter (JMC, Newton, IA, U.S.A). The soil corer was kept sharpened and was hammered into the soil with a 680g rubber mallet, so that any roots would be cut through and intact cores would result. If the corer hit rocks or other barriers to full depth, I moved 10cm away and retried. When full depth cores could be obtained (most cores), the top of the disposable collection tube was made even with top of soil, to result in cores as close as possible to 30cm. When thatch was present (rarely), I moved it aside to get the corer even with the top of the soil surface. In the cases where rocky soils or shallow parent material limited coring, soil depth was noted for each individual core that could be obtained. After collection, cores were placed in a plastic cooler with ice packs, and were placed and stored in a freezer (~ -20°C) upon return to the laboratory.

Soil core processing, bulk density estimation, and particle size analysis:

Bulk density estimations were made based on the known volume and depth of the core sampling tubes. Because tubes are transparent, any incomplete cores (if rocks or

roots caused soil to be pushed aside instead of cut through, for example) could be detected before processing and avoided. Frozen cores were pushed out of the sampling tubes with a wooden dowel, and kept intact as far as possible. The depths of O horizons (if present), and of any other obvious horizonation, were noted for each individual core, and the color of each horizon was determined using a Munsell soil color book. Any thatch, e.g. plant litter, not belonging to soil O or A horizons, was removed. Most cores came out intact and were cut into depth increments (0-5, 5-15, and 15-30cm) with a knife. Depth increments from all usable cores from each site were combined, weighed, and their lengths recorded if any cores were not complete. Any coarse fragments larger than 2 cm in any dimension were removed at this time, and their weights recorded. Once the depth increments were combined, they were thawed and homogenized, and a representative subsample removed and dried to determine moisture content. Coarse fragments larger than 2mm and smaller than 2cm were removed by sieving prior to soil particle size analysis (see below), and their weights recorded. The volume of coarse fragments was then estimated by assuming a density of 2.65 $g\text{-}cm^{-3}$ for all. Soil bulk density was estimated from the weight and volume of soil in each depth increment, excluding the weight and estimated volume of coarse fragments.

Particle size analysis was conducted using the pipet method, with pre-treatment to remove organic matter and including fine clay analysis (United States Department of Agriculture, Natural Resources Conservation Service, 2004).

Soil chemical analyses:

Soil percent C, H, and N were quantified separately from the three soil depth increments at the University of Maryland Environmental Sciences and Technology Analytical Laboratory using a LECO CHN-2000 analyzer (LECO Corporation, St. Joseph, MI, U.S.A.). Active soil C was assessed following methods given by Weil et al. (2003) using a Bausch and Lomb 2500 spectrophotometer (Bausch and Lomb,Rochester, NY, U.S.A.). Other soil chemical testing was conducted by the University of Delaware Soil Testing Program, including: soil pH, cation exchange capacity (pH7), and Melich-3 extractions for of P, K, Ca, Mg, Mn, Zn, Cu, Fe, S, B, Al, As, Cd, Cr, Ni and Pb.

Using bulk density measurements, total soil C per weight of soil, and volume of soil cores, total soil C per area was calculated for 0 – 5, 5 – 15, and 15 – 30 cm depth increments; the three were summed for total C per area to 30 cm depth.

Soil temperature measurements:

Additional funding for temperature loggers and ozone monitors was received after the soil samples had been collected. Therefore, these measurements were made several months after soil samples were collected, with the assumption (which would be needed regardless of timing) that relative differences among sampling sites measured in any single year are representative of differences over all the years the soils have developed. At 60 of the original 62 sampling locations, temperature loggers (Hobo Pendant Temperature Data Logger 8K-UA-001-08, Onset Computer Corporation, Cape Cod, MA, U.S.A) were attached to galvanized steel hex-head lag screws (1/4 x 4 inch U.S. size) with a short length of fluorescently colored nylon mason twine, and the assemblage

buried at 8cm depth. Placement of temperature loggers began in August of 2009, but took much longer than expected because of pleasant weather conditions that prevailed for most of the autumn—crowded golf courses meant that I could not get safe access to sampling sites after ~630AM (in contrast to conditions during soil coring, which were generally cold and rainy, with very few golfers on the courses). Therefore, all temperature loggers were not placed until Dec. 5, 2009; loggers collected temperature measurements hourly until May 24, 2010 when data capacity was reached. A GPS unit (Trimble GeoXT) along with the written site descriptions were used to place the temp loggers (onset HOBO pendant temp, part #UA-001-08) in the center of each rectangular sampling area (one at each golf course). Each logger was attached to a 3 ½ inch zinc-plated steel hex bolt using nylon, fluorescent-colored mason's twine and buried at 8cm depth from the soil/turf surface.

Temperature loggers were subsequently collected by using a GPS unit to locate within ~1m of the loggers' buried locations. Then a metal detector (Garrett Ace 250) was used to locate the exact burial location. A pitchfork was used to lift the turf and retrieve the loggers. Of the 60 loggers placed, 54 were found. The remaining six were in soils where the metal detector gave false positive signals, possibly due to iron-bearing parent material, but the loggers were never found. Two of the retrieved loggers were damaged by the pitchfork, so that a total of 52 of the 62 courses have available temperature data.

Passive Ozone Monitors:

With the number of study sites, active ozone monitoring equipment was not a possibility, but there are several means of passively monitoring ozone. I chose indigo carmine (over potassium iodide and other compounds) as the most affordable and non-

toxic option based on its use in similar public applications (Grosjean et al., 1995; Scheeren and Adema, 1996; Franklin et al., 2004). I designed my own monitors based on these researchers' findings and materials I could obtain, including components for physical protection from rain and sunlight and a diffusion barrier to insulate the reactive compounds from rapid atmospheric changes. An image of the complete monitor apparatus can be seen in the instructions sent to course managers in Appendix 1. Four inch diameter (U.S. standard size) round black plastic flowerpots, suspended upside down with a small piece of thin plastic covering the drainage holes, protected the monitors from rain and sun. Clear plastic holders for 47mm-diameter filter papers (Millipore Petrislide ™ part PDMA04700, EMD Millipore, Billerica, Massachusetts,U.S.A.) were used to suspend the indigo carmine-laden filter papers; their lids were cut with a lathe to be open in front with only a thin ring remaining to hold the filter in place. To the outside/front of the lids, 2 layers of filters, as described by Franklin et al. (2004) and attached with silicone glue, formed the diffusion barrier. Indigo carmine-soaked cellulose filter papers were prepared as described by Franklin et al. (2004). Using a Konica-Minolta CR-200 Chroma Meter colorimeter (Konica Minolta, Ramsey, NJ, U.S.A.), the color coordinates *L*, *a*, and *b* were recorded as in Franklin et al. (2004), and then the monitor set-ups were mailed or brought to each participating golf course. The passive ozone monitors were exposed to the outdoors for two weeks, starting on Aug. 23 or 24, 2009, and then returned by mail. Upon oxidation by ozone and possibly by other oxidizing gases, dye color changes from deep blue to yellow/white. Color coordinates were reassessed upon return and the color change calculated as: $(\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$.

Duplicate passive monitors were placed for one, two, and three week periods, also starting on Aug. 23 or 24, 2009, at the site of a tower in Beltsville where EPA's Clean Air Status and Trends (CASTNET) tower has active ozone monitor equipment running continuously. Cumulative measurements from this tower were downloaded for these periods to calibrate the duplicate passive monitors with actual ozone exposures. Additional monitors—indoors for a zero-exposure control, at the top of the Beltsville tower, and near the tower but inside tree canopies---were placed to get a better sense of effects, and duplicate monitors were placed at several golf courses as well. Tree canopy protection reduced ozone exposure of filters. Duplicate monitors at golf courses agreed only at a coarse level—the overall range in ozone exposure relative to Beltsville ranged from 0.38 – 2.17 (unitless ratio); duplicate A readings were 0.38 and 0.52; duplicate B readings were 1.18 and 1.48; duplicate C readings were 0.86 and 1.12; duplicate D readings were 0.95 and 0.97. These duplicate monitors were placed within a few feet of each other; the lack of agreement may be due to inconsistent deposition or dissolution of dye on the filter, differences in exposure due to trees or other microsite differences, or other unknown differences. For the multiple regression analysis below, relative ozone exposure—the ratio of each site measurement divided by two-week measurements made at the Beltsville tower site, was used as an explanatory variable. Where duplicates were available, the average reading was used.

Approximation of tropospheric [CO_2]/fossil fuel emissions over study sites:

Landscan 2008 (UT-

Battelle, LLC. http://www.ornl.gov/sci/landscan/landscan_documentation.shtml)measure ments were used as a proxy for continuous [CO2] measurements over the study sites.

Landscan is a representation, on a 1km grid scale, of ambient population—representing residents and people moving through an area in cars—assembled from census information landcover and other spatial data. Landscan values may capture the combination of automobile and residential fossil fuel (natural gas and fuel oil) emissions where they occur, as well as decreased photosynthesis due to land development. Similar estimates have been made using spatial imagery of night-time light pollution (Doll et al.).

Statistical analysis:

Complete explanatory data, including temperature and ozone, were available for 39 of the 62 courses. My statistical approach was to model the main effects of explanatory factors on responses, using linear multiple regression following Faraway (2002), with the goal of explaining the most variation in responses with a small number of explanatory variables that were conserved in a variety of models. Steps were i) explore the main effects and individual two-factor interactions of various factors and combinations of factors in multiple regression, retaining factors exhibiting consistent relationships dependent variables ii) examine potential models and factor combinations for collinearity, lack of fit, high residuals and high leverages, iii) transform factors and revise models as needed, and iv) use stepwise regression to finalize models. I started with factors that were expected to have significant impacts, such as temperature and Landscan values, and then added and removed factors repeatedly. For significant factors, the significance level and effect magnitude and direction were recorded and compared among different model configurations. Factors that did not maintain similar effects among different models were not retained in final models. I then used stepwise multiple

regression, working both forwards and backwards, to suggest explanatory factors that might be eliminated. This function uses Akaike's "an information criterion" (AIC) to evaluate different combinations of explanatory factors in models.

Results:

General:

The minimum, maximum, and mean values of all measurements are given in Table 2. After preliminary regression modeling, the soil of one golf course was determined to be an outlier and was excluded from subsequent models. The site of this course, a historic urban park established in 1928, currently adjacent to Interstate 95, was a dumping area for debris from the Great Baltimore Fire of 1904 (personal communication, course staff). Total C density was much higher in the soil of this course than at any other study site (164.4 Mg C/hectare in the upper 30 cm of soil; the next highest value was 126.6 Mg C/ha), and when included in regression models, both the residual variance and leverage of total soil C from this site were large enough to qualify it as an outlier. Soil contained visible pieces of charcoal and brick, and total C in this soil is likely to be influenced more by the presence of charcoal than by the factors acting in the other courses. Aside from this outlier, courses were only excluded from regression analyses when explanatory data were missing.

Total and active C are were found to be tightly correlated (correlation coefficient = 0.71, Fig. 7) . When modeling total C, inclusion of active C as a predictor improved the adjusted coefficient of determination (adj-R^2; adjusted for added numbers of predictor variables) by 10%. In contrast, when modeling active C, inclusion of the natural log of total C improved adj- R^2 by 54%. Therefore, with the goal of modeling the most variability in responses, active C was modeled with the natural log of total C included as an explanatory variable. This does not give us any information about soil C cycling between active and total C pools, however, only a statistical relationship.

