Abstract. The process of urbanization may alter the ability of microorganisms to supply nutrients to plants. However, both the composition and structure of soil biological communities, and the extent of variation within these communities, is not clear in urban areas. Therefore, baseline information regarding the impact of urban land management practices on soil microbial communities is essential to improving individuals’ ability to manage urban soils and the plants they support. This study examined soil microbial communities over five urban land uses with different degrees of urbanization in metropolitan Milwaukee, Wisconsin, U.S. The objectives were to 1) determine if differences exist in bacterial and fungal community composition, biological activity, and the soil physical and chemical environment across five urban land uses, and 2) determine if differences in the bacterial and fungal compositions compare to differences in the soil’s physical and chemical characteristics. Bulk density, soil organic matter, pH, magnesium, sodium, total nitrogen, and C:N ratio displayed significant differences between streets and forests. Microbial biomass did not differ between land uses, and the differences in bacterial and fungal community composition reflect only a small portion of the total microbial pool. The decomposition of transposed leaf litter showed significant decline in C:N ratio over time, but no statistical differences between land use were observed. The results display a highly redundant microbial assemblage, and suggest that in locations with adequate levels of soil carbon and where parent material and soil forming processes are homogeneous, urbanization and landscape management have less impact on soil microbiology than expected.

Key Words. PLFA; Soil; Soil Bacteria; Soil Fungi; Soil Microbiology; TRFLP; Urbanization; Wisconsin.

Nutrient cycling, most notably carbon (C) and nitrogen (N), are primarily regulated by the composition, activity, and relative abundances of soil bacteria and fungi. These organisms are thought to be influenced by the type and quantity of organic inputs and the physical and chemical environment in which they live (Zak et al. 2003; Lauber et al. 2008). It is widely believed that changes to the organic inputs or the soil’s physical and chemical environment will alter the composition and structure of the soil microbial community, and presumably alter the products of the processes these organisms regulate (Faeth et al. 2005; Lauber et al. 2008; Hall et al. 2009).

The urbanization of rural lands tends to alter historic vegetation patterns (Faeth et al. 2005; Nowak et al. 2007) and induce changes in the soil’s physical and chemical properties (Craul et al. 1999; Scharenbroch et al. 2005). For example, the process of urbanization has been reported to alter both the quantity (i.e., tree density, Nowak et al. 2007) and composition (e.g., tree species, leaves and woody debris versus grass clippings) of organic inputs into the soil (Craul et al. 1999; Faeth et al. 2005; Scharenbroch et al. 2005). Urbanization has also been demonstrated to alter bulk density, soil aggregation and porosity, temperature and moisture regimes, and soil pH (Craul et al. 1999). Additionally, the by-products of industrial activity—most notably the heavy metals lead, cadmium, mercury, zinc, copper, and chromium—tend to accumulate in the soils of heavily industrialized urban areas (Fantroussi et al. 1999; Hinojosa et al. 2005; Ayansina and Oso 2006; Yang 2006; Park et al. 2010). The degree of soil alteration associated with urbanization appears to be related
to 1) intensity of the urbanization [e.g., single-family residential versus city center (Pouyat et al. 2007)], 2) the extent of land management activities required to maintain the landscape in its current urban land use (Fantroussi et al. 1999; Pouyat et al. 2007), and 3) the time elapsed since the initiation of the land-use conversion (Scharenbroch et al. 2005). Generally, changes to the soils' physical and chemical properties are most pronounced in areas that support a high density of urban infrastructure (e.g., roads and buildings) or have recently undergone a significant land-use change.

The process of urbanizing and the subsequent maintenance of formerly rural landscapes are believed to negatively affect the soil biology (McDonnell et al. 1997; Kaye et al. 2005; Newbound et al. 2012; Xu et al. 2014). Unfortunately, the direct and persistent effects of urbanization on soil microbial communities remains largely unstudied when compared to the number of investigations involving the impact on the soils' chemical or physical properties. Furthermore, there are no reports within the scientific literature that define the ideal or desired structure and composition for a soil's microbial community. Consequently, urban landscape managers attempting to mitigate or correct the effects of urbanization on soils' biological communities, and the processes they regulate, lack essential baseline information upon which to make soil management decisions. Therefore, the goal of this study was to compare soil microbial communities across various urban landscapes and to identify key soil characteristics that may aid in evaluating the state of the biology in urban soils. It is hypothesized that the combination of changes in edaphic characteristics induced by the process of urbanization, associated landscape management activities, and the time elapsed since the initiating land-use conversion event will result in significant changes in the abundance, structure, and activity of the soils' microbial community. Specifically, the objectives were to 1) determine if differences exist in bacterial and fungal community composition, biological activity, and the soil physical and chemical environment across five urban land uses, and 2) determine if differences in the bacterial and fungal compositions compare to differences in the soil's physical and chemical characteristics.

