# WHY ARE TOTAL SOIL RESPIRATION MEASUREMENTS HIGHER IN URBAN FORESTS THAN RURAL FORESTS?

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*Abstract.* Long-term monitoring has shown that total soil respiration (TSR) (the production of carbon dioxide  $(CO_2)$  by roots and microorganisms) is higher in a series of urban forests than a series of rural forests in the Baltimore, MD metropolitan area. These differences may be due to differences in: root density, the quantity and quality of soil organic matter, microbial biomass, soil moisture and temperature, vegetation characteristics, distance from trees, leaf litter amount and the abundance of earthworms. Through field and laboratory measurements we determined which of these factors were most influential to total soil respiration. The results show that faunal (earthworm) biomass is higher in urban sites than in rural sites. Higher TSR measurements may thus be due to earthworm respiration as well as to earthworm stimulation of root respiration. Earthworms were most abundant in wet soils suggesting that if climate change results in changes in soil moisture, earthworm effects on soil respiration may be altered.

## INTRODUCTION

Atmospheric levels of carbon dioxide have reached their highest levels in over 750,000 years and are increasing exponentially. Although the burning of fossil fuels contributes to these high levels, the majority of atmospheric  $CO_2$  comes from naturally occurring processes, such as soil respiration. Annual soil  $CO_2$  effluxes (via decomposition) are roughly 10 times larger than fluxes associated with the combustion of fossil fuels (Mooney et al., 1987) and soil organic matter contains double the amount of carbon as the atmosphere (Schlesinger, 1991; Schlesinger and Andrew, 2000). There is great interest in determining if warmer climates caused by climate change will retard or accelerate these natural fluxes.

The process of soil respiration is the emitting of carbon dioxide into the atmosphere by plant roots, heterotrophic and autotrophic microorganisms (fungi and bacteria), and soil fauna (e.g. earthworms) (Hogberg and Read, 2006). Variation in soil  $CO_2$  flux is dependent on: topography, root density, quantity and quality of soil organic matter, microbial biomass, soil moisture and temperature, vegetation characteristics, distance from trees, leaf litter amount, and the composition of soil fauna communities (Chimner, 2004; Ryan and Law, 2005; Fisk et al., 2004).

Soil respiration is one of the largest sources of terrestrial carbon flux (Raich and Potter, 1995). The balance of terrestrial carbon storage is determined by many factors, mainly soil respiration and vegetation photosynthesis. This carbon balance may change with natural and human disturbances. There is potential in soils to mitigate climate change and sequester carbon, or release carbon and increase the amount of  $CO_2$  in the atmosphere (Tang et al., 2009). Key human disturbances of concern in relation to soil carbon sequestration are the effects of urbanization on atmosphere chemistry (Grimm et al., 2008) and invasive species, such as earthworms (Bohlen et al., 2004). These disturbances can affect the plant, microbial and faunal components of total soil respiration and soil C balance.

Long-term research as part of the NSF funded urban long-term ecological research (LTER) project in Baltimore (the Baltimore Ecosystem Study, BES) has addressed the effects of urbanization and invasive species on total soil respiration in forests. Groffman et al. (2006, 2009) found that urban forest soils respire more than rural forests and suggested that multiple factors (temperature, moisture, atmospheric chemistry, earthworms) could be responsible for this difference. Szlavecz et al. (2006), working in the same sites, found that earthworm abundance was higher

in urban forests compared to rural forests, and that the majority of the earthworms were exotic species from Europe and Asia. Studies in urban forests are relevant to broader global change questions in that these ecosystems provide a glimpse of how future forests may look and behave. Urban forests experience human disturbances, are hotter, polluted, and have high concentrations of  $CO_2$  and invasive species.

In this study, our main objective was to determine if differences in earthworm abundance underlie the higher TSR in the urban forests of Baltimore. Using the same BES long-term study sites, we measured soil  $CO_2$  efflux, soil moisture, root and microbial biomass, soil organic matter, and earthworm abundance. Three of these sites were located in close proximity to the urban interior of Baltimore City, and another four located in the rural fringe of Baltimore County. These seven sites provided the essential urban to rural gradient needed to determine the natural and human-induced disturbances to TSR.