Fiure. 7. the relationship between active and total C, to 30 cm depth

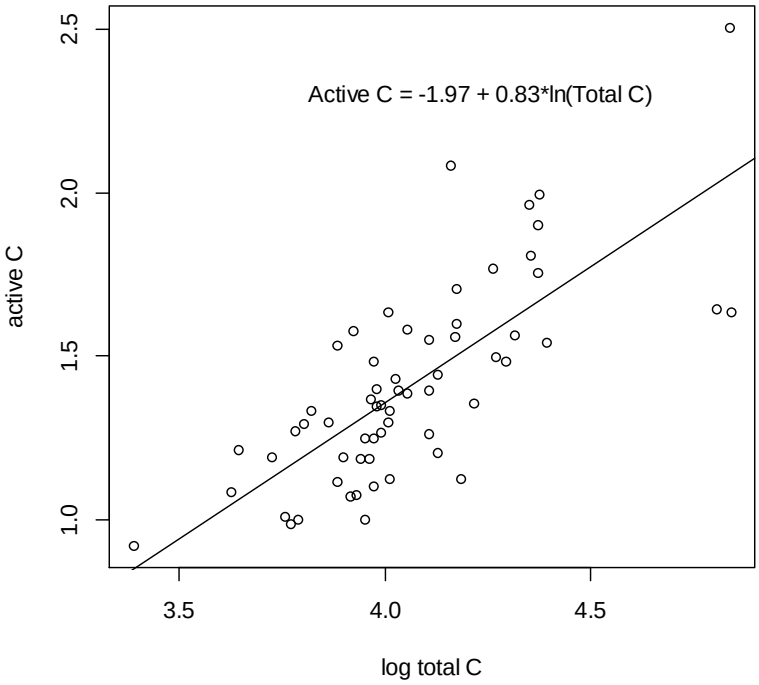


Figure 8. Relationships between mean hourly temperatures and daily minimum and maximum temperatures; overall means obscure the differences in February- and May-only (lower plots).

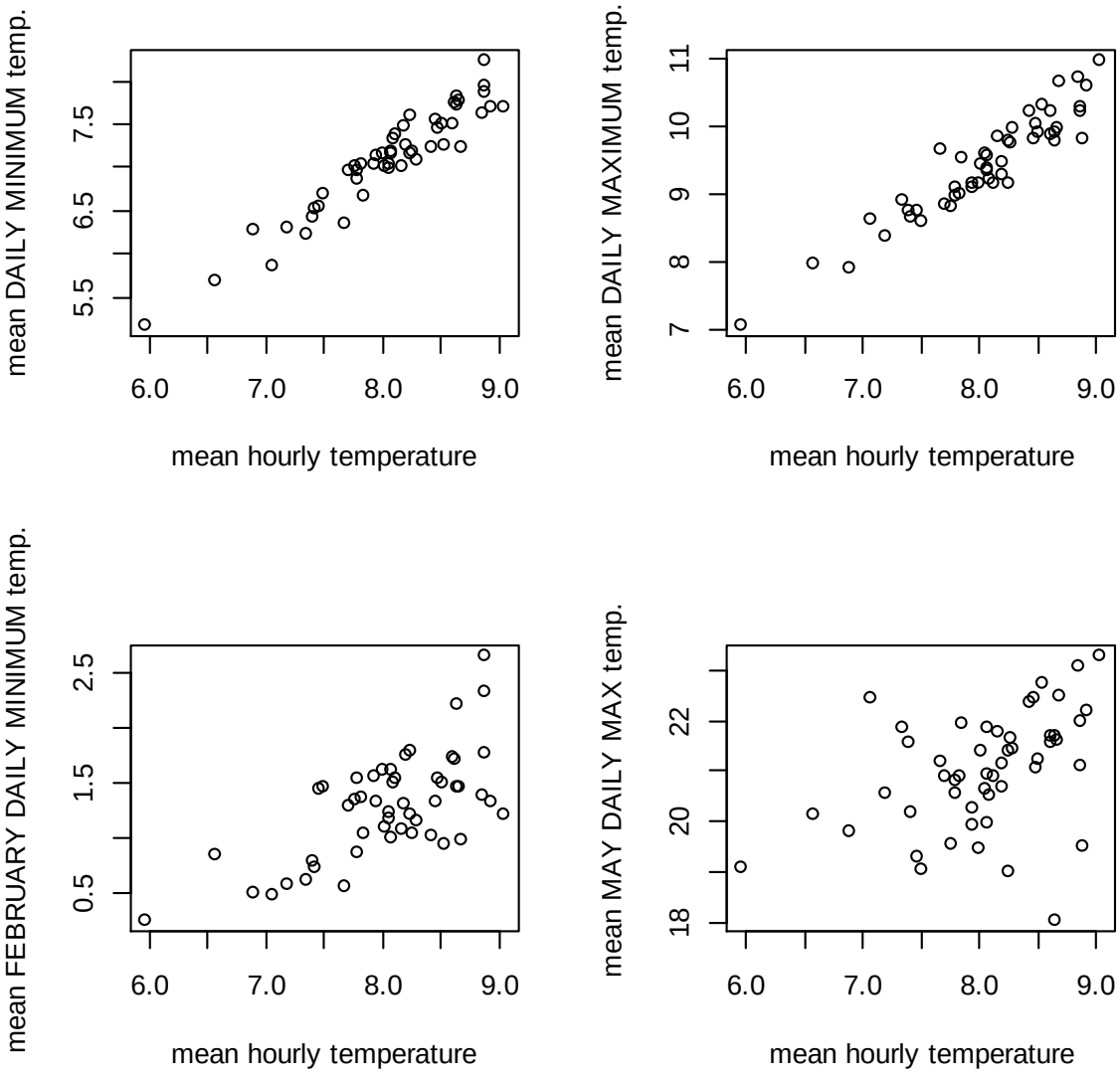


Table 2. Minimum, maximum, and mean values for golf course study measurements

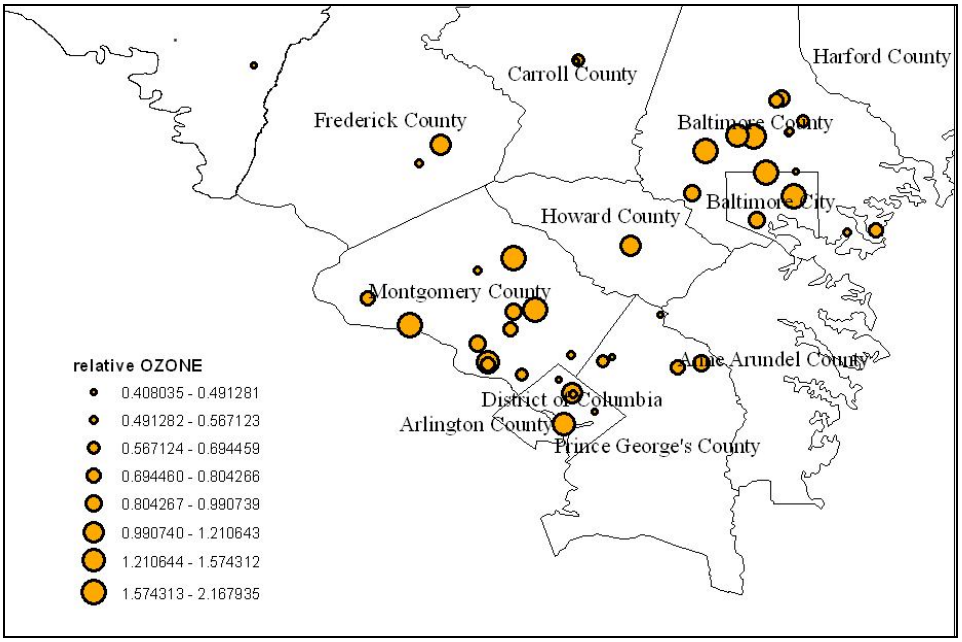
Variable	Lat	Long	Course age	Ozone exposure relative to BEL116 tower	Ave hourly temp	Ave daily min temp	Ave daily max temp	Ave daily min temp, Feb 2010 only	Ave daily max temp, May 2010 only	%sand in soil	%silt in soil	%clay (tot)in soil	%fine clay	Active C(Mg/Ha) to 30 cm	Total C (Mg/Ha) to 30cm	C:N 30cm
Min	-77.72	38.88	25	0.38	6.0	5.2	7.1	0.3	18.0	11.3	16.2	6.1	0.9	0.9	29.6	9.3
Max	-76.42	39.67	118	2.17	9.0	8.2	11.0	2.7	23.3	73.2	75.1	36.9	15.7	2.5	126.6	20.3
Mean	-77.0	39.24	62	0.93	8.1	7.1	9.4	1.3	21.0	37.8	42.4	19.8	6.5	1.4	60.4	11.9

Variable	Soil pH (water)	P	K	Ca	Mg	Mn	Zn	Cu	Fe	B	S	Al	As	Cd	Cr	Ni	Pb	CEC	Bulk density to 5 cm (g/cm3)
Min	4.4	4.1	32.0	155.4	38.3	6.0	0.65	0.32	32.9	0.10	11.6	507	ND	0.02	ND	0.54	0.36	3.9	0.48
Max	7.3	200.6	304.8	1982.6	125.8	161.5	48.9	33.2	477.5	0.75	73.9	1080	0.12	0.74	0.17	3.73	20.3	27.9	1.19
Mean	5.7	20.8	104.2	794	422.1	59.4	3.9	2.0	92.9	0.35	31.9	754	0.28	0.06	1.45	1.00	3.90	12.8	0.93

While overall mean hourly temperature was linearly correlated with mean daily-minimum and mean daily-maximum temperatures over all dates of measurement, the mean daily-minimum temps from February 2010 only and mean daily-maximum temps from May 2010 only (the coolest and warmest months available) were not tightly correlated with overall mean hourly temperature (Fig. 8) . This may be due to differences among microsite qualities. All five temperature variables were investigated for with response data, but only minimum daily temperature from February 2010 was found to be useful as an explanatory variable.

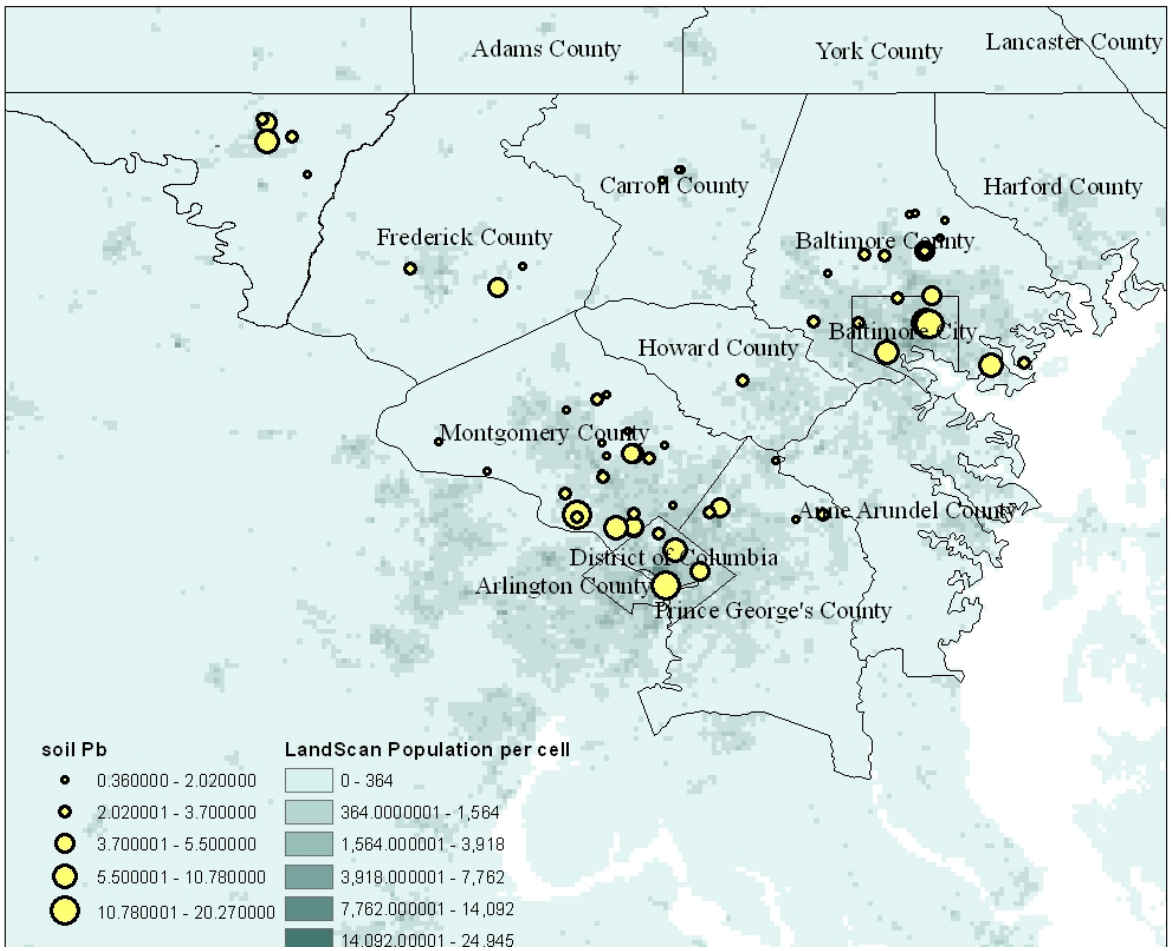
Of the 45 courses where ozone monitors were placed and returned, relative ozone exposure (expressed as a proportion of exposure at the EPA's CASTNET tower in Beltsville) ranged from 0.38 to 2.17 with a mean of 0.92 (Table 2, Fig. 9). Ozone was found to vary considerably over short distances, as evidenced by variability in neighboring courses (Fig. 9).