**MATERIALS AND METHODS**

**Experimental Design**

Study sites were located within a 12 km radius in metropolitan Milwaukee, Wisconsin, U.S. The soils within the Milwaukee area are typically deep, moderately well to well drained, and slightly alkaline, consisting of fine textured loess deposits over alkaline glacial till. Common soil series in this area include Morley, Blount, Varna, and Elliot; however, a majority of the soil in the City of Milwaukee has not been classified as a result of anthropogenic influences (Soil Survey Staff).

The urban land-use designations in this study were urban street side terraces [Streets (ST)], new (<5 years since development) residential landscapes receiving intensive management [New Managed (NM)], old (>25 years since development) residential landscapes receiving intensive management [Old Managed (OM)], old residential landscapes receiving minimal management [Old Unmanaged (OU)], and forested lands [Forests (FR)]. Intensively managed study sites received regular fertilizer applications and pesticide treatments over the last 25 years (OM) or 5 years (NM) (pers. comm.: Nick Crawford, Crawford Tree and Landscaping, April 2009), whereas the unmanaged properties have no record of receiving fertilizer or pesticide applications over the past 25 years (pers. comm.: property owners, April 2009). Based on exposure to anthropogenic influences (management level, vehicular traffic loads, and housing densities) and previous findings that soils recover from disturbances over time (Scharenbroch et al. 2005), the order of land-use designations from most- to least-urbanized, in this study, was assumed to be: ST > NM > OM > OU > FR. The vegetative communities in all locations, with the exception of FR sites, consisted of turfgrass areas with landscape trees of various species. The FR sites had mixed hardwood vegetation dominated by *Quercus* sp. >25-years-old with a leaf litter surface layer devoid of turfgrass.

Five urban study sites were randomly selected for each land use from a pool of potential locations, resulting in 25 urban sites. Each study site was subdivided into 25 m² soil sampling areas. Within each study site, three soil cores (2.5 cm × 25 cm) were removed from the near center.
of five randomly selected sampling areas and homogenized into one representative soil sample. Approximately half of the homogenized soil samples were placed in paper bags, air dried, passed through a 2 mm sieve, and stored at room temperature until physical and chemical analysis. The remaining soil was immediately placed on ice, frozen within 12 hours, and stored at -20°C prior to biological analysis.

**Physical and Chemical Soil Analysis**

Physical characteristics measured for each site included: percent sand, silt, and clay measured by the hydrometer method (Gee and Bauder 1986); soil organic matter (SOM) content (percent loss on ignition, Kalra and Maynard 1991); percent volumetric water content at field capacity (pressure plate technique at one third bar, Gardner 1986); and bulk density as measured by averaging two 68.7 cm³ oven-dried soil samples (Blake and Hartage 1986) independently collected with an ICT International model 0200 Soil Bulk Density Sampler (ICT International, Australia) at two randomly selected undisturbed locations in each study site. Chemical analyses consisted of total carbon (C), and total nitrogen (N) as measured on a CE 2000 Carbon nitrogen analyzer (Carlo Erba), 2:1 water:soil pH (electrode), extractable phosphorus (Bray), ammonium acetate extracted calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), and cation exchange capacity (CEC), by summation of the base cations Ca, Mg, K, and Na.