# Study Site

The BES LTER plots used by Groffman et al. (2006) and Szlavecz et al. (2006) are located in or near the Gwynns Falls watershed in Baltimore City and County, MD. In 2000, the population in the watershed was roughly 356,000 within watershed, with people. Land use varies the the lower region dominated bv residential/commercial/industrial development and the upper region with a mixture of low density residential areas and agricultural and forested lands.

The Gwynns Falls watershed lies in two physiographic provinces, the Piedmont plateau and the Atlantic Coastal Plain. BES plots are situated on the Piedmont portion of the watershed. This plateau is composed of old igneous and metamorphic rocks.

There are four rural plots at Oregon Ridge Park, two urban plots at Leakin Park, and one urban plot at Hillsdale Park. Typical forest vegetation in the plots is composed of *Liriodendron tulipifera*, *Quercus alba*, *Acer rubrum*, *Fraxinus pennsylvanica*, *Ulmus Americana*, *Betula nigra*, and *Platanus occidentalis* (Brush et al., 1980). Annual precipitation is ~1060mm per year (Doheny, 1999). Annual air temperature ranges from 12.8 to 14.5 °C. (NOAA, 2000).

# METHODS

Soil respiration has been measured monthly since 1998 on the BES plots using an *in situ* flux chamber method (Bowden et al., 1990; Bowden et al., 1991). Measurements of soil  $CO_2$  efflux consisted of placing three 287mm diameter by 40mm high polyvinyl chloride (PVC) cylinders onto permanently installed PVC soil collars directly prior to measurement. Following placement of the cylinders on the collars, 9 mL gas samples were taken at 0, 10, 20, and 30 min thereafter from gas sampling ports in the center of the cylinder top using fine-needle polypropylene syringes. Samples were then analyzed for  $CO_2$  by gas chromatography with thermal conductivity detection. Flux calculations were from the linear rate of change in gas concentration, the internal volume of the cylinder and soil surface area.

Soil cores (three per plot) were collected with a bulb corer. These soil samples were taken within 10 to 20 m of the soil collars where soil respiration is measured. Measurements of root and microbial biomass, soil organic matter, soil moisture and microbial respiration were obtained from these cores. All roots were hand removed from the soil cores (a 15 minute time limit established), rinsed with deionized water, then dried at 60 °C for 48 hours, and weighed. Soil moisture content was obtained by drying at 60°C for 48 h (McInnes et al., 1994). The amount of soil organic matter was determined by loss on ignition at 450 °C for 4 h (Nelson and Sommers, 1996). Amounts of inorganic N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) in the soils were determined by extraction with 2 *M* KCl followed by colorimetric analysis with a flow solution analyzer (Lachat Quikchem 8000, Clackamas, OR).

Amounts of microbial biomass C and N were measured with the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976). Soils were fumigated to kill and lyse microbial cells. The fumigated sample was

then inoculated with 0.1g of fresh soil, and the microorganisms from the fresh soil grew using the dead cells as substrate. The flushes of  $CO_2$  and 2 *M* KCl extractable inorganic N (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) released by the growth activity of the cells during the 10 day incubation at field moisture content were assumed to be directly proportional to the amount of C and N in the microbial biomass of the original sample. A proportionality constant (0.41) was used to calculate biomass C from the  $CO_2$  flushes. Carbon dioxide and inorganic N concentrations were measured as described above. Inorganic N flush data were not corrected with a proportionality constant.

Inorganic N and CO<sub>2</sub> production were also measured in unfumigated control samples. These incubations provided estimates of microbial respiration and potential net N mineralization and nitrification. Quantification of microbial respiration was from the amount of CO<sub>2</sub> that evolved over the 10 day incubation. The amount of potential net N mineralization and nitrification was calculated from the accumulation of  $NH_4^+$  plus  $NO_3^-$  and  $NO_3^-$  alone during the 10 day incubation.