Figure 9, relative ozone exposure at study sites



Soil lead also varies considerably among sample locations (Fig. 10, with Landscan 2008 values shown in background grid cells).

Figure 10. Soil lead content and Landscan gridcell values across study locations



Final models of total and active soil C:

After excluding the single outlier, significant relationships of total and active C with explanatory factors could be modeled (Table 3; Figs. 11 and 12). Two soil quantities, cation exchange capacity at ph7 (CEC) and soil lead (Pb) content, together explained 37% of the variance in total soil C (both were positively correlated with total C). Addition of relative ozone exposure and minimum February temperature (both positively correlated with total C), and of fertilization status (negatively correlated with total C) to the multiple regression model increased the adj-R^2 to 58%. Multicollinearity was not detected by pairwise comparisons of this set of predictor variables; all pairwise correlation coefficients were ≤ 0.37 (whereas correlation coefficients approaching or greater than 0.7 could be problematic).

For active C, a large part of the variability was explained by total C (adj-R^2 = 54%). Including soil pH and relative ozone exposure (positively correlated with active C) and fertilization status and course age (negatively correlated with active C) in the model raised the adj-R^2 to 78%. Again, no pairs of predictor variables were highly correlated (all pairwise correlation coefficients were ≤ 0.47).

Figure 11. Residuals of the total C multiple regression, showing relationships with mean individual explanatory variables after other model factors are accounted for. a) February-only daily minimum temperatures, b) fertilization status, c) CEC, d) relative ozone exposure, e) soil lead concentration, f) soil clay content.

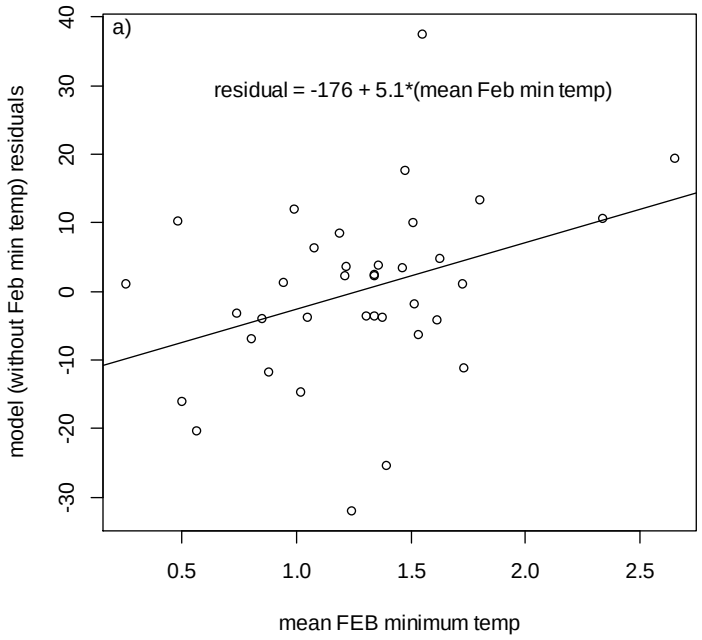
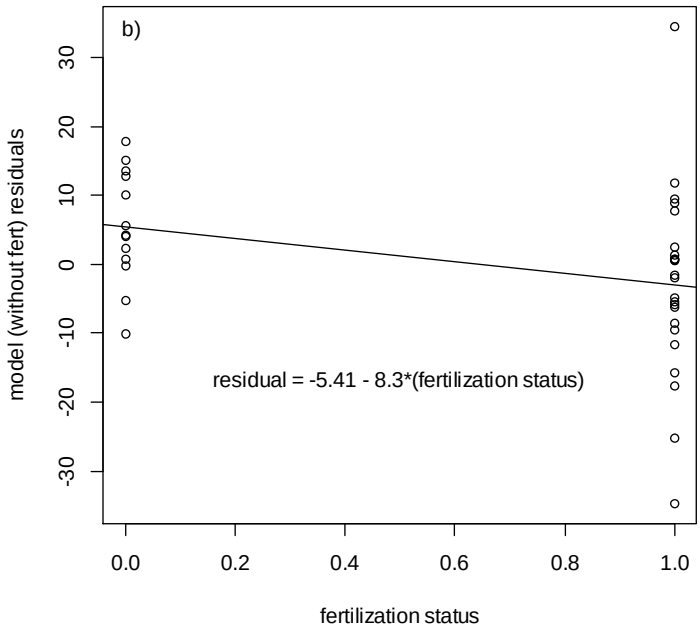


Figure 11 continued



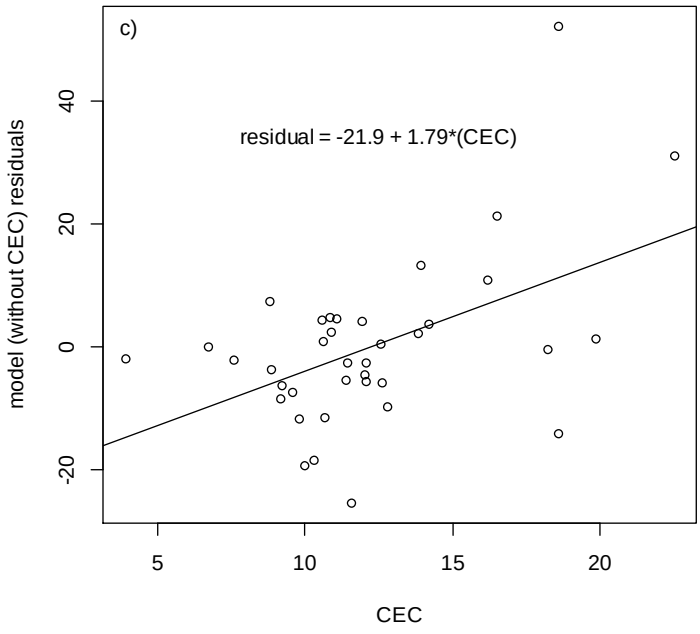
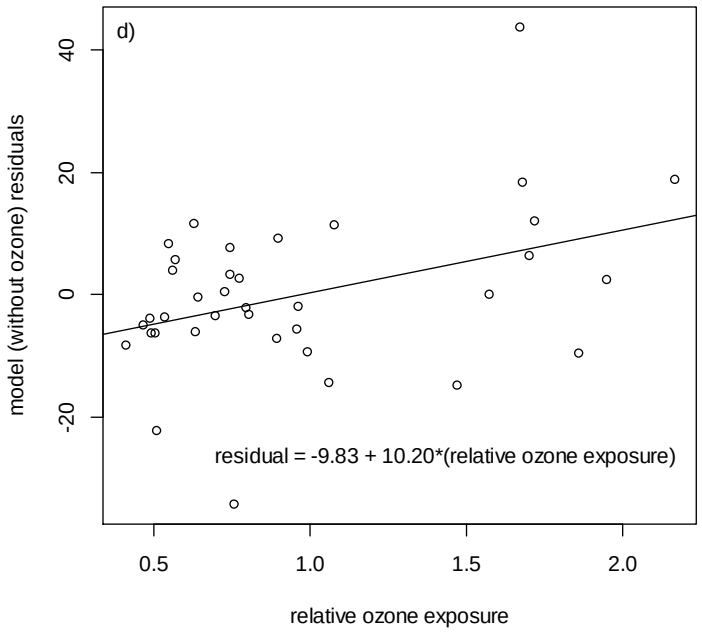


Figure 11 continued



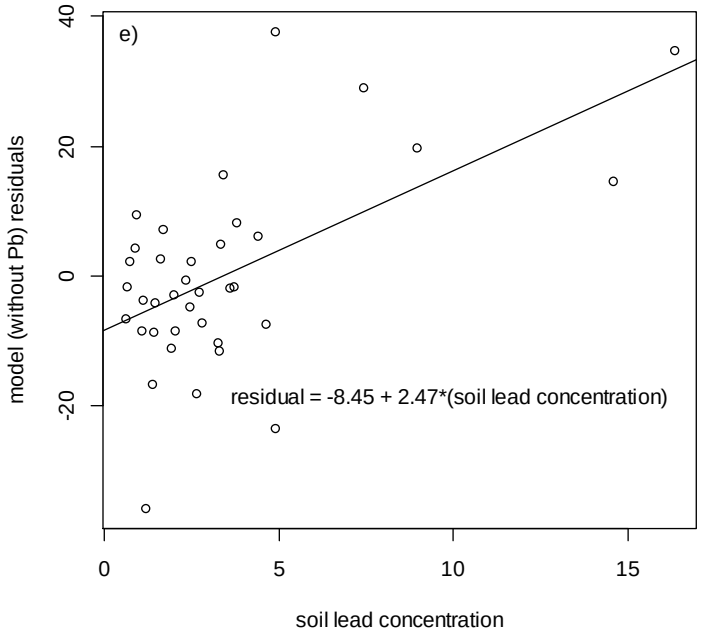


Figure 11 continued

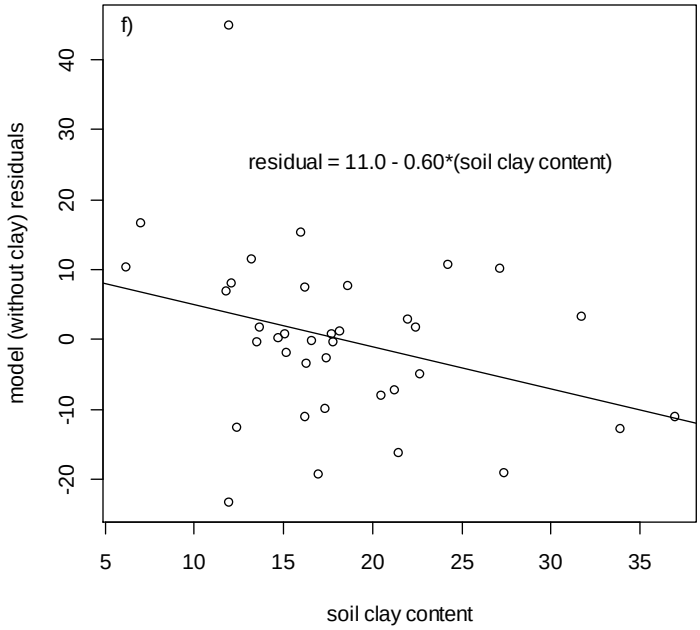


Figure 12. Residuals of the active C multiple regression, showing relationships with mean individual explanatory variables after other model factors are accounted for. a) log(total soil C content), b) fertilization status, c) relative ozone exposure, d) course age, e) soil pH