**Molecular Community Fingerprint Analysis with TRFLP**

Terminal restriction fragment length polymorphism (TRFLP) was used to profile the bacterial and fungal communities (Liu et al. 1997). All molecular analyses were performed in the University of Wisconsin–Stevens Point Molecular Conservation Genetics Laboratory (MCGL). Microbial DNA was extracted from 0.25 g of soil using a variation of the modified Burgmann method (Thakuria et al. 2008). The variation consisted of replacing the final polyvinylpolypyrrolidone spin column with a modified gel purification method according to Zhang et al. (2009). Reaction mixtures for bacterial 16S rDNA PCR amplification contained 1 µl template DNA, 1× Taq buffer (New England Biolabs, Ipswich, Massachusetts, U.S.), 600 µM dNTPs, 3.0 mM MgCl₂, 0.25 mg bovine serum albumin (BSA), 150 µM each primer, and 2.5 U Taq DNA polymerase (New England Biolabs) in a final volume of 50 µl. The primers were 6-FAM labeled 8f (6FAM-AGAGTTTGATCCTGGCTCAG) and 926r (CCGTCAAATCCTTTRAGTTT) (Hackl et al. 2004). Bacterial PCR conditions consisted of a 5-min hot start at 95°C, followed by 25 cycles of 95°C/1 min, 53°C/1 min, 72°C/1 min with a final extension of 72°C/15 min. For fungal 18s rDNA amplification, the PCR reaction contained 1 µl template DNA, 1× Taq buffer (New England Biolabs), 600 µM dNTPs, 2.0 mM MgCl₂, 0.25 mg BSA, 100 µM each primer, and 2.5 U Taq DNA polymerase (New England Biolabs) in a final volume of 50 µl. The primers were PET labeled fun18S1 (PET-CCATGCATGTTAAGTTAA) and fung5 (GTAAAGTCTGGTTCC) (Anderson and Cairney 2004). Fungal PCR conditions consisted of a 5-min hot start at 95°C, followed by 35 cycles of 95°C/30 sec, 53°C/30 sec, 72°C/1 min with a final extension of 72°C/15 min. Three PCRs of each sample were performed on a GeneAmp 9700 (Applied Biosystems, Foster City, California, U.S.), and the resulting products were pooled to minimize bias. The pooled products were cleaned and concentrated using a DNA Clean & Concentrator-25 Kit (Zymo Research, Irvine, California, U.S.) following the manufacturer’s protocol.

Multiple single digests of the concentrated bacterial and fungal PCR products were completed using 10 U of restriction enzyme for two hours at 37°C. The bacterial products were digested with HhaI, MspI, and RsaI (New England Biolabs) and renamed B1, B2, and B3, respectively. Similarly, fungal PCR products were digested with AluI, MspI, and RsaI (New England Biolabs) and renamed F1, F2, and F3, respectively. Terminal restriction fragments (T-RF) were visualized using a 3730xl Genetic Analyzer (Applied Biosystems) with LIZ™ 1200 internal size standard (Applied Biosystems). Fragments were sized using Genemapper v4.0 analysis.
software (Applied Biosystems) and a custom 2 bp binning panel (Rees et al. 2004) with a minimum peak height detection of 50 fluorescent units.

**Microbial Biomass and Microbial Community Lipid Analysis (PLFA/FAME)**

Lipid analyses were completed using a hybrid procedure of phospholipid fatty acid (PLFA) and fatty acid methyl ester (FAME) analysis (Balser 2001) at the University of Wisconsin–Madison. Approximately 3.0 g of milled, freeze-dried soil was extracted and analyzed according to WalDROP and FIRESTONE (2004). The bacterial fatty acid standards 9:0 (nonanoic methyl ester) and 19:0 (nonadecanoic methyl ester) (Sigma, St. Louis, Missouri, U.S.) with MIDI peak identification software (MIDI, Newark, Delaware, U.S.) were used to identify peaks. Fatty acids were excluded if present in less than 3 samples or if less than 0.5 mol%.

**Litter Decomposition**

Fifteen *in situ* litter decomposition bags containing approximately 1.0 g of air-dried white oak (*Quercus alba*) leaves were buried at randomly selected locations in each study site. The litter bags were constructed of gray 1.0 mm fiberglass screen (25 cm × 20 cm) and inserted vertically in the upper 25 cm of the soil profile. Prior to installation, approximately 5 mg of leaf tissue was removed from each litter bag and analyzed for initial total C, total N, and C:N ratio using an NC 2100 Soil analyzer (Alantech, Atlanta, Georgia, U.S.) with Clarity 6.12.2006 software (2006 DataApex Ltd, Czech Republic). Three buried litter decomposition bags from each property were removed 10, 30, 60, 90, and 120 days after installation. The bags were washed in deionized water, to remove soil particles, and air dried. The remaining leaf material was pulverized and analyzed for total C, total N, and C:N ratio. Between sampling period changes in total C, total N, and C:N ratio of transposed litter were analyzed using repeated measures ANOVA in statistical analysis software (SAS v9.2, Cary, North Carolina, U.S.). Differences among land uses within a sample period were analyzed in SAS using ANOVA with Duncan’s multiple range test (Duncan’s MRT).

