Woody plant-soil relationships in interstitial spaces have implications for future forests within and beyond urban areas

Journal:	*Ecosystems*
Manuscript ID	Draft
Types:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Mejía, Gisselle; Dartmouth College, Environmental Studies; CUNY Graduate Center, Earth and Environmental Sciences
Key Words:	Nitrogen cycling, Carbon cycling, Woody plant community, Urban land-use change, Urban-residential interface, Novel ecosystems

Journal: Ecosystems

Manuscript type: Article

Title: Woody plant-soil relationships in interstitial spaces have implications for future forests within and beyond urban areas

Shortened version: Woody plant-soil relationships in urban areas

Gisselle A. Mejía[1,2]*, Peter M. Groffman[2,3,4], Meghan L. Avolio[5], Anika R. Bratt[6,7], Jeannine Cavender-Bares[8], Noortje H. Grijseels[9], Sharon J. Hall[10], James Heffernan[6], Sarah E. Hobbie[8], Susannah B. Lerman[11], Jennifer L. Morse[12], Desiree L. Narango[13], Christopher Neill[14], Josep Padullés Cubino[15], Tara L.E. Trammell[16]

[1]Department of Environmental Studies, Dartmouth College, 6182 Steele Hall
Hanover, NH 03755, USA, gisselle.a.mejia@dartmouth.edu.
[2]Department of Earth and Environmental Sciences, The Graduate Center of the City University of New York, 365 5th Ave, New York, NY 10016, USA
[3]Advanced Science Research Center at the Graduate Center of the City University of New York, 85 St. Nicholas Terrace, New York, NY 10031, USA, pgroffman@gc.cuny.edu
[4]Cary Institute of Ecosystem Studies, 2801 Sharon Turnpike, Millbrook, NY 12545, USA
[5]Department of Earth and Planetary Sciences, Johns Hopkins University, 227 Olin Hall, Baltimore, MD, USA, meghan.avolio@jhu.edu
[6]Nicholas School of the Environment, Duke University, 9 Circuit Drive, Box 90328, Durham, NC 27708, USA, james.heffernan@duke.edu
[7]Department of Environmental Studies, Macalester College, Olin-Rice 158A, St. Paul, MN, 55105-1899, USA abratt@macalester.edu
[8]Department of Ecology, Evolution & Behavior, University of Minnesota, 1987 Upper Buford Circle, St. Paul, MN 55108, USA, Jeannine Cavender-Bares: cavender@umn.edu; Sarah Hobbie: shobbie@umn.edu
[9]School of Sustainability, Arizona State University, Walton Center for Planetary Health, 777 E University Dr, Tempe, AZ 85281, USA, noortjegrijseels@gmail.com
[10]School of Life Sciences, Arizona State University, P.O. Box 874601, Tempe, AZ 85287–4601, sharonjhall@asu.edu
[11]USDA Forest Service Northern Research Station, 160 Holdsworth Way, Amherst, MA 01003, USA, susannah.b.lerman@usda.gov
[12]Department of Environmental Science and Management, Portland State University, 1825 SW Broadway, Portland, OR 97201, USA, jlmorse@pdx.edu
[13]Vermont Center for Ecostudies, PO Box 420, Norwich, VT 05055, dnarango@vtecostudies.org
[14]Woodwell Climate Research Center, 149 Woods Hole Road Falmouth, MA, 02540-1644, USA, cneill@woodwellclimate.org
[15]Centre for Ecological Research and Applied Forestries (CREAF), Campus de Bellaterra Edifici

C 0819, Cerdanyola del Vallès, Spain, padullesj@gmail.com
[16]Department of Plant and Soil Sciences, University of Delaware, 161 Townsend Hall
Newark, DE 19716, USA, ttram@udel.edu

*Corresponding author
Gisselle A. Mejía
mejia.gisselle@gmail.com

Author contributions

Gisselle A. Mejía and Peter M. Groffman, equally conceived of study methodology, conducted formal analysis, data visualization and writing – original draft.