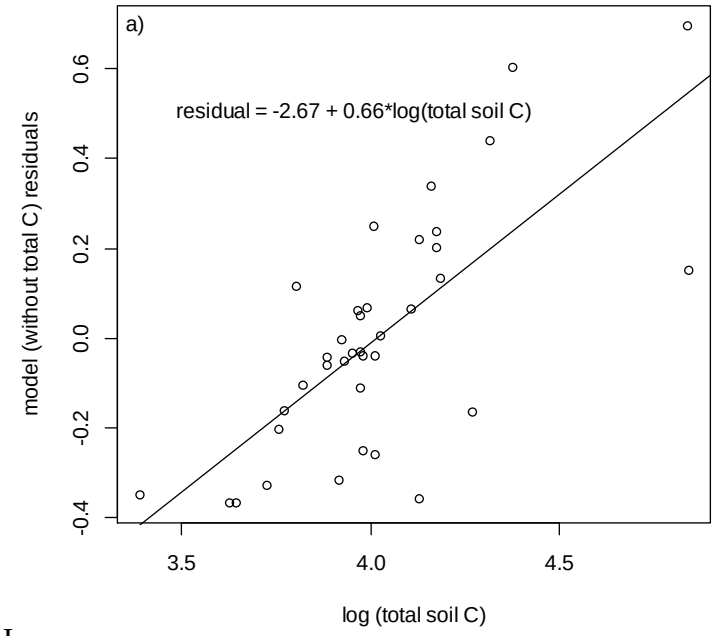
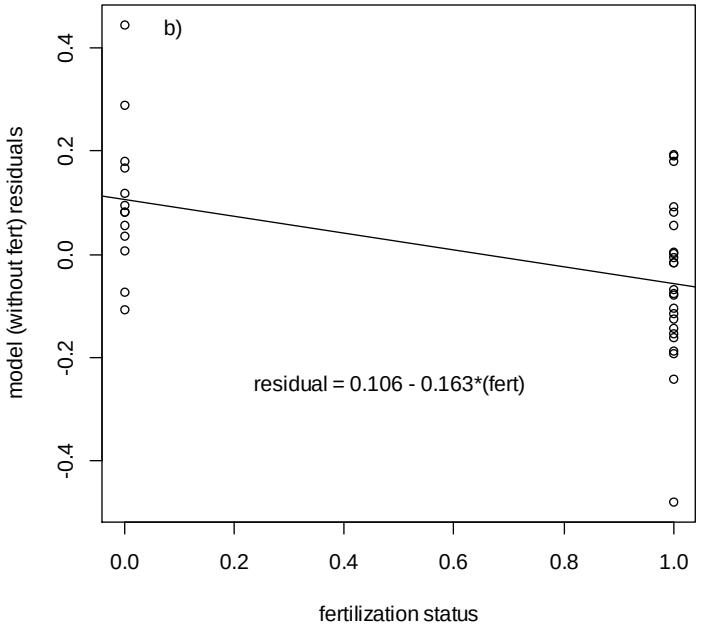
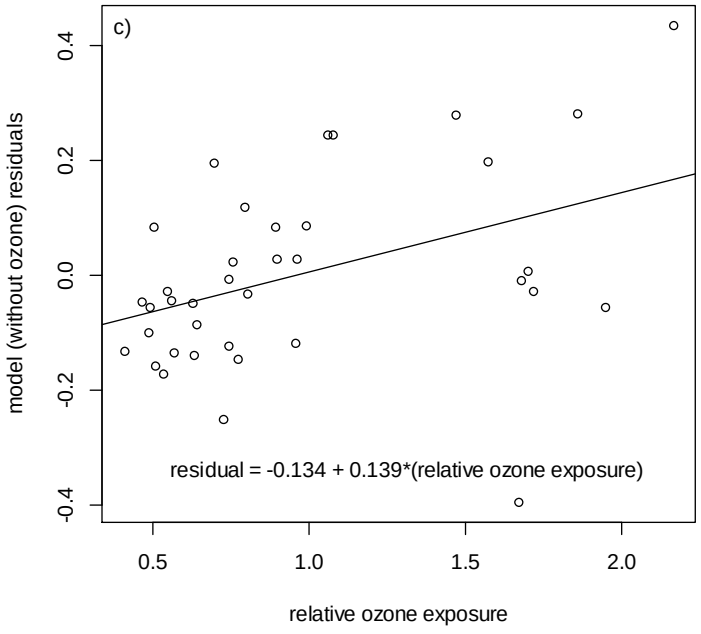


Figure 12 continued





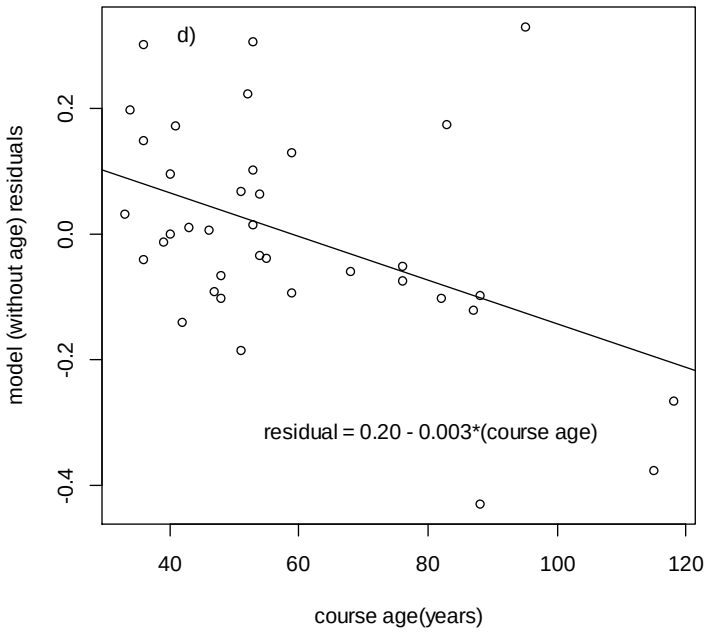


Figure 12 continued

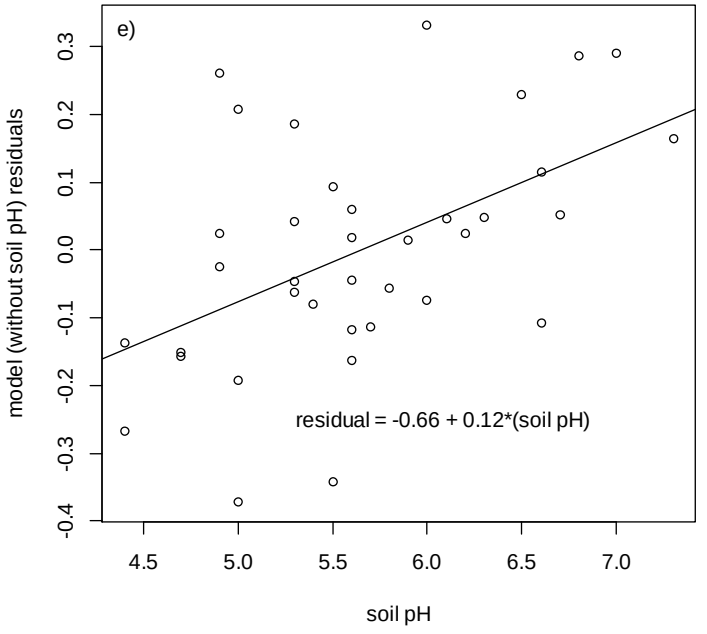


Table 3. Final regression models: partial correlation coefficients and effect size confidence intervals

	Factor:	Pb***	CECpH7***	OZONE*	aveMINfeb*	Clay*	Fert†
Total soil C (model***): adj R2 = 0.58	partial R^2	0.39	0.31	0.18	0.16	0.15	0.10
	95% CI	1.69 – 4.40	1.08 - 3.59	3.00 - 22.19	1.33 - 12.82	−1.54 - −0.12	−9.23 - 0.35
	Std 95% CI	0.37 – 0.98	0.23 – 0.77	0.08 – 0.58	0.06 - 0.58	−0.53 - −0.04	−0.47 - 0.02
	Factor:	Log(total soilC)***	Soil pH***	OZONE***	Course age***	Fert**	
Active soil C (model***): adj R2 = 0.76	partial R^2	0.69	0.30	0.27	0.26	0.19	
	95% CI	0.64 - 1.00	0.07 - 0.20	0.10 - 0.32	−0.007 - −0.002	−0.26 - −0.05	
	standardized 95% CI	0.57 – 0.89	0.16 – 0.46	0.16 – 0.52	−0.58 - −0.17	−0.42 - −0.09	

† P<0.10; * P<0.05; ** P<0.01; *** P<0.001

The squares of the partial correlation coefficients (partial R^2) and 95% confidence intervals of effect size (CI, calculated as estimated effect size ± 1.96×effect size standard error) for raw and standardized data are presented. The square of the partial correlation indicates how much remaining variation in response is correlated with a factor, after other covarying factors are accounted for. Standardized CI confidence interval units are standard deviations of response per standard deviation of factor. Unstandardized CI units are measured units of response (Mg C hectare^{-1}) per measured unit of factor (°C, % moisture, or unit pH).

Discussion:

In the temperate, mesic, managed turfgrass soils of the study, expectations were that soils with warmer mean temperatures would store less total carbon and have greater proportions of labile C, operationally defined by active C. Instead, soil carbon storage did not vary across a mean temperature increase of ca. 2.8°C, but increased as minimum February daily temperature rose from ca. 0°C to 2.2°C. These measurements of soil C reflect the net balance of plant inputs and decomposition over more than 25 years of soil development, but these two processes were not separately measured. Nevertheless, these findings lend support to the idea that soil C decomposition in temperate field settings is limited less by reaction kinetics (since we did not see a decrease in soil C with increasing mean temperature) than by other factors (e.g. substrate availability, oxygen availability, moisture, etc.). The range in temperature among our sites is small relative to that in many experimental designs, where treatments of 5°C or larger temperature increases are common. Therefore, these results don't negate any experimental findings and may not extrapolate to larger temperature increases; however they do highlight what may be most important in temperate field settings in the near future.

Our measurements of active C represent an operationally defined quantitythe amount of C oxidized by a weak potassium permanganate solution in 12 minutes. This measurement had meaning in agricultural field settings, being well correlated with substrate-induced soil respiration and with total soil C, and was a better indicator of no-till versus conventional till soils better than total soil C (Weil et al., 2003). In our study, active C and total C were highly correlated to eachother, and both quantities were correlated with some factors in common (relative ozone exposure and fertilization status.

However, each was also related with other factors uniquely (total soil C with soil lead, CEC, and February minimum temperature; active C with course age and soil pH). If the active C measurements are interpreted to represent soil C that is not bound to chemical sites or mineral surfaces in the soil matrix, then the differential responses of active and total C can provide insight into the mechanisms at work in the study soils.

Positive relationship of total soil C with minimum February temperature:

The positive correlation of total soil C with increasing daily minimum February-only temperature is the opposite of what would be expected based only on the linear effects of reaction kinetics (i.e. that in a system with no limitations, increasing temperature stimulates decomposition due to increasing energy available for soil C substrate chemical breakdown). This expectation may not be applicable in some field settings for at least two reasons. The first has already been mentioned: in intact soils, microbial enzymatic breakdown of soil C substrate does not constitute a system with no limitations; rather, the availability of soil C substrate (as well as of oxygen and sometimes water) is often limiting, because it is not easily accessible in the soil matrix. Secondly, this expectation is based on a linear relationship with temperature, which overlooks the fact that a crucial threshold is crossed when air and/or soils freeze or thaw.

Many experiments suggest that freeze/thaw events disrupt soil organic matter and promote mineralization by physical and/or chemical disruption. In a boreal setting, dissolved organic C (DOC) increased in stream runoff waters after deep soil freezing (Ågren et al., 2012), which the authors attributed to exclusion of DOC from ice crystals as soil water freezes. Phillips et al. (2012) compared dynamics of sterilized and intact cores from northern U.S. prairie soils during freezing and thawing; even sterilized (no

microbial respiration) cores emitted CO_2 below freezing temperatures, which the authors attribute to physical disruption of soil pores by ice formation, and in intact cores there was evidence of increases in both microbial activity and substrate availability after thawing. Prieme and Christensen (2001) detected large pulses of CO_2 from intact soil cores after freezing and thawing; these were largest in grassland soil cores, which was attributed to root exudates from living grasses stressed by freeze.