**Statistical Analysis**

One-way analysis of variance (ANOVA, $\alpha = 0.05$) and Duncan’s MRT were conducted using SAS to compare soil physical and chemical properties among land uses. All physical and chemical attributes were normalized according to Clarke and Gorley (2006). The physical and chemical properties of each sample were compared with a Euclidian distance matrix using Plymouth Routines in Multivariate Ecological Research v6 (PRIMER v6, Lutton, UK) (Clarke and Gorley 2006). Analysis of similarities (ANOSIM) and non-metric multi-dimensional scaling (MDS) (2D stress limit of <0.20) with cluster overlay (PRIMER v6) were used to make inferences regarding differences in the edaphic properties of the soils among land uses. Pairwise land-use comparisons were evaluated on the edaphic properties if the global R value was significant ($\alpha = 0.05$, 999 permutations, Clarke and Warwick 2001). Pairwise R values below 0.50 indicated edaphic variables within the land-use categories were not separated, values between 0.50 and 0.70 were overlapping, and values above 0.70 were well separated (Clarke and Warwick 2001).

Estimates of total microbial biomass [sum of total microbial lipids (Balser 2001)] in each land use were compared using a one-way ANOVA ($\alpha = 0.05$). Individual lipids were standardized by dividing the respective lipid abundances (µmol/g soil) by the total lipid abundance within the sample (Balser 2001). Individual peaks within the TRFLP bacterial and fungal profiles were standardized by dividing by the sum of all peak heights within that profile (Rees et al. 2004). Simpson diversity, Shannon diversity, Pielou’s evenness, and Margalef species richness (Clarke and Gorley 2006) were calculated from the standardized TRFLP and PLFA/FAME profiles (PRIMER v6). One-way ANOVA ($\alpha = 0.05$) and Tukey’s Honestly Significant Difference (Tukey’s HSD) were used to evaluate differences in biodiversity measures among land uses for the samples. For multivariate analysis, standardized TRFLP and PLFA/FAME profiles were square-root transformed and analyzed with a Bray-Curtis similarity matrix using ANOSIM and MDS (2D stress <0.20) with cluster overlay in PRIMER v6 (Rees et al. 2004).
RESULTS

Soil Physical and Chemical Properties

Land use had a significant effect on two physical and seven chemical measurements. The bulk density of ST and NM were 45%–50% higher than FR (P < 0.001, Table 1). Soil organic matter in the ST and NM were 40% lower than OU and FR (P = 0.005, Table 1). Soil pH (P < 0.001), total N (P = 0.015), extractable K (P = 0.006), Mg (P < 0.001), Na (P < 0.001), and P (P = 0.026) (Table 2) were all significant. Soil pH decreased consistently from ST to FR (Table 2) where the ST and NM land uses differed from the other land uses. Conversely, total N and Mg increased from ST to FR and extractable Na was 230% or higher in ST than all other land uses (Table 2). The combined edaphic characteristics of the ST were most similar to NM, and were well separated from those of the OM, OU, and FR (Table 3; Figure 1). The remaining land-use comparisons were either overlapping, or not well separated.

### Table 1. Mean values ± standard error (n = 5) for physical properties of soils collected on May 17th and 18th, 2009 from: street side terraces (ST), new managed (NM), old managed (OM), old unmanaged (OU), and forest (FR) properties in metropolitan Milwaukee, Wisconsin, U.S. Variables with significant ANOVA (α = 0.05, n = 5) were analyzed with Duncan’s MRT; the differences among the land uses are indicated by lowercase letters.

<table>
<thead>
<tr>
<th>Land use</th>
<th>Bulk density (g/cm³)</th>
<th>Soil organic matter (%LOI)</th>
<th>CEC (mmol/kg)</th>
<th>Water at field capacity (%)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>1.51 ± 0.03 a</td>
<td>6 ± 0.6 a</td>
<td>207 ± 5.0 a</td>
<td>21 ± 1 a</td>
<td>39 ± 2 a</td>
<td>42 ± 1 a</td>
<td>20 ± 3 a</td>
</tr>
<tr>
<td>NM</td>
<td>1.41 ± 0.07 a</td>
<td>5 ± 0.5 a</td>
<td>217 ± 5.8 a</td>
<td>23 ± 2 a</td>
<td>35 ± 5 a</td>
<td>43 ± 3 a</td>
<td>23 ± 2 a</td>
</tr>
<tr>
<td>OM</td>
<td>1.11 ± 0.03 b</td>
<td>8 ± 0.4 ab</td>
<td>211 ± 13.3 a</td>
<td>31 ± 3 a</td>
<td>28 ± 4 a</td>
<td>45 ± 7 a</td>
<td>28 ± 7 a</td>
</tr>
<tr>
<td>OU</td>
<td>1.09 ± 0.05 b</td>
<td>10 ± 1.0 b</td>
<td>218 ± 16.2 a</td>
<td>28 ± 3 a</td>
<td>34 ± 31 a</td>
<td>43 ± 5 a</td>
<td>24 ± 5 a</td>
</tr>
<tr>
<td>FR</td>
<td>0.97 ± 0.08 b</td>
<td>10 ± 1.6 b</td>
<td>199 ± 19.2 a</td>
<td>30 ± 4 a</td>
<td>35 ± 10 a</td>
<td>54 ± 6 a</td>
<td>11 ± 4 a</td>
</tr>
</tbody>
</table>