Meghan L. Avolio, Jeannine Cavender-Bares, Sharon J. Hall, James Heffernan, Sarah E. Hobbie, Susannah B. Lerman, Jennifer L. Morse, Christopher Neill, and Tara L.E. Trammell jointly conceived and supervised the experimental designs, data collection methods, and contributed to funding acquisition and writing – review and editing of the manuscript.

Anika R. Bratt, Noortje Grijseels, and Josep Padullés Cubino contributed to data curation, project administration, investigation and writing – review and editing of the manuscript.

Desiree L. Narango contributed to data curation and writing – review and editing of the manuscript.

Highlights

- There are marked differences in woody plant composition between interstitial areas – at residential-wildland interface – and natural reference areas across cities.
- Differences in woody plant composition were related to variations in soil Nitrogen (N) availability.
- Novel communities in interstitial areas have implications for management of ecosystem services that rely on native biodiversity in forests within and beyond urban areas.

Abstract

Relatively unmanaged interstitial areas at the residential-wildland interface can support the development of novel woody plant communities. Community assembly processes in urban areas involve interactions between spontaneous and cultivated species pools that include native, introduced (exotic/non-native) and invasive species. The potential of these communities to spread under changing climate conditions has implications for the future trajectories of forests within and beyond urban areas. We quantified woody vegetation (including trees and shrubs) in relatively unmanaged "interstitial" areas at the residential-wildland interface, and in exurban reference natural areas in six metropolitan regions across the continental United States. In addition, we analyzed soil N and C cycling processes to ensure that there were no major anthropogenic differences between reference and interstitial sites such as compaction, profile disturbance, or fertilization, and to explore effects of novel plant communities on soil processes. We observed marked differences in woody plant community composition between interstitial and reference sites in most metropolitan regions. These differences appeared to be driven by the expanded species pool in urban areas. There were no obvious anthropogenic effects on soils, enabling us to determine that compositional differences between interstitial and reference areas were associated with variation in soil N availability. Our observations of the formation of novel communities in interstitial spaces in six cities across a very broad range of climates, suggests that our results have relevance for how forests within and beyond urban areas are assessed and managed to provide ecosystem services and resilience that rely on native biodiversity.

Keywords: Nitrogen cycling, carbon cycling, woody plant community, urban land-use change, urban-residential interface, novel ecosystems

Introduction

Urban expansion in the continental U.S. has created large areas of urban, suburban, and exurban land use intermixed with remnant native ecosystems and agricultural land (Pouyat and others 2007). These land-use patterns are similar across different climate regimes and biomes, creating ecological homogenization at regional and continental scales (Groffman and others 2017). For example, plant communities and functional diversity in residential yards converge across the continental United States (U.S.) due to similarities in human preferences and management (Polsky and others 2014; Locke and others 2019; Padullés Cubino and others 2019b). Beyond residential yards, interactions between human decisions and natural processes of community assembly have the potential to affect less populated areas surrounding the dense urban core (Groffman and others 2014). In relatively unmanaged interstitial spaces (i.e., remnant or spontaneously forested areas surrounded by residential development), native and introduced (exotic/non-native) vegetation (introduced to an area outside its native ecosystem and reproduced spontaneously; USDA, NRCS 2023) have the potential to mix and assemble into new or novel communities (Hobbs and others 2009; Andrade and others 2021). These areas are common throughout metropolitan areas but are especially common in large and expanding suburban and exurban land uses at the residential-wildland interface (Brown and others 2005).

In this study, we addressed the question of whether analysis of forests that have spontaneously assembled in in urban interstitial spaces provide insight into how global environmental change will affect the forests of the future. We ask if the complex mix of anthropogenic factors affecting these spaces (altered climate and atmospheric chemistry, altered disturbance regimes, altered species pool) are analogous to factors playing out across the globe at lower intensity (McDonnell and Pickett 1988). If so, the novel communities that assemble in these spaces may provide a glimpse of the forests that may become widespread across the world.