In soils of the study region, milder winter temperatures may increase soil C through several mechanisms: lack of physical disruption of soils and soil organic matter, lack of freeze-out and subsequent leaching or runoff of DOC, lack of plant freezing stress, and possibly stimulation of cool-season grass growth at a time when water is not limiting. In our models, active soil C did not increase with February minimum temperature as total C did. This may be because the active C pool is already accessible to soil microbial biomass, such that disruption by freezing has no further effect. The lack of correlation with active C suggests that increased plant inputs are not the cause of the rise in total soil C under warmer February daily minimum temperatures. If increased plant inputs were the main mechanism, we would expect to see a response of both active and total C. Furthermore, minimum daily temperatures occur almost exclusively during dark hours when plants are not photosynthesizing.

This finding suggests that more detailed expectations/modeling of the effects of temperature on soil carbon are needed. In soils that don't freeze, such as in the tropics and in many incubation experiments, warming temperatures do appear to result in decreased soil carbon in field settings, butin temperate zones and higher latitudes, freezing effects may be more important. These responses are likely to vary with

ecosystem traits such as plant community type (e.g. deciduous forest, rainforest, grassland). Studying these two aspects of temperature separately and interactively, in controlled experiments, would be of great interest.

Positive correlation of total C with soil lead (Pb) levels:

The positive correlation between soil lead levels and total C is not surprising, although it has not been at the forefront of soil carbon research. Pingitore et al. (2009) state that "Pb-humates," their designation for high-affinity complexes that form between lead and soil organic matter in surface soils, have been well-characterized .This affinity between lead and organic matter suggests that lead atoms chemically stabilize soil organic C like other positive soil species (e.g. Ca++) and surfaces (e.g. clays, amorphous oxides) do. While it is possible that this correlation is because soils with intrinsically high levels of total C attract and retain more lead, this is unlikely to be the main mechanism because the source of lead in the study sites is likely to be previous atmospheric deposition from leaded gasoline (i.e. sites are far from any structures with leaded paint, industrial pollution, and other potential solid sources). Because lead is not very mobile once in soils (see next), it its more likely that historically deposited soil lead is causing an increase in total soil C. In soil mobility studies, lead moved downwards through soil profiles only in association with, and at the same rate as, soil organic matter, whereas cesium moved both in association with organic matter as well as more rapidly with soil aqueous phases (Dorr and Munnich, 1989). Among common heavy metals in soils, lead has been shown to have the strongest affinity for "clays, peat, Fe oxides, and usual soils" (Bradl, 2004). These findings suggest that lead binds to soil organic matter

more strongly and more permanently than other heavy metal species. Particulate organic matter was shown to adsorb lead and other heavy metals much more than the whole soil (Guo et al., 2006). In the study soils, it seems likely that as plant-derived C was added to soils with high lead content, it became enriched with lead and was stabilized by it.

In addition to this chemical stabilization of soil C, lead may also inhibit soil microbial activity and/or reduce soil microbial biomass (Muhammad et al., 2005; Nakatsu et al., 2005; Lazzaro et al., 2006), thus reducing decomposition rates in soils with high lead levels. However, this mechanism is not supported here, because if overall decomposition was inhibited, then both active and total soil C should increase with increasing soil lead levels. Positive impacts of other heavy metals (e.g. arsenic, cadmium, chromium, nickel, and copper) might also be expected, but were not observed in this study. As mentioned previously, these other metals are not thought to have as strong an affinity for soil C as lead. Additionally, they may not be present in sufficient concentrations to have observable impacts in the study soils.

Because the lead in the study soils was most likely deposited after emission by automobiles burning leaded gasoline, soil lead content is tied to roads and vehicular traffic (Yesilonis et al., 2008)and may be correlated with [CO_2] levels, N and S deposition, ozone exposures, and potentially many other important factors. However, no correlations of soil lead levels with these variables were detected in these data.

Positive correlation of total and active C with relative ozone exposure:

As with temperature, the positive correlations of total and active soil carbon with relative ozone exposure did not match our initial expectations. Agricultural crop plants grown under elevated ozone exhibit decreased photosynthesis, early senescence of leaves,

and decreased growth and yields (Booker et al., 2009). Ozone causes oxidative damage to plant tissues/molecules, and plants may close their stomates more under elevated ozone; modeling results indicate that these impacts on plant productivity will decrease the amount of carbon sequestered in terrestrial ecosystems (Sitch et al., 2007).

Ozone exposure, like soil lead, is likely to be correlated with a number of other factors that are very important for plant growth. The precursors of tropospheric ozone formation include nitrogen oxides and volatile organic compounds, often the result of fossil fuel combustion, and hot weather and bright sunlight promote ozone formation in the atmosphere. Ozone formation can be inhibited when nitrogen oxides are present in high concentrations, and wind can distribute precursors and ozone itself over long distances. As with soil lead, no pairwise correlations between the passive ozone exposure measurements and other factors were detected (correlation coefficients between ozone and soil S, C:N ratio, Pb levels, and Landscan values were all less than 34%, not shown), although we can't be certain that the ozone measurements aren't capturing variability due to undetected correlated factors (e.g. nitrogen deposition, [CO_2]). While this result is unexpected and co-varying factors cannot be ruled out, there is some evidence that support this finding. Two studies have found that increased ozone exposure decreased soil microbial biomass as detected by phospho-lipid fatty acid analysis (Kanerva et al., 2008; Manninen et al., 2010). Decreased microbial biomass, which may be associated with corresponding decreases in microbial decomposition of soil organic matter,may result from decreased quantity and/or quality of plant litter entering the soil. The quality of plant litter in particular might be expected to decrease after a pulse of high ozone, since ozone causes oxidative damage to aboveground plant tissues. This idea is

supported by fact that both total and active C are positively correlated with ozone—high ozone site soils contain more active C, which is not chemically or physically protected, suggesting that microbial decomposition in general is suppressed.

This result also highlights an important limitation of some experimental studies of ozone effects. Many of these experiments apply uniformly high ozone treatments for the duration of the experiment. In contrast, the sites included in this study have been exposed to locally and temporally varying ozone for many years. The effect of short-duration exposure to elevated ozone may be very different than the effects of chronic exposure—plants may recover from short-term exposure by repairing oxidative damage at the chloroplast or leaf level, whereas prolonged exposure may cause leaves to senesce (die and fall off of the plant). When premature leaf senescence occurs, the plant's investment in the leaves, as well as returns of photosynthate from those leaves, are lost. Therefore, another aspect of ozone exposure that should be considered in models and expectations is plant community type. For instance, turf grasses grow continuously from protected meristems (sites of new cell production) whenever resources are available and temperatures are appropriate. Mowed or grazed turf grasses are not likely to be as strongly impacted as broadleaved plants by damage to exposed leaf parts, as grass leaves will be removed and regrown whether damaged or not. In contrast, in many temperate woody and herbaceous broadleaved plant species, more leaves are produced earlier in the season, and resources are allocated to woody, reproductive, and/or storage structures later in the season. In these types of plants, high ozone exposure can cause entire leaves to senesce early, and they may not be replaced easily or at all depending on the growth stage of the plant and the availability of resources.

Negative correlations of active and total C with fertilization status:

The expectation for the effect of fertilization would be for increased soil C, through increased plant growth and thus plant inputs to soils. However, as with almost every expectation relating to soil C dynamics, there is no real consensus. A recent meta-analysis shows that the expectation for increased soil C with increased fertilization holds in agricultural systems (albeit, with a small increase, just +3.5%) but does not hold for forest or grassland soils (Lu et al., 2011). In a well known, very-long-term agricultural study ("the Morrow Plots"), decades of mineral fertilizer application was associated with decreased soil C (Khan et al., 2007), and the authors state that fertilizer N stimulated soil microbial decomposition of plant residue and soil C, so that despite increased plant production, declining soil C results. In a four year grassland study, mineral N fertilization significantly decreased the amount of labile soil C , although total soil C increased slightly (Dijkstra et al., 2005); the authors suggest that plant inputs were stimulated more than organic matter decomposition.

It is also possible that causation is reversed--that the courses applying fertilizer on their roughs do so because their soils have lower soil organic matter content and thus lower innate fertility. I know anecdotally, however, that many of the courses apply fertilizer throughout (i.e. on fairways, greens, and roughs) simply because they are private/country club courses with higher greenskeeping budgets, and/or because fertilizer application is part of their traditional management regime. Similarly, many courses with roughs in poor shape do not fertilize, because there is no budget for fertilizer. Therefore,

fertilization status here is not likely to reflect plant nutrient status. This is evidenced by C:N ratios in the top 30cm of the study soil profiles, which were not correlated with fertilization status (data not shown).

Positive correlation of total C with cation exchange capacity (CEC):

CEC is the sum of the exchangeable cations that a soil can absorb. While soil organic matter contributes greatly to CEC, the amount of soil organic matter retained by a soil will be higher when soil mineral and chemical properties give it a high CEC. This is because multivalent cations, attracted to negatively charged sites on soil mineral surfaces, also attract and bind negatively charged sites on organic molecules and thus stabilize them. Therefore this relationship is as expected, and also reinforces that measurements of active C represent soil C that is not associated with chemical and/or mineral sites—if this was not the case, we would expect active C to increase with CEC.

Positive relationship of active C with soil pH:

The rate of decomposition of straw was found to be lower at pH 7.5 than at pH 5.5 (Roper and Smith, 1991). In contrast, the amount of particulate organic C decreased with pH in mountain grasslands (Leifeld et al., 2008). Therefore, there is no clear expectation for decomposition of active C in soils of varying pH. However, it is likely that plant inputs to soil increased with soil pH, which ranged from 4.4 to 7.3 in study soils. Moving upward across this range in soil pH, phosphorus availability improves greatly, and aluminum and iron toxicity disappear (Haynes and Mokolobate, 2001). Therefore, this relationship is likely due to increased plant productivity.

Slight negative relationship between total C and soil clay content:

This correlation is opposite of what was expected—clay is thought to stabilize soil C through cation bridging. Clay content also highly tied with soil pore spaces and water retention; as soils go from coarse to silt-loam and clay-loam textures, plant-available water reaches a maximum, and then drops as clay content increases further. Therefore, increasing clay content is likely correlated with a parabolic soil moisture function, and the multiple regression model is attempting to model both using only clay content. It has been demonstrated that correlations revealed by multiple regression analyses may be mistaken in magnitude and direction when important predictors are missing (Faraway, 2002), so this relationship can't give useful information.

Study limitations and other factors:

It is important to note that the lack of a detectable correlation of active or total soil C with Landscan values does not conclusively demonstrate that there is no effect of [CO_2] on soil C. There may be an effect that is too small or too variable to be detected in these data, or that has already been captured by other explanatory variables in the models. Furthermore, Landscan values may not give an adequate representation of actual [CO_2] for a number of reasons; power plant emissions are not included in Landscan, and the mixing of atmospheric gases is not well characterized at the scale of the Landscan grid-average levels over neighboring grid squares may better reflect well mixed atmospheric levels

Along with the lack of direct measurements of [CO_2], an important limitation of this study is that it is missing measurements of soil moisture. Soil moisture in particular is likely to have large impacts on plant productivity and carbon inputs to soil, as well as on soil oxygen status and microbial respiration and decomposition.

Conclusions:

The results of this study demonstrate that this observational approach can detect soil C responses to environmental factors, including short term climate changes. They also lend further support to the idea that small increases in temperature may not negatively impact soil C storage in temperate systems, and suggest that soil lead, ozone exposure, and fertilization status may have important effects in intact soils.

In the Chesapeake Bay watershed, turfgrass was estimated to cover 1.26 million acres in 2003, and that quantity was expected to increase over time. Of this area, golf courses constituted 3% and residential lawns constituted 70% (National Environmental Education & Training Foundation (NEETF) and Center for Watershed Protection(CWP), 2003). In Maryland, the land area in turfgrass is larger than the combined areas growing corn, soybeans, and wheat. Therefore the trends detected in this study represent large proportions of state and regional soils and may have important consequences for soil C sequestration as well as for models of C cycling. In Baltimore, MD, U.S.A., soil lead was found to be elevated in soils within 100m of major roads (Yesilonis et al., 2008). While ozone levels have improved over recent years, the American Lung Association gave Prince George's County, MD, U.S.A. a grade of F for ozone pollution for 2011 (http://www.stateoftheair.org/2012/states/maryland/prince-george-s-24033.html). Lead

contaminated soils and high ozone areas may therefore constitute large areas of the region, making the positive impacts of lead and ozone on soil C storage notable for modeling and sequestration calculations.