### Table 2. Mean values ± standard error (n = 5) for chemical properties of soils collected on May 17th and 18th, 2009 from: street side terraces (ST), new managed (NM), old managed (OM), old unmanaged (OU), and forest (FR) properties in metropolitan Milwaukee, Wisconsin, U.S. Variables with significant ANOVA (α = 0.05, n = 5) were analyzed with Duncan’s MRT; the differences among the land uses are indicated by lowercase letters.

<table>
<thead>
<tr>
<th>Land use</th>
<th>pH (mg/kg)</th>
<th>P (mg/kg)</th>
<th>K (mg/kg)</th>
<th>Mg (mg/kg)</th>
<th>Na (mg/kg)</th>
<th>C (g/100g)</th>
<th>N (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>8.3 ± 0.2 a</td>
<td>1.7 ± 0.7 a</td>
<td>71 ± 9 c</td>
<td>311 ± 16 a</td>
<td>280 ± 62 a</td>
<td>5.0 ± 0.3 a</td>
<td>0.15 ± 0.03 a</td>
</tr>
<tr>
<td>NM</td>
<td>8.0 ± 0.0 ab</td>
<td>7.2 ± 0.7 a</td>
<td>112 ± 10 bc</td>
<td>484 ± 31 b</td>
<td>66 ± 25 b</td>
<td>4.4 ± 0.6 a</td>
<td>0.18 ± 0.02 a</td>
</tr>
<tr>
<td>OM</td>
<td>7.8 ± 0.1 bc</td>
<td>4.3 ± 1.2 a</td>
<td>137 ± 7 ab</td>
<td>520 ± 14 bc</td>
<td>84 ± 47 b</td>
<td>4.2 ± 0.5 a</td>
<td>0.23 ± 0.03 ab</td>
</tr>
<tr>
<td>OU</td>
<td>7.7 ± 0.1 cd</td>
<td>18.0 ± 7.1 b</td>
<td>162 ± 19 a</td>
<td>557 ± 30 bc</td>
<td>38 ± 13 b</td>
<td>4.9 ± 0.7 a</td>
<td>0.34 ± 0.05 b</td>
</tr>
<tr>
<td>FR</td>
<td>7.4 ± 0.1 d</td>
<td>6.2 ± 1.9 a</td>
<td>90 ± 27 bc</td>
<td>575 ± 29 c</td>
<td>41 ± 10 b</td>
<td>4.1 ± 0.7 a</td>
<td>0.33 ± 0.06 b</td>
</tr>
</tbody>
</table>

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Analysis of Bacterial Communities
The intensity of urbanization had little effect on biodiversity within the bacterial community. Eight of the twelve biodiversity comparisons were insignificant (data not shown); however, FR had higher average richness and Shannon diversity than OU. Differences in the bacterial community profiles among the land uses were detected with ANOSIM and MDS. The global R values were all significant ($P = 0.001$) but generally low for all three bacterial profiles, ranging from 0.269 (B3) and 0.461 (B1). Pairwise comparisons within the B1 and B2 profiles indicated bacterial communities in FR, OM, and NM are well separated from one another (R > 0.70) (Table 3). However, the distinctive clusters represented by the NM, OM, and FR profiles were grouped in close proximity to one another, suggesting large-scale similarities exist among these communities (Figure 2).

Analysis of Fungal Communities
No consistent land-use effects on fungal diversity indices were observed among all three fungal profiles. However, increased urbanization appeared to decrease richness. There were also significant differences in the diversity of seven of the twelve fungal land-use comparisons (data not shown). Specifically, ST had lower average fungal richness, evenness, Shannon diversity, and Simpson diversity than all other land uses in the F1 profiles. In the F2 profiles, OU had lower richness than FR. In the F3 profiles, ST had lower richness than OM and FR. The ANOSIM global R values were all significant ($P \leq 0.014$), but low (F1= 0.371, F2 = 0.200, and F3 = 0.187). The F1 fungal community profiles showed the NM sites were well separated from the OM and FR sites, and overlapped with the OU and ST profiles (Table 3). The separations between NM and the other land uses were the product of highly similar fungal communities within the NM sites (Figure 2). All other comparisons were not significant.