Understanding processes of community assembly in interstitial spaces requires an understanding of the factors sorting for cultivated and spontaneous species pools in urban ecosystems (Knapp and others 2012; Pearse and others 2018; Lopez and others 2018, Blouin and others 2019; Padullés Cubino and others 2019a; Padullés Cubino and others 2019b; Padullés Cubino and others 2020; Cavender-Bares and others 2020). Native vegetation originates from the pool of continental flora interacting with regional climatic drivers, resulting in assemblages adapted to the regional biome. These native species interact with spontaneous (i.e., self-propagated) and cultivated species pools introduced by humans (Pearse and others 2018; Avolio and others 2021). The former results from the natural dispersal of regional flora that adapt to or persist in the urban environment, and the latter include pools introduced from the horticultural industry – primarily determined by human preferences and policies. Cultivated and horticultural species – including both native and introduced (exotic/non-native) species – that escape and establish on their own can also become part of the urban spontaneous pool, dispersing and mixing with regional and continental native flora to create novel communities (Aronson and others 2016; Pearse and others 2018; Blouin and others 2019; Cavender-Bares and others 2020; Avolio and others 2021). The potential for these novel communities to spread under changing climatic conditions has implications (e.g.,for ecosystem services, social benefits, and potential for biodiversity conservation) for the future trajectories of forests within and beyond urban areas (Hobbs and others 2006, 2009; Johnson and Handel 2016).

Novel ecosystems can differ in diversity, composition, age, and structure from native ecosystems, and therefore, potentially exhibit different ecosystem functions (Hobbs and others

2009). In urban areas, novel ecosystems can consist primarily of species that are adapted to or persist under urban-associated stresses (e.g., urban heat, air, soil, light, and noise pollution). Introduced (e.g., exotic/non-native) – including invasive species – are disproportionately represented in urban species pools (Avolio and others 2015; Pregitzer and others 2019). Invasive species tend to thrive in nutrient-rich soils, often escape natural enemies, and are frequently quick to establish in unmanaged lands (Ehrenfeld 2003; Carreiro and Tripler 2005; Heneghan 2004; Johnson and Handel 2016). The presence of these species has implications for the ecosystem functions and services that rely on native biodiversity to support food webs (Narango and others 2018; Tallamy and others 2021), water resources (Richardson and van Wilgen 2004; van Wilgen and others 2008; Le Maitre and others 2000); and soil conservation (Scott and others 1998).

Evaluation of community and ecosystem processes in interstitial areas must consider soil properties. Urban soils are very heterogeneous (Pouyat and others 2007). For example, while many urban soil profiles have been markedly altered by physical, chemical, and biological disturbance such as compaction, atmospheric deposition, and invasion by exotic earthworms (Pouyat and others 2010; Herrmann and others 2020), others are relatively undisturbed (Raciti and others 2011, Trammell and others 2020a; Ryan and others 2022). These alterations, as well as natural variation in the properties of relatively unaltered soil profiles, have marked implications for plant community development and ecosystem function (Frelich and others 2019). Soil properties are thus potentially important drivers of novel plant community structure and function in interstitial areas and must be considered when evaluating the potential of these novel communities to spread within and beyond urban areas. More practically, if soils in interstitial areas have been disturbed by site-specific activities such as tillage, fertilization, compaction, or pollution, the value of these areas as locations for analysis of how forests that have spontaneously assembled in in urban interstitial spaces provide insight into how global environmental change will affect the forests of the future is reduced.