Field collections of earthworms were taken where Szlavecz et al. (2006) had previously sampled (three samples per plot) by using a mustard solution which irritates the earthworms causing them to protrude out of the soil. One cup of Coleman's Mustard powder was mixed with four gallons of water in a five gallon bucket. Each 25 x 25 cm sample quadrant was poured with four gallons of this mustard solution. This mustard solution was poured slowly to soak the quadrant. Earthworm extraction of each quadrant was given 30 minutes. The animals were then killed in 75% ethanol, set in 4% formalin for several days and preserved in 75% ethanol (Szlavecz et al., 2006).

# Statistical Analysis

Differences between urban and rural sites were evaluated using one-way analysis of variance. Relationships between variables were explored with Pearson Product Moment Correlations. SAS statistical software was used for all analyses (SAS Institute, 1988).

## RESULTS

In the seven sites sampled, mean root biomass ranged from 4 to 12 mg g<sup>-1</sup> of dry soil, and was significantly (p < 0.10) higher in rural than urban plots (Figure 1). There were strong relationships between root biomass and soil organic matter (SOM) (r = 0.88, p <= 0.01) and microbial biomass C (r = 0.73, p < 0.10) and N (r = 0.91, p < 0.01).

Microbial biomass C ranged from 383 to 1247 mg C kg<sup>-1</sup> and was higher in rural (mean = 949) than urban (mean = 692) sites, but the differences was not statistically significant (Figure 2). Microbial biomass N ranged from 53 to 121 mg N kg<sup>-1</sup> and was significantly (p < 0.10) higher in rural (mean = 94) than urban (mean = 67) sites (Figure 2).

SOM ranged from 0.07 to 0.13g g<sup>-1</sup> of dry soil with no significant differences between rural (mean = 0.10) and urban (mean = 0.08) plots. There were significant relationships between SOM and microbial respiration (r = 0.68,  $p \le 0.10$ ) and microbial biomass N (r = 0.67,  $p \le 0.10$ ).

Earthworm biomass ranged from 171 to 603 g m<sup>-2</sup> and was higher in urban (mean = 251) than rural (mean = 201), but the differences were not statistically significant (Figure 3). Earthworm density ranged from 61 to 371 individuals m<sup>-2</sup> and was higher in urban (mean = 350) than in rural (mean = 272), but the differences were not statistically significant (Figure 3). Mean soil moisture content ranged from 0.21 to 0.26g g<sup>-1</sup> of wet soil and averaged 0.24 in both rural and urban plots. There was significant correlations between soil moisture content and earthworm biomass (r = 0.89, p < 0.01) and density (r = 0.73, p <= 0.10) (Figure 4, 5).

Due to time and lack of expertise, we were not able identify what earthworm species were collected.

# DISCUSSION

The objective of this study was to determine the reasons for the difference in TSR between urban and rural plots that has emerged from long-term BES monitoring (Groffman et al., 2006, 2009). This monitoring has shown that urban plots consistently show higher respiration than rural plots and this difference has persisted over 10 years of monitoring during wet and dry and warm and cool years.

Given that there are three components of TSR; root, microbial and faunal respiration, we attempted to make measurements that would shed light on each of these components. Our results showed that root and microbial biomass were lower in urban sites, making these variables unable to explain the higher TSR measurements in the urban sites. The only variables that were higher in the urban plots than the rural plots was earthworm biomass and density, suggesting that differences in soil fauna community composition and activity are responsible for the long-term differences in TSR in the BES sites.

Earthworms could increase TSR through direct and indirect effects. The direct effect would be through their respiration activity and the indirect effect would be the ability of earthworms to increase fine roots and microbial respiration. The mixing caused by earthworms might have enhanced resource availability and aeration for microbial activity as suggested by Li et al. (2002). In addition, the burrowing of earthworms may have increased the porosity of the soils for gas diffusion as reported by Lange et al. (2009). On the other hand, burrowing of earthworms could sequester organic carbon (Don et al., 2008), which reduces microbial activity.