PLFA/FAME Estimates of Microbial Biomass and Community Analysis
Three of the four biodiversity indices revealed significant differences among land uses (data not shown) with ST resulting in lower evenness, Shannon diversity, and Simpson diversity than FR and OM. However, total microbial biomass did not differ significantly ($P = 0.579$) among the land uses. The global R value (0.359) was significant ($P = 0.001$) but low (F1= 0.371, F2 = 0.200, and F3 = 0.187). The F1 fungal community profiles showed the NM sites were well separated from the OM and FR sites, and overlapped with the OU and ST profiles (Table 3). The separations between NM and the other land uses were the product of highly similar fungal communities within the NM sites (Figure 2). All other comparisons were not significant.

Litterbag Decomposition Analysis
The biological decomposition of the transposed leaf litter was consistent in all land uses. Over time, the C:N ratio of transposed leaf litter declined significantly ($P = 0.001$) in all land uses, with an average C:N ratio across all sites following 0-, 10-, 30-, 60-, 90-, and 120-day incubation of 66.80, 52.72, 43.35, 37.51, 34.67, and 34.49, respectively. The declines in C:N ratio, however, did not differ among land uses in any of the sampling periods, where $P = 0.649, 0.521, 0.524, 0.116$, and 0.097 for the 10-, 30-, 60-, 90-, and 120-day sampling periods, respectively.

DISCUSSION
The detected differences in microbial communities among the land uses were not consistent across all TRFLP or lipid community profiles, and tended to be most pronounced in comparisons between the least (forest) and the most (street) urbanized landscapes. Regardless, even at the extremes of human
influence there remained a great deal of similarity among the TRFLP-generated bacterial, fungal profiles, and FAME/PLFA microbial community profiles. Consequently, the composition of the bacterial and fungal communities did not appear to be substantially altered by the degree of urbanization or the intensity of landscape management. These findings are consistent with those of Girvan et al. (2003), Kaye et al. (2005), and Newbound et al. (2012), who also observed a high degree of similarity in the composition of bacterial assemblages from landscapes with different land management histories, but were within close geographic proximity to one another with similar underlying soil characteristics.

![Figure 2. The 2-D MDS plots from the TRFLP bacterial communities B1, B2, and B3, and fungal communities F1, F2, and F3 as measured from soils collected on 17 and 18 May 2009 from: street side terraces (.), new managed (▲), old managed (Δ), old unmanaged (●), and forest (○) properties in metropolitan Milwaukee, Wisconsin, U.S. Each sample property is represented by its land use. The outlined circles indicate groupings with 65% similar bacterial or fungal populations created from a Bray-Curtis similarity cluster diagram in PRIMER v6.](image-url)
and origins. Each site in the present study was also located in a small geographic region with similar soil-forming processes and climatic regime. This resulted in limited soil texture differences (i.e., percent sand, silt, and clay) and no change in soil water-holding capacity. The strength of the assertion that geographic proximity and soil-forming factors strongly regulate the composition of soil microbial communities is reinforced by Xu et al. (2014), who examined the composition of soil microbial communities in urban parks within 16 Chinese cities that encompassed a wide distribution of latitudes and longitudes. The authors concluded that geographic location and the corresponding differences in temperature and precipitation, in addition to soil pH, better explained the variability in microbial community structure than urbanization. In this study, researchers observed reductions in SOM, total N, elevated soil pH, bulk density, and extractable Na with increasing urbanization, all of which are similar to the results reported by McDonnell et al. (1997) and Scharenbroch et al. (2005). Individually and collectively, these conditions have been demonstrated to alter microbial community profiles (Balser 2001; Lauber et al. 2008; Rousk et al. 2010; Xu et al. 2014) and/or biological functioning (Rietz and Haynes 2003; Pavao-Zuckerman and Coleman 2007; Hall et al. 2009; Rousk et al. 2009).

The observed differences in pH and sodium in the Milwaukee sites were likely too small to have a significant influence on the native microbial communities. Rousk et al. (2010) identified pH as an influence to the relative abundance and diversities of soil bacteria; however, the influential range of values (4 to 8) was far greater than those observed in the Milwaukee sites [7.4 to 8.2 (Table 2)]. Similarly, Högberg et al. (2007), Lauber et al. (2008), and Singh et al. (2008) correlated pH to bacterial communities, but again the pH values (<5) were acidic. Acidic soils with pH of 4.5 and below have been documented as having an inhibitory influence on microbial function (Rousk et al. 2009); however, this is very different than the alkaline Milwaukee soils used in the present study. The elevated sodium levels measured in the street-side locations is attributed to winter salt additions on all area roads (pers. comm.: R. Krouse, City of Milwaukee). Omar et al. (1994) observed a decrease in bacterial and fungal counts only after the addition of more than 5% NaCl to a garden soil. Despite the significant increase in Na levels observed in the Milwaukee street-side locations, the 5.8% exchangeable Na percentage was far less than a 5% total NaCl, and is not high enough to interfere with the structure and function of the microbial communities.