This study evaluated woody plant community composition and soil microbial carbon (C) and nitrogen (N) cycle processes in interstitial (relatively unmanaged) and natural reference ecosystems (representative of regional biomes) in six metropolitan areas across the U.S. (Baltimore, MD; Boston, MA; Los Angeles, CA; Miami, FL; Minneapolis-St. Paul, MN; Phoenix, AZ). We tested whether woody plant community composition in interstitial sites differed from that in natural reference sites and whether soil properties were related to those differences. Woody community composition was measured to identify whether plant assemblages included combinations of introduced (e.g., non-native/exotic) and native species that differed from assemblages in natural reference sites. We measured basic soil properties (moisture, bulk density), soil microbial biomass C and N content, basal respiration, inorganic N pools, potential net N mineralization and nitrification, and denitrification potential and visually inspected soil profiles to ensure that there were no major anthropogenic differences between reference and interstitial sites such as compaction, profile disturbance, or fertilization, and to explore effects of novel plant communities on soil processes. We aimed to answer two questions: 1) How does woody plant community composition differ between interstitial and natural reference sites? 2) Are these differences in vegetation associated with variation in soil C and N cycling processes? We hypothesized that: 1) Woody plant community composition in interstitial sites would differ from that in reference sites, with higher proportion of introduced species and 2) soil N cycling would be altered in interstitial sites, with higher N pools and rates of N cycling processes in the sites with plant communities most distinct from those in reference sites. Results were evaluated in terms of implications for how forests within and beyond urban areas are assessed and managed to provide ecosystem services and resilience that rely on native biodiversity.

Methods

Site selection

We sampled interstitial and reference sites in six major U.S. Metropolitan Statistical Areas (cities): Boston, MA (BOS), Baltimore, MD (BAL), Los Angeles, CA (LAX), Miami, FL (MIA), Minneapolis-St. Paul, MN (MSP) and Phoenix, AZ (PHX) that represent different ecological biomes and/or major climatic regions across the U.S. (Trammell and others 2016). Within each region, between four and six natural areas that represented the dominant ecological biome(s) were selected as reference sites. The natural areas were located in protected areas with native vegetation (including trees, shrubs, and cacti) and were located 1 km or more from other sites. Reference ecosystems include mature (over 75-yr-old) oak and tulip poplar forests (BAL); mature (roughly 100-yr-old) northern oak-dominated hardwood forests (BOS); remnant southern California chaparral (LAX); coastal upland pine rockland, subtropical hardwood hammock, coastal hammock, and pine flatwoods (MIA); oak savanna on sandy outwash, tallgrass prairie and bluff prairie on moraine, and maple-basswood forest on moraine (MSP); and native Sonoran Desert (PHX).

Interstitial sites were sampled (n = 4 to 6) on public lands within each metropolitan area. These sites were located in relatively unmanaged areas (generally absent of intensive human intervention such as plowing, mowing, irrigation, fertilization) with vegetation that had developed spontaneously. In addition, the selection criteria included sites with natural soil profiles similar in texture and landscape position to those in the reference areas, without signs of anthropogenic soil disturbance. In some cities, e.g., Minneapolis St.-Paul this required distributing sites across different soil parent materials. Unmanaged patches that fit these criteria were located within the same region as the refence sites, either on the edge of the city, at the interface with suburban residential land in public parklands or woodlands (for more detailed description, see Padullés Cubino and others 2020; Lerman and others 2021a). Soil taxonomy was identified using USDA Natural Resource Conservation Service (NRCS) maps for each native reference and interstitial site in each city (Table S1).

Woody vegetation sampling

Within each interstitial and native reference site, we established three 8-m radius plots to assess tree (including shrubs, and cacti in Phoenix) density, basal area, height and condition (e.g., live/dead). Plot locations were randomly selected with a Geographic Information System (GIS) mapping tool before field sampling. We sampled all individuals above 1m height with a stem greater than 2.54 cm diameter at breast height (DBH) in each plot. We used the USDA Forest Service i-Tree Eco v6.0 manual (https://www.itreetools.org/) as a reference for recording species in the field. Woody plant stems were split into understory (< 10 cm DBH) and overstory (> 10 cm DBH). Species identifications were cross-checked with World Flora Online (formerly The Plant List), a comprehensive open-access database containing 400,000 recorded plant species contributed by various institutions (http://www.worldfloraonline.org/), the online tools for standardizing taxonomic names Taxonomic Name Resolution Service version 5.0 (Boyle and others 2013; https://tnrs.biendata.org/), and Integrated Taxonomic Information System (ITIS) online database (https://itis.gov/citation.html). Species were also classified according to origin (native or introduced) based on whether the species was considered native or introduced to the state it was sampled in according to the USDA PLANTS database (https://plants.usda.gov).