In climate change studies, observational studies have been recommended for elucidating broad trends, whereas controlled experiments good for determining mechanisms (Schnitzer et al., 2008). This has proved to be the case here; results could be understood in the context of current experimental research, and direct our focus in new directions.

Chapter 4: overall conclusions

One goal of this research was to explore the use of urbanization as a surrogate for future levels of climate change in field studies. Traditional experimental climate change studies (i.e. experiments with actively imposed, controlled treatments) have yielded excellent information, but suffer many limitations such as small overall number of study sites/systems, low statistical power, large-step-increase treatments, inability to represent complex interactions, and a reliance on disturbed soil to overcome high variability. I wanted to demonstrate that studies using urbanization as a substitute for treatments could overcome some or all of these limitations, and so be a complement to the body of more traditional climate change research,. I carried out two studies to this effect.

The first study took place in the context of an existing experiment created to study the responses of weedy plant community development in response to urbanization. Plots with a common, initially homogenized soil and seedbank were established in a rural, suburban, and urban location along a single transect and allowed to develop for five years, during which time temperature, [CO_2], ozone exposure, vapor pressure deficit and other environmental measurements were made. In the sixth year I made repeated measurements of the length of arbuscular mycorrhizal fungal hyphae in the soils of these plots. I also made one-time measurements of arbuscular mycorrhizal spores and phospho-lipid fatty acid profiles. Towards the urban side of the transect, temperatures and [CO_2] increased, and so did cumulative plant biomass production and plant secession. The suite of these changes was expected to increase the abundance of arbuscular mycorrhizal spores and hyphae, but the opposite was true. Similarly, increased plant biomass and/or accelerated plant community succession away from weedy annual

colonizer species were expected to impact the abundances of both spores and hyphae, but instead, only soil factors—soil acidity, temperature, and moisture—had predictive value in multiple regression modeling of hyphae and spores of one species. This constitutes a new type of study system for arbuscular mycorrhizal research, one in which both plant host and environmental variables vary. This is both a limitation and a strength of the study: this complexity may capture interactions that cannot be mimicked in more tightly controlled experimental studies, but it is difficult to make clear correlations with so many varying factors. In this study, the small number of replications and a single transect resulted in a large number of both response and predictor variables, but there were not enough data points to adequately model all of them.

The purpose of the second study, which I designed and carried out in entirety, was specifically to try a new approach to soil carbon/climate change research. It was not clear if any responses would be detectable with this approach, or if underlying soil variability and other differences among sites would mask any responses. A large number of study sites (relative to the sample size or number of blocks in most controlled experimental studies), 62, were established in the roughs of golf courses established for 25 years or longer, located around the state of Maryland and the District of Columbia. These long-undisturbed soils all grew similar mixtures of cool season grasses and had similar low levels of management. I measured active and total soil carbon and many soil, environmental, and management variables, and used multiple regression to look for patterns. One strength of the study was the use of small, buried temperature probes that recorded hourly temperatures at each site, and were also able to yield temperature summary values such as minimum and maximum temps for certain periods. While there

is innate variation in soils and parent materials across the study area, the large number of sites, similarity in plant and management factors, and large number of potential explanatory variables allowed that variability to be incorporated in and explained by the regression modeling.

Widely expected negative effects of warming mean annual temperature on soil carbon were not found in this study. In contrast, a there was a small positive correlation between minimum daily temperatures from February and total soil carbon (not active carbon), suggesting that freeze/thaw disruption of soil organic matter is an important process in soils of this region. Similarly, ozone exposure and soil lead had unexpected positive correlations with soil carbon.

The validation of this approach, in which treatments were not controlled but provided by the environment, was one of the main purposes of this study and may be more important than the specific findings. The effect sizes and variances that were measured in this study could be used to conduct a statistical power analysis to determine how small the effect of [CO_2] on soil C (if any) might be in order to escape detection in this study; the five years of continuous [CO_2] measurements made by George et al. (2007) at the transect locations of the first dissertation study could be used to estimate the magnitude and variance of [CO_2] differences. However, this would be more complicated than a simple effect-size power analysis because of the spatial and temporal variability of [CO_2] and its unknown properties over a finer scale.

This method was inexpensive and can contribute data, new hypotheses, many more study sites and higher replication to the body of traditional research, and can serve

as a means to validate expectations derived through traditional methods. Broad implementation of studies using this type of experimental design is economically feasible and could provide critical information for carbon cycling research and modeling. Studies of this type are also good for outreach. My project involved meeting with staff at the courses, most of whom were excited about the research and very willing to help with ozone monitors and in any other way they could. I also interacted with many golfers while I worked on the courses, and almost all were curious about the study. Many of the people I met were quite skeptical of climate change science, who nevertheless were interested in the process and in how I might interpret the findings. Getting citizens involved in studies like this is good for science literacy and for the project. If I could return to the golf course study in the future, I would try to secure funding for stainless steel air-sampling canisters so that [CO_2] could be measured directly by mass spectrometry (Ross Salawitch, personal communication, 7/19/2012). This would be an ideal way to get people involved in the science of climate change research.

With more funding (still small relative to the operating budgets of large controlled experiments) there are a number of improvements I could make to the golf course study. I would have collected samples to one meter in depth (or as deep as the profile goes, if shallower than one meter); there may be different dynamics of soil C deeper in the profile that may strengthen or negate my findings in the upper 30 cm of soil. Also, in order to use the soil C measurements in regional or global inventories of soil C, or to use them in models, one meter cores are standard. I would also use probes to continuously measure soil moisture in the upper soil profile at each sampling location. These measurements, like the detailed temperature measurements, would likely yield a lot of information about

soil C dynamics and may improve the statistical models. Once soil moisture measurements are available, the effects of soil texture (if present in these soils) may become more apparent. Measurements of soil mineral particle specific surface area and of soil mineralogy may be more useful than soil texture in predicting soil C stocks, because they would give a better picture of available soil C binding surfaces in each soil (CEC may be capturing this adequately, but there is no way to know until these measurements are made and compared). More precise measurements of [CO_2] and of ozone exposure would be of value, and so would measurements of annual plant biomass production, root and soil respiration, and photosynthesis rates under a variety of conditions. Characterization of soil microbial biomass, respiration, and community composition would indicate if the proposed mechanism of microbial inhibition is valid and would allow refinement of this hypothesis (e.g. overall reduction in biomass, change in community composition, and/or slowed rates of respiration).

This study could also, potentially, be combined with more active experimental treatments. Many of the golf courses have plenty of space and are open to scientific research, so plant communities or environmental variables might be managed in small plots. However, it is difficult to leave any equipment at ground level on a golf course. Alternatively, more sampling locations might be added at each course and/or more courses might be included in a future study, to try to get more measurements at different levels of several variables.

What do the results tell us to expect under future climate change? Because fossil fuel consumption and emissions are not likely to slow anytime soon, it seems likely that [CO_2] will continue to rise, global warming will continue, and the 2˚C limit to global

warming that many have proposed will almost certainly be surpassed. In the near future, in temperate grasslands, my results suggest that there will not be a precipitous loss in soil C based on warming temperature, and there may be increased storage as soil freezing frequency decreases and ozone exposures increase. I can't speculate on the effects of $[CO_2]$ at any concentration based on my results, and excessively high $[CO_2]$ will alter so many processes (photosynthesis, stomatal conductance, soil moisture, possibly soil acidity of cation leaching, pH of rainwater) that predictions are difficult. I often feel disheartened at the lack of progress in limiting $[CO_2]$; the results of this study could serve as a small hopeful point—that if emissions can be reined in soon, in the interim temperate soils may help to buffer rising $[CO_2]$ by storing more C.

Appendix 1: instructions mailed with passive ozone monitors

The monitor should be placed outside for exactly 2 weeks, starting sometime between 7 PM Monday 8/23 and 11 AM Tuesday 8/24. Inside the assembly are blue filter papers. They are dyed with indigo carmine, which is similar to what colors denim, and is not toxic.

Instructions:

1. Keep the filters inside the zip lock bags, indoors, away from heat and direct sun, until they are ready to be hung up.

2. The fronts of the clear plastic filter holders are not solid – they are open, with 2 kinds of filters glued on to allow air flow. Therefore, hold them by the edges if possible.

3. When it is time to hang up the monitor, pull down on the string ends inside the flowerpot, so that you can hook the two filter holders on to the string. Then push the metal washer down the string to tighten, so that the clear plastic rain shield lays close over the holes in the flowerpot. Call me anytime for questions/problems.

4. Please jot down the approx time and day the monitor was put outside here _____ and if possible, collect it at the same time and day, 2 weeks later (**Mon.9/7 or Tues. 9/8**).

5. How and where to hang:

I have taped a hook to each flowerpot, if needed. Hanging from a tree branch, against a tree trunk, or in a similar protected place is ideal. However, good air flow is needed, so please avoid dense stands of trees and enclosures if possible. Hang at shoulder height, between 5 and 6 feet high. If possible, don't hang the monitor near the maintenance building/lot, because exhaust from the equipment can give a false reading. Any location within several hundred feet of the soil sampling location is ok. Facing away from the direction of play might help the monitors survive for 2 weeks…

6. After the 2 weeks: please unhook the two clear filter holders from the string, put them back in the zip lock bag, and mail to me in the padded envelope. The rest of the assembly—you can save it for me, use the parts, recycle, or toss, whatever is easiest for you.

THANKS VERY MUCH FOR YOUR HELP.

Appendix 2: sample of R code and approach to multiple regression analysis

```
#EMH/golf stats, June 30, 2012
# "#" indicates comments—R disregards anything entered after the #
#Include "golfmaster.txt", text file of data to see
#output shown in smaller font.  Use Courier New and single space to
paste #from R to word.  Anything not prefaced with "#" is code.

rm(list=ls(all=TRUE))
#this removes previously used variables from R, useful
#for me because I have several files with similar/same names of ind
variables

golfmaster <- read.table(file.choose(), header = T)
#this is how you read in a #text file.  See attached text file
golfmaster.txt #for proper format for R.

head(golfmaster) #shows top few lines of the data file
# make subset of complete sets only: using logical loop, courtesy of
Matt #Kramer @ USDA biometrical consulting service—is.na means any cell
that is #empty or has na
tl <- logical(62)
for (i in 1:62) {
  if (!is.na (sum (golfmaster[i,8:12]))) {
    tl[i] <- TRUE   #now only complete rows retained by the vector t1.
  }
 }

gms1 <- golfmaster[tl,]  #gms1 is now the subset with no missing data.

#use when comparing resids or other strings among models—e.g. if model
#includes temperature or other incomplete variable, it will
automatically #exclude missing values, but then
#you can't plot resids from that model against the dependent variable
unless #you use the file of completes as data source….



library(MASS)  # I can't remember if I still need this package, may be
old

#STEP 1, play around with potential regression models, take notes of
what is #sig and stays sig, and keeps
#direction of correlation .  using scaled values (variance = 1, mean
=0) to #eval effect sizes easier
#########################################################################
################################
#use gms1 (complete sets only) to explore

fullEMH <- lm(data = gms1, scale(hyphpercm2) ~ juliandate + x + y +
scale(age) + scale(soilpH) + scale(avesilt))
summary(fullEMH)
#only age, negative effect

lm(formula = scale(hyphpercm2) ~ juliandate + x + y + scale(age) +
    scale(soilpH) + scale(avesilt), data = gms1)
```