Furthermore, the differences detected in soil organic matter, total N, and bulk densities also resulted in a minimal influence on the microbial communities. There is evidence suggesting that vegetative community differences observed in urban areas result in reduced SOM and increased soil bulk densities (Scharenbroch et al. 2005; Scharenbroch and Lloyd 2006; Nowak et al. 2007). Similarly, reductions in soil organic matter were observed in areas with greater human influence; however, the reduction in SOM did not reduce total soil carbon. Differences in the amount and composition of soil carbon substrates can initiate a shift in microbial biomass, community composition, and microbial activity (Goldfarb et al. 2011). However, if there are limited changes to soil carbon additions, a sustainable and generally homogeneous carbon substrate will be maintained in the soil. Ultimately, if resources (carbon substrates) are not limiting and selective pressures are low, microbial diversity would remain unchanged, and a redundant microbial community would result (Zhou et al. 2002).

Figure 3. The 2-D MDS plot created from the total microbial community as analyzed by PLFA/FAME from soils collected on 17 and 18 May 2009 from: street side terraces (◼), new managed (▲), old managed (Δ), old unmanaged (●), and forest (⏺) properties in metropolitan Milwaukee, Wisconsin, U.S. Each sample property is represented by its land use. The outlined circles indicate groupings with 80% similar microbial communities created from a Bray-Curtis similarity cluster diagram in PRIMER v6.
In general, variations to microbial communities appear to be more pronounced when urbanization occurs in native landscapes, where primary production is severely limited by extremes in temperature and moisture (Xu et al. 2014). Native limitations to primary production and plant communities are often overcome during the creation and management of urbanized landscapes through irrigation and fertilization; thereby stimulating a buildup of soil carbon that largely differs from the native landscape. The size and quality of the soil carbon pool, in particular, has been shown to influence both microbial biomass and ecosystem functioning, particularly nitrogen mineralization (Kaye et al. 2005). Kaye et al. (2005) detected limited differences in soil microbial assemblages attributed to the increased urbanization; however, they additionally observed statistically higher microbial biomass in urban landscapes relative to the surrounding agricultural areas. They attributed this increase in microbial biomass to elevated soil carbon storage and enhanced carbon cycling rates in the urban landscape as compared to agricultural sites. Contrary to Kaye et al. (2005), the present study found no changes in soil microbial biomass or total carbon across the different land uses; therefore, it would appear the statistical differences observed in SOM are not of sufficient magnitude to generate a significant difference in soil carbon or large scale shifts in the microbial composition, and likely did not limit the growth of the microbial community. Within the land-use categories with the highest levels of urbanization in this study, the soil carbon pools and accompanying chemical and physical environments are capable of supporting a high level of microbial diversity and potentially, associated ecological functioning; as verified by statistically similar outcomes of in situ litter decomposition.

Despite being a coarse measure of microbial activity, the comparable degradation of a moderately difficult to decompose organic substrate (Quercus alba leaves) suggests the biological communities in the different urban areas have a comparable capacity to decompose organic material. Although bulk densities were elevated in the highly urbanized soils, any oxygen deficiencies associated with high bulk densities did not limit microbial activity in the degradation of the transposed leaf litter; this indicated the presence of a potentially resilient microbial assemblage. Furthermore, using the forested land use as a model for a sustainably functioning soil, the similarity in litter decomposition suggests the microbial communities in the different Milwaukee landscapes is similar in activity to that of the area forests. Therefore, because there were no differences in the observed microbial activity and biomass, and only subtle changes in the microbial communities, it is reasonable to conclude that the microbial composition and activity was ubiquitous in the Milwaukee area sites encompassed by this study.

**CONCLUSION**

This study was unable to detect large scale, consistent differences in bacterial or fungal community structure and microbial biomass along an urbanization gradient, despite using a variety of well-documented and optimized procedures. Although subtle variances in microbial community profiles were observed within the urbanized landscapes, the microbial communities were largely redundant. Any significant differences observed in soil properties did not reflect large scale differences in microbial composition or biomass, which is likely attributed to the high levels of soil organic matter and carbon resources throughout the locations encompassed in this study. Therefore, because these soils appear to have the capacity to decompose organic matter equally within a similar geographic and climatic region, the impact of urbanization and landscape management practices does not appear to dramatically alter soil microbiology where parent material, soil-forming processes, and soil carbon resources are homogeneous.