Introduced species are defined as reproducing spontaneously in the wild without human help and tend to persist, and invasives are defined as (1) non-native (or alien) to the ecosystem under consideration and (2) a species whose introduction causes or is likely to cause economic harm, environmental harm, or harm to human health (USDA, NRCS, 2023). One reference site in Minneapolis-St. Paul (Cedar Creek Ecosystem Science Reserve BU 103) did not have woody plant species present (Table S1).

Soil sampling and laboratory analysis

Two soil cores up to 30 cm depth were collected at random locations along transects at each site using a 3.3 cm diameter soil corer, enclosed in plastic sleeves with end caps, put into coolers, and shipped on ice to the Cary Institute of Ecosystem Studies, Millbrook, NY, USA, where they were stored at 4° C (up to 21 days) until they could be processed. In the laboratory, analysis followed procedures described by Raciti and others (2011) and Ryan and others (2022). Soil cores were first visually inspected for evidence of obvious anthropogenic alteration of the soil profile and then divided into 0-10 cm and 10-30 cm sections. Coarse roots and rocks (>2 mm) were removed by hand. The separated roots and rocks were dried at 105° C, and rock volumes were estimated using an assumed density of 2.7 g/cm^3. Water content was measured via gravimetric analysis, where soil samples were dried for 48 hours at 105° C. Dried samples were used to calculate bulk density (BD) as (total dry mass - rock mass) / (total volume - rock volume). Soil organic matter content was measured by loss on ignition at 450^o C. Cores were not available for one reference site in Los Angeles (Zuma Canyon), and one reference site in Miami (Pine Ridge Sanctuary; Table S1).

Soil exchangeable nitrate (NO_3^-) and ammonium (NH_4^+) were extracted in 2 M KCl and analyzed colorimetrically using a Lachat Flow Injection Analyzer. Potential net N mineralization and nitrification, and basal respiration were measured in a 10-day laboratory incubation of soils at room temperature and field moisture. Soils were placed in glass jars fitted with rubber septa to allow for sampling of headspace gas. After incubation, the headspace of the jars was sampled and analyzed for carbon dioxide (CO_2) by gas chromatography with a thermal conductivity detector, and soils were extracted for NO_3^- and NH_4^+. Potential net N mineralization was calculated as the total accumulation of inorganic N, nitrification was calculated as the accumulation of NO_3^-, and respiration was calculated as the accumulation of the CO_2 during the incubation (Robertson and others 1999).

Microbial biomass C and N content were measured using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976). Soil samples were fumigated with chloroform for up to 24 hrs to lyse microbial cells, inoculated with 0.1 g fresh soil, and incubated for 10 days in mason jars with fitted rubber septa. Microbial biomass C was calculated from the production of CO_2 in the fumigated samples using a proportionality constant (0.41). Microbial biomass N was not corrected with a proportionality constant, and values are just the inorganic produced over the 10-day incubation of fumigated samples.

Rates of potential denitrification were measured using the denitrification enzyme assay (Smith and Tiedje 1979; Groffman and others 1999). Soil subsamples were amended with NO_3^-, glucose, chloramphenicol, and acetylene, and incubated anaerobically for 90 minutes. Gas samples were removed after 30 and 90 minutes and analyzed for nitrous oxide (N_2O) by gas chromatography with an electron capture detector.