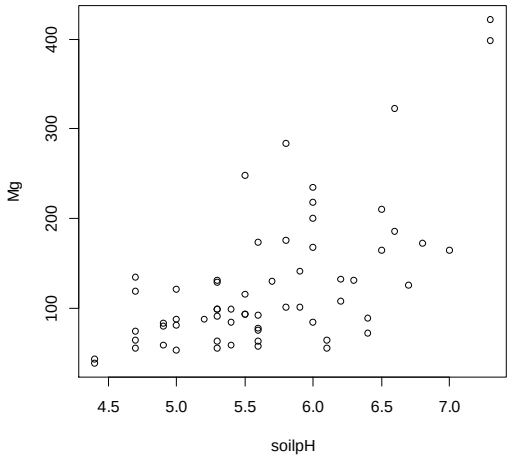
```
Residuals:
     Min       1Q   Median       3Q      Max
-1.42547 -0.58160 -0.08231  0.40750  2.33252

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)    65.495324  72.064364   0.909    0.370
juliandate      0.007583   0.010567   0.718    0.478
x               0.386829   0.627423   0.617    0.542
y              -0.925714   0.955068  -0.969    0.340
scale(age)     -0.358935   0.170050  -2.111    0.043 *
scale(soilpH)  -0.292820   0.178706  -1.639    0.111
scale(avesilt) -0.075422   0.176818  -0.427    0.673
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.9563 on 31 degrees of freedom
Multiple R-squared: 0.2337,     Adjusted R-squared: 0.08543
F-statistic: 1.576 on 6 and 31 DF,  p-value: 0.1873
```

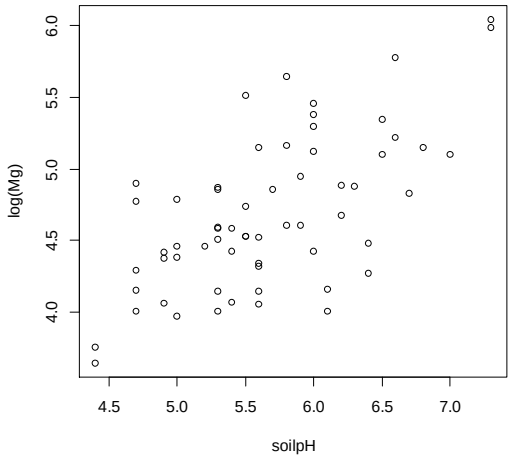
```
#try others—temp, silt, etc.
EMH0<-lm(data = gms1, scale(hyphpercm2) ~ scale(soilpH) +
scale(avesilt) + scale(aveMAXmay) + scale(age))
summary(EMH0)
#soil pH and age sig, both neg
lm(formula = scale(hyphpercm2) ~ scale(soilpH) + scale(avesilt) +
    scale(aveMAXmay) + scale(age), data = gms1)

Residuals:
     Min       1Q   Median       3Q      Max
-1.53161 -0.67538 -0.08662  0.61355  2.45480

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)       2.205e-16  1.493e-01   0.000   1.0000
scale(soilpH)    -3.755e-01  1.589e-01  -2.364   0.0241 *
scale(avesilt)   -1.951e-01  1.533e-01  -1.272   0.2123
scale(aveMAXmay) -2.192e-01  1.566e-01  -1.400   0.1709
scale(age)       -3.391e-01  1.552e-01  -2.185   0.0361 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.9201 on 33 degrees of freedom
Multiple R-squared: 0.245,      Adjusted R-squared: 0.1535
F-statistic: 2.677 on 4 and 33 DF,  p-value: 0.04886

#betttter; remove juliandate, not needed.  Max may temp ns but not too
far off either, continue to explore.

EMH1<-lm(data = gms1, scale(hyphpercm2) ~ scale(soilpH) +
scale(avetotclay) + scale(aveMAXmay) + scale(age))
summary(EMH1)
#same

EMH2<-lm(data = gms1, scale(hyphpercm2) ~  scale(soilpH) + scale(age) +
scale(avetemp) + scale(Zn) + scale(P))
summary(EMH2)
#nope, only silt and (borderline) pH.

EMH3<-lm(data = gms1, scale(hyphpercm2) ~  scale(soilpH) +
scale(avetotclay) + scale(LANDSCAN) + scale(relOZONE) + scale(age))
summary(EMH3)
 #worse and worse
```

```
EMH3<-lm(data = gms1, scale(hyphpercm2) ~  scale(soilpH) +
scale(avesilt) + scale(age) + scale(aveMAXmay))
summary(EMH3)
#neigher CEC or CA can sub for soil pH, which is having negative assoc-
-opposite of in transect manuscript, but
#this is a much larger range

EMH4<- lm (data=gms1, hyphpercm2 ~ soilpH + avesilt + age + aveMAXmay)
summary(EMH4)

#now wer're getting somewhere
EMH5<- lm (data=gms1, scale(hyphpercm2) ~ scale(soilpH) + scale(Mg) +
scale(age) + scale(aveMAXmay))
summary(EMH5)
```

```
lm(formula = scale(hyphpercm2) ~ scale(soilpH) + scale(Mg) +
    scale(age) + scale(aveMAXmay), data = gms1)

Residuals:
     Min       1Q   Median       3Q      Max
-2.03800 -0.47656 -0.04256  0.35343  2.37471

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)       4.368e-16  1.381e-01   0.000 1.000000
scale(soilpH)    -9.146e-01  2.435e-01  -3.757 0.000668 ***
scale(Mg)         6.515e-01  2.384e-01   2.732 0.010026 *
scale(age)       -3.439e-01  1.435e-01  -2.396 0.022388 *
scale(aveMAXmay) -4.121e-01  1.646e-01  -2.503 0.017435 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.851 on 33 degrees of freedom
Multiple R-squared: 0.3541,     Adjusted R-squared: 0.2758
F-statistic: 4.522 on 4 and 33 DF,  p-value: 0.005049
```

```
#not significant/needed: P, Fe, Al, Pb, Zn, lat and long, BUT Mg is
highly #sig. it is .63 cor with pH but improves the degree of sig

plot(soilpH, Mg)#higher pH soils sometimes have  higher Mg, but not
always
#so not multicollinearity.  Log transform Mg makes them look even less
#tightly correlated.
```

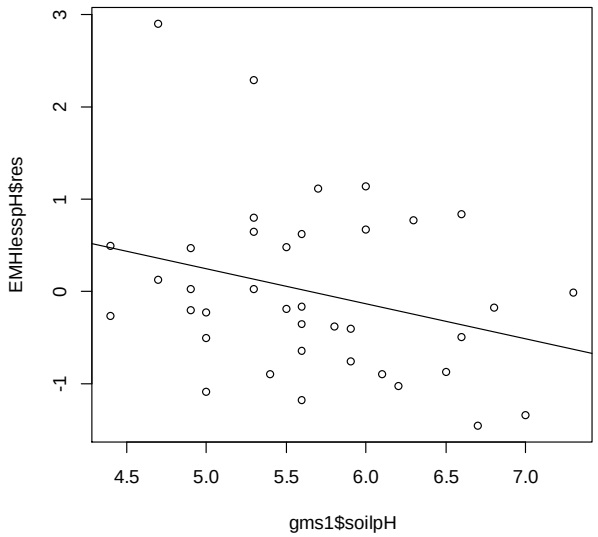




```
EMH6<- lm (data=gms1, scale(hyphpercm2) ~ scale(Mg) + scale(soilpH) +
scale(avetotclay) + scale(age) + scale(aveMAXmay))
summary(EMH6)
#maybe try using pHsquared instead of Mg?
#doesn't improve R2 value as  much as using both pH and Mg though

EMH6a<-  lm (data=golfmaster, scale(hyphpercm2) ~  scale(soilpH)^2 +
scale(avesilt) + scale(age) + scale(aveMAXmay))
summary(EMH6a)

EMH6b<-  lm (data=gms1, scale(hyphpercm2) ~ scale(Mg) + scale(soilpH)
+ scale(age) + scale(aveMAXmay) + scale(avesand))
summary(EMH6b)
#sand--maybe, active C, not total C
#tried/not shown, not useful for model:  NOT CALCIUM, K, NOT MIN FEB
TEMP, still not tot clay, NOT Pb or As,Not iron, NOT totalC, NOT c to N
, NOT OZONE
EMH8<-  lm (data=gms1, scale(hyphpercm2) ~ scale(Mg) + scale(soilpH) +
scale(CtoNprof) + scale(age)
+ scale(aveMAXmay))
summary(EMH8)
##############################################################################
###########################################
so, i guess lets stick with model 6-no, no. 7
EMH7 <-  lm (data=golfmaster, scale(hyphpercm2) ~  scale(soilpH)  +
scale(log(Mg)) + scale(age) + scale(aveMAXmay) +
scale(avesand))
summary(EMH7)

lm(formula = scale(hyphpercm2) ~ scale(soilpH) + scale(log(Mg)) +
    scale(age) + scale(aveMAXmay) + scale(avesand), data = golfmaster)

Residuals:
     Min       1Q   Median       3Q      Max
-1.42108 -0.54690 -0.03085  0.43029  2.03111

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      -0.1104     0.1051  -1.050 0.299277
scale(soilpH)    -0.6094     0.1515  -4.022 0.000218 ***
scale(log(Mg))    0.4155     0.1426   2.914 0.005534 **
scale(age)       -0.2212     0.1065  -2.076 0.043611 *
scale(aveMAXmay) -0.2843     0.1098  -2.590 0.012894 *
scale(avesand)    0.2750     0.1070   2.570 0.013535 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7396 on 45 degrees of freedom
  (11 observations deleted due to missingness)
Multiple R-squared: 0.3836,     Adjusted R-squared: 0.3151
F-statistic: 5.601 on 5 and 45 DF,  p-value: 0.0004276
```

```
attach(golfmaster)#this means now R will go to golfmaster for data
unless I
#specify otherwise
cor(Mg, soilpH) #=0.63, approaching  the .7 mentioned as problematic
but not
#other correlations ok, low
cor(soilpH, age)
cor(gms1$soilpH, gms1$aveMAXmay)
cor(gms1$soilpH, gms1$avesand)
```

```
cor(gms1$aveMAXmay, gms1$avesand)

stepAIC(EMH7, direction = c("both"))
#this is a way to do stepwise mult #regression model selection with AIC

plot(EMH7)
plot(EMH6$res, ylab = "residuals", main ="index plot of residuals")
# overall resids look ok, no big obvious pattern
```