**LITERATURE CITED**


Keith N. Turnquist (corresponding author)
Wisconsin Cooperative Fishery Research Unit
College of Natural Resources
University of Wisconsin–Stevens Point
800 Reserve Street
Stevens Point, Wisconsin 54481, U.S.
kturnqu@uwsp.edu

Les P. Werner. Forestry
College of Natural Resources
University of Wisconsin–Stevens Point
800 Reserve Street
Stevens Point, Wisconsin 54481, U.S.

Brian L. Sloss
College of Natural Resources
University of Wisconsin–Stevens Point
800 Reserve Street
Stevens Point, Wisconsin 54481, U.S.

Résumé. Le processus d’urbanisation peut affecter la capacité des micro-organismes à fournir des éléments nutritifs aux plantes. Toutefois, tant la composition et la structure des communautés biologiques des sols, que l’étendue des variations au sein de ces communautés ne sont pas claires en milieu urbain. Par conséquent, l’information de base concernant l’impact des pratiques de gestion des sols en milieu urbain sur les communautés microbienne de ces mêmes sols est essentielle afin d’améliorer la capacité des gestionnaires à gérer les sols urbains et les plantes qui y croissent. Cette étude a analysé les communautés microbien en de cinq terrains urbains aux usages variés et ce, en fonction de différents degrés d’urbanisation dans la région métropolitaine de Milwaukee dans l’état du Wisconsin, États-Unis. Les objectifs étaient de 1) déterminer s’il existe des différences dans la composition des communautés bactériennes et fongiques, dans l’activité biologique et dans les propriétés physico-chimiques du sol dans les cinq différents types d’usage des sols urbains, et 2) déterminer si les différences dans les compositions bactériennes et fongiques se comparaient aux différences dans les propriétés physico-chimiques des sols. Il existe des différences significatives entre les sols longeant les rues et les sols des forêts dans la composition de la densité volumétrique, la matière organique du sol, le pH, le magnésium, le sodium, l’azote total et le ratio C : N. La biomasse microbienne ne différait pas en fonction de l’usage des sols, et les différences dans la composition des communautés bactériennes et fongiques n’affectent qu’une petite partie du bassin microbienn global. La décomposition de la litière foliaire transposée affichait une baisse significative dans le rapport C : N sur une période de temps, mais on n’a observé aucune différence statistique selon les différents usages des sols. Les résultats affichait un assemblage microbien hautement redondant, et suggèrent que dans les endroits présentant des niveaux adéquats de carbone dans le sol, et où la roche mère et les processus de formation du sol sont homogènes, l’urbanisation et l’aménagement paysager de surface ont moins d’impact que prévu sur la microbiologie du sol.


Resumen. El proceso de urbanización puede alterar la capacidad de los microorganismos para suministrar nutrientes a las plantas. Sin embargo, no está claro en las zonas urbanas tanto la composición y estructura de las comunidades biológicas del suelo, como el grado de variación dentro de estas comunidades. Por lo tanto, la información básica sobre el impacto de las prácticas de manejo de zonas urbanas en las comunidades microbianas del suelo es esencial para mejorar la capacidad de los individuos para manejar los suelos urbanos y las plantas que soportan. Este estudio examinó las comunidades microbianas del suelo en más de cinco usos de terrenos urbanos con diferentes grados de urbanización en el área metropolitana de Milwaukee, Wisconsin, Estados Unidos. Los objetivos fueron: 1) determinar si existen diferencias en
la composición de la comunidad bacteriana y fúngica, actividad biológica y el medio ambiente físico y químico del suelo a través de cinco usos del suelo urbano, y 2) determinar si existen diferencias en las composiciones de bacterias y hongos en comparación con las diferencias en las características físicas y químicas del suelo.

La densidad aparente, la materia orgánica del suelo, pH, magnesio, sodio, nitrógeno total, y la relación C: N muestran diferencias significativas entre las calles y los bosques. La biomasa microbiana no difirió entre los usos del suelo, y las diferencias en la composición de la comunidad bacteriana y fúngica reflejan sólo una pequeña porción de la alberca microbiana total. La descomposición de hojarasca transpuesta mostró disminución significativa en la relación C: N en el tiempo, pero no se observaron diferencias estadísticas entre los usos del suelo observados. Los resultados muestran un conjunto microbiano muy redundante, y sugieren que en lugares con niveles adecuados de carbono en el suelo y donde el material parental y procesos de formación del suelo son homogéneos, la gestión del paisaje urbano tiene menos impacto de lo esperado en la microbiología del suelo.