The strong correlation between earthworm biomass and density and soil moisture content (Figure 4, 5) suggests that earthworm effects on TSR are most likely to be important in wet soils and wet years. Over the long term, climate change that results in wetter soils could encourage earthworm invasion and produce noticeable effects on TSR. Interactions between invasive species and climate change are a great challenge to predicting the future structure and function of ecosystems in many regions.

In addition to affecting TSR, the prevalence of earthworms in urban forest soils might explain why root and microbial biomass and SOM were lower than that of rural forest soils. Earthworms can consume roots and microorganisms and their physical disturbance can cut roots. According to Snyder et al. (2009), earthworms and microorganisms compete for N, which might explain why microbial biomass N was lower in urban forests soils (Table 1). A study by Burtelow et al. (1998), also showed that earthworm castings are capable of elevating denitrification, which may also contribute to decreases in microbial biomass N. The declines in root and microbial biomass might affect other ecosystem nutrient cycling functions in addition to TSR, e.g., nitrous oxide and methane flux, nitrogen availability and loss.

The variables measured in this study can be compared with previous studies on these plots and elsewhere in the Baltimore metropolitan area. The amount of microbial biomass N present in this study is lower than what was found in previous measurements on these plots by Groffman et al. (2006) and the amount of root biomass in this study is much lower than that observed by Gift et al. (2010) in riparian forest sites elsewhere in Baltimore. The amount of SOM found in this study was similar to that observed by Gift et al. (2010). It was quite dry at the time of sampling these plots, perhaps explaining why microbial N and root biomass were in low production.

Earthworm biomass and density exceed the amount collected by Szlavecz et al. (2006). This may be due to our method of earthworm extraction with the mustard powder solution, instead of the mild (0.2%) formaldehyde solution used by Szlavecz et al. (2006). Although our numbers are higher than that of Szlavecz et al. (2006), we found the same trend of higher numbers of earthworms in urban plots than rural plots (Szlavecz et al., 2006).

Earthworm effects on TSR are clearly worthy of further study. Microcosms could be used to plant and microbial respiration and leaf litter decomposition with or without the presence of earthworms to see whether earthworms in urban forests are increasing or decreasing soil  $CO_2$  efflux as described by Snyder et al. (2009).

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#### APPENDIX



FIGURE 1. Mean root biomass of urban and rural plots.



FIGURE 2. The mean microbial biomass C and N of the urban and rural plots.



FIGURE 3. Earthworm biomass and density of urban and rural forests in the Baltimore metropolitan area.



FIGURE 4. Earthworm biomass vs. soil moisture content. 'U' meaning urban forests and 'R' meaning rural forests.



FIGURE 5. Earthworm density vs. soil moisture content. 'U' meaning urban forests and 'R' meaning rural forests.

TABLE 1. Total soil respiration variables of urban and	rural forest soils in the Baltimore metropolitan area.
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Land Use	Variable	Mean
Rural	Microbial Respiration (µg C g <sup>-1</sup> d <sup>-1</sup> )	16.8
	C Microbial Biomass (µg C g <sup>-1</sup> dry soil)	949
	N Microbial Biomass (µg N g <sup>-1</sup> dry soil)	94.4
	SOM (g g <sup>-1</sup> dry soil)	0.10
	Root Biomass (mg g <sup>-1</sup> dry soil)	8.6
	Soil Moisture Content (%)	0.24
	Earthworm Biomass (g m <sup>-2</sup> )	201
	Earthworm Density (individuals m <sup>-2</sup> )	272
Urban	Microbial Respiration (µg C g <sup>-1</sup> d <sup>-1</sup> )	8.5
	C Microbial Biomass (µg C g <sup>-1</sup> dry soil)	692
	N Microbial Biomass (µg N g <sup>-1</sup> dry soil)	66.7
	SOM (g g <sup>-1</sup> dry soil)	0.08
	Root Biomass (mg g <sup>-1</sup> dry soil)	5.3
	Soil Moisture Content (%)	0.24
	Earthworm Biomass (g m <sup>-2</sup> )	251
	Earthworm Density (individuals m <sup>-2</sup> )	350