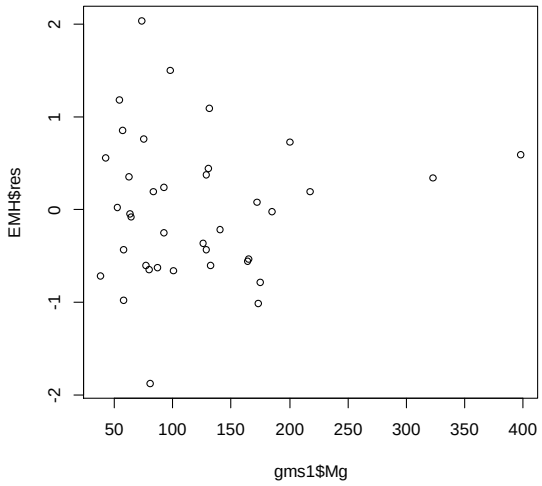


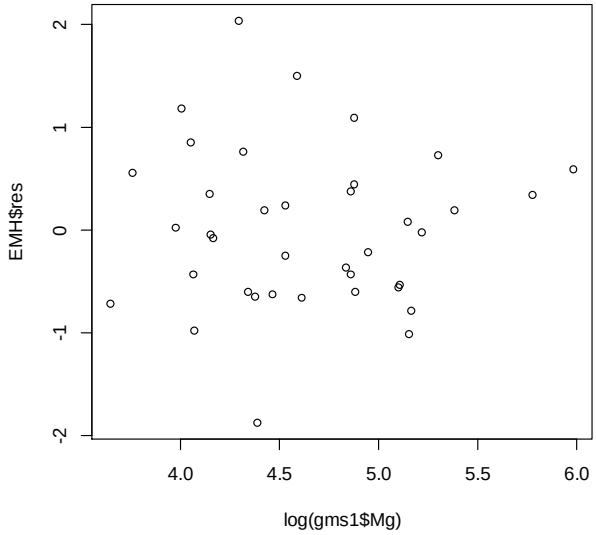

```
#now need to look at the resids of the full model against each factor
and the
#then graph resids of (model - factor1) vs factor 1 to really view what
the model is doing

EMH<-  lm (data=gms1, scale(hyphpercm2) ~ scale(log(Mg)) +
scale(soilpH)  + scale(age) + scale(aveMAXmay) +
scale(avesand))
summary(EMH)

# Mg--without log(Mg) in model, back to just age and soil pH, and adj
R2 of 15%.  not bad actually, but still.
EMHlessMg<-  lm (data=gms1, scale(hyphpercm2) ~) + scale(soilpH)  +
scale(age) + scale(aveMAXmay) +
scale(avesand))
summary(EMHlessMg)
plot(gms1$Mg, EMH$res)#it is heteroskedastic, shaped like backwards
megaphone= decreasing variance with incr Mg
# pH
EMHlesspH<-  lm (data=gms1,scale(hyphpercm2) ~ scale(log(Mg))  +
scale(age) + scale(aveMAXmay) +
scale(avesand))
summary(EMHlesspH)#without pH, everything loses all sig except sand in
whole dataset.  not sure what that means lets see.
plot(gms1$soilpH, EMH$res) #look good
plot(gms1$soilpH, EMHlesspH$res) #with temp removed from model, can see
positive relationship
```

```
abline(lm(EMHlesspH$res~gms1$soilpH))

# sand
EMHlesssand <-   lm (data=gms1, scale(hyphpercm2) ~ scale(log(Mg)) +
scale(soilpH)  + scale(age) + scale(aveMAXmay))
summary(EMHlesssand)  #no big deal for rest to remove sand
plot(gms1$avesand, EMH$res) #res look fine
plot(gms1$avesand, EMHlesssand$res) #but still relationship looks
pretty good
abline(lm(EMHlesssand$res~gms1$avesand))

# age
EMHlessage <-  lm (data=gms1, scale(hyphpercm2) ~ scale(log(Mg)) +
scale(soilpH)  + scale(aveMAXmay) +
scale(avesand))
summary(EMHlessage)#no big deal to remove age
plot(gms1$age, EMH$res) #looks fine
plot(gms1$age, EMHlessage$res) # still a nice looking relationship
abline(lm(EMHlessage$res~gms1$age))

# max may temps
EMHlessmaxtemp<-   lm (data=gms1, scale(hyphpercm2) ~ scale(log(Mg)) +
scale(soilpH)  + scale(age) + scale(avesand))
summary(EMHlessmaxtemp)
plot(gms1$aveMAXmay, EMH$res) #look good
plot(gms1$aveMAXmay, EMHlessmaxtemp$res) #with temp removed from model,
can see positive relationship
abline(lm(EMHlessmaxtemp$res~gms1$aveMAXmay))


##########################################################################
#########################
#partial correlations and CI

#   is recalculating t values from lm output, which is just estimate /
s.e..  #could just use t value column 3

t.valuesEMH8 <- summary(EMH8)$coefficients[,1] /
summary(EMH8)$coefficients[,2]
#squared t values = f values.  f dist = ratio of two chi squared dists.
partcorrEMH <- sqrt((t.valuesEMH8) / ((t.valuesEMH8) +
EMH8$df.residual))
partcorrEMH
partcorrEMH^2
round(partcorrEMH^2, 3)

###"model" is your multiple regression fit, i.e.

###"model" is your multiple regression fit, i.e.

#final EMH8 is unscaled version of final EMH7, so--yes, close to same
except for intercept of course.
```

```
nd(partcorrEMH^2, 3)
(Intercept)      soilpH     log(Mg)         age   aveMAXmay     avesand
      0.240       0.315       0.219       0.172       0.189       0.099
>
```

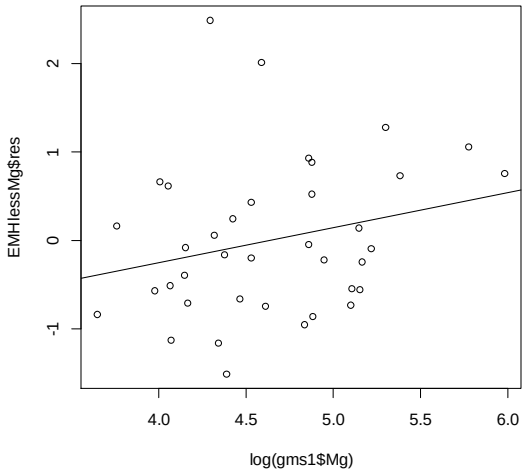
```
##############################try again with scaled, should be same part
corr

Unscaled CI:
#95% CI for the effect sizes obtained by estimate +/- 1.96*std.error
upperCIEMH <- summary(EMH8)$coefficients[,1] +
1.96*summary(EMH8)$coefficients[,2]
lowerCIEMH <- summary(EMH8)$coefficients[,1] -
1.96*summary(EMH8)$coefficients[,2]
round(lowerCIEMH, 3)
round(upperCIEMH, 3)
nd(lowerCIEMH, 3)

Unscaled CI: 95%
(Intercept)      soilpH     log(Mg)         age   aveMAXmay     avesand
    209.749     -61.841      14.487      -0.846     -11.250      -0.023
> round(upperCIEMH, 3)
(Intercept)      soilpH     log(Mg)         age   aveMAXmay     avesand
    883.188     -20.036      69.344      -0.115      -1.845       1.122
>
Standardize CI
 (lowerCIscaleEMH,3)
 (Intercept)    scale(soilpH)   scale(log(Mg))     scale(age) scale(aveMAXmay)
scale(avesand)
  -0.316           -0.906            0.136         -0.430           -0.499
0.065
> round(upperCIscaleEMH, 3)
 (Intercept)    scale(soilpH)   scale(log(Mg))    scale(age) scale(aveMAXmay)
scale(avesand)
0.096           -0.312            0.695           -0.012           -0.069
0.485
>


upperCIscaleEMH <- summary(EMH7)$coefficients[,1] +
1.96*summary(EMH7)$coefficients[,2]
lowerCIscaleEMH <- summary(EMH7)$coefficients[,1] -
1.96*summary(EMH7)$coefficients[,2]
round(lowerCIscaleEMH,3)
round(upperCIscaleEMH, 3)
_____________________________________________________________________________
> round(lowerCIprop, 5)
(Intercept)      soilpH     log(Mg)         age   aveMAXmay     avesand
  144.07222   -43.04281     8.27489    -0.57450    -7.95130     0.14479
> round(upperCIprop, 5)
(Intercept)      soilpH     log(Mg)         age   aveMAXmay     avesand
  657.90885   -14.83865    42.26063    -0.01655    -1.10062     1.07450
```

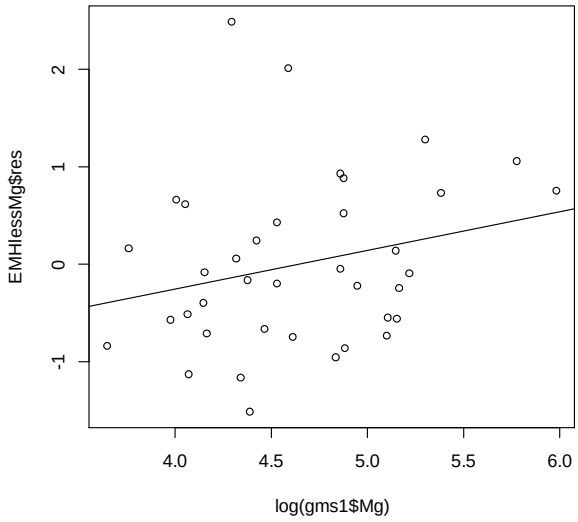


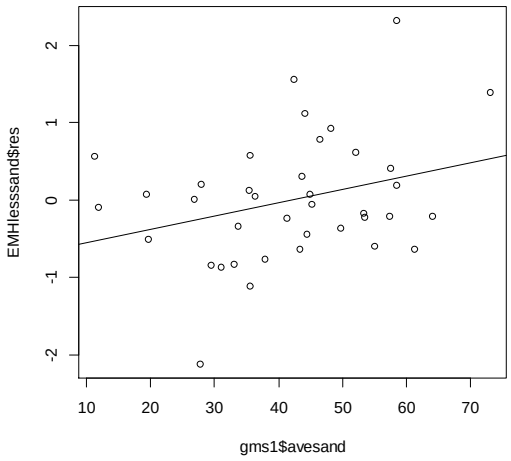


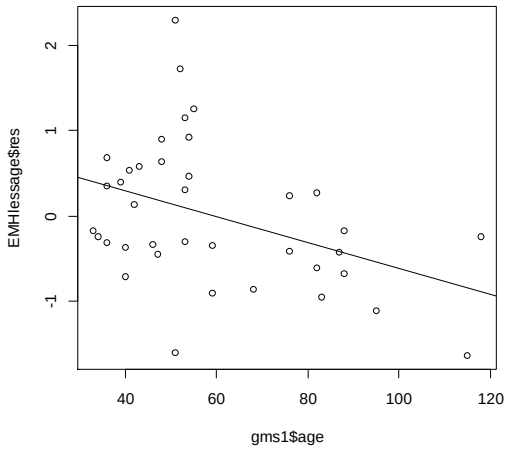
transforming to log(Mg) improves heteroskedacity

Relationships with Mg and transformed log(Mg), etc.


EMHlessMg$res
log(gms1$Mg)


EMHlessMg$res
log(gms1$Mg)

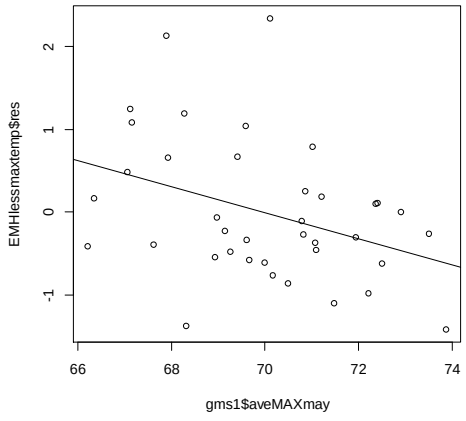

EMHlesssand$res
gms1$avesand

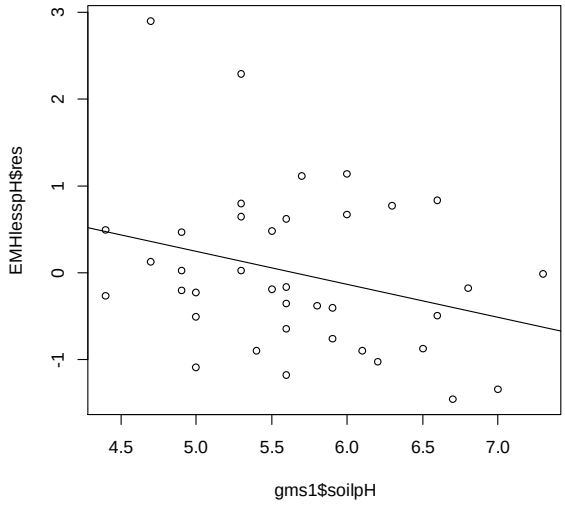

EMHlessage$res
gms1$age

###removing Max May temp does reduce sig of Mg and sand

```
lm(formula = scale(hyphpercm2) ~ scale(log(Mg)) + scale(soilpH) +
    scale(age) + scale(avesand), data = gms1)

Residuals:
    Min      1Q  Median      3Q     Max
-1.4107 -0.5216 -0.2443  0.4263  2.3356

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)    -7.502e-17  1.470e-01   0.000   1.0000
scale(log(Mg))  4.133e-01  2.419e-01   1.709   0.0969 .
scale(soilpH)  -6.271e-01  2.423e-01  -2.588   0.0142 *
scale(age)     -3.276e-01  1.530e-01  -2.141   0.0397 *
scale(avesand)  2.173e-01  1.536e-01   1.415   0.1664
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.9063 on 33 degrees of freedom
Multiple R-squared: 0.2674,     Adjusted R-squared: 0.1786
F-statistic: 3.012 on 4 and 33 DF,  p-value: 0.03187
```

But clearly this is a much looser relationship:





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