Data analysis

All analyses were performed in R (Version 3.3.3; R Core Team 2019). To prepare the species data for site-, and city-level comparison, we first characterized woody plant structure in each plot in terms of species' relative abundance, frequency, dominance, and importance, and in terms of species diversity (richness, evenness) to examine biodiversity patterns. Relative abundance (RA) for each woody plant species, i.e., the proportion of individuals of a particular species to the total number of individuals in a plot, was determined per plot. At each site, frequency was recorded as the percentage of plots in which a species was found, and relative frequency (RF) was calculated as the proportion of total frequency of all species to the total frequency per plot. Relative dominance (RD) was calculated as the proportion of basal area per species to the total basal area per plot. Basal area was calculated as (π x $(DBH/2)^2$) and values were converted from cm^2 to m^2. The importance value index (IVI), which presents the ecological importance and dominance of a species, was calculated as the sum of RA, RF and RD (Curtis and McIntosh 1951). Woody plant species diversity (richness and evenness) was calculated using the *codyn* package in R (Hallett and others 2016) for each site. Species richness was calculated as the overall number of species, and community evenness was calculated as the inverse of Simpson's D. The relative proportion of introduced species was calculated as the percent abundance for canopy (DBH $>$ 10 cm) and sapling (DBH $<$ 10 cm) layers. All plot-level values were averaged for each interstitial and reference site.

To evaluate differences in species composition (i.e., the identity of species present in a community) among interstitial and reference areas, we used non-metric multidimensional scaling (NMDS) from the *vegan* package (Oksanen and others 2020). NMDS is an unconstrained method that uses the pairwise dissimilarity of species composition and reduces dimensional space to better assess compositional differences between sites (Legendre and Legendre 2012).

We used Bray-Curtis dissimilarity, which is a semi-metric index of distance between species vectors and quantifies the compositional dissimilarity between sites based on species abundance data (Legendre and Legendre 2012). Species contributions to vegetation patterns were determined using Pearson correlation coefficients between species abundance and NMDS dimensions with the ‘scores’ function included in the *vegan* package in R (Brown 2019; Oksanen and others 2020). The ‘betadisper’ function was used to calculate the homogeneity of group variances (distance between centroids and group means for interstitial and reference sites). Finally, the ‘adonis’ function– a permutational analysis of variance – was applied using 999 permutations to test compositional differences (differences in centroid locations) between reference and interstitial sites.

To characterize and compare soil characteristics between interstitial and reference sites, we averaged whole-core (0-30 cm) estimates of microbial biomass C and N, basal respiration, NO_3^- NH_4^+, potential net N mineralization and nitrification, denitrification potential, soil organic matter content, and bulk density per site. We used bulk density values to convert all soil parameters to an areal basis (g/m^2). For each soil parameter, departures from normality were determined using Levene’s test, and to determine variances within land-use type in each city. We compared whole-core differences between interstitial and reference sites between sites using the non-parametric Wilcoxon rank sum test for each soil parameter.

To determine multivariate patterns in soil parameters across interstitial and reference sites in each city, we conducted principal component analysis (PCA) using the ‘prcomp’ function in R. The data was standardized (divided by their standard deviation) prior to performing the PCA analysis. Loadings were extracted for all soil parameters in relation to each principal component to explain soil patterns in ordination space. The soil parameters used in the analyses were coded as microbial biomass C (BiomassC), microbial biomass N (BiomassN), basal respiration (Respiration), NO_3^- (NO3), NH_4^+ (NH4), total inorganic nitrogen (TIN), potential net N mineralization (Mineralization), potential net nitrification (Nitrification), denitrification potential (DEA), organic matter content (OM), and bulk density (BD).

To investigate relationships between woody plant species composition and soil parameters, we also ran a canonical correspondence analysis (CCA), a constrained ordination method to analyze associations between environmental variables and community composition data, for each city. We computed the variance inflation factor (VIF) using the 'vif.cca' function to check for redundancy amongst predictor variables (soil parameters). Conventionally, VIF > 10 indicates high levels of redundancy among the predictor variables that affect model fit (Zuur and others 2009). The variables which contributed to VIF > 10 were removed and the model was run again. Analysis of variance (ANOVA) was conducted to test significance effects of individual predictors (soil parameters), and of the full model using 999 permutations.

Results

Question#1: Does woody plant community composition differ between reference and interstitial areas?

Across cities, there was no consistent difference in mean woody plant species richness between reference and interstitial sites (Table 1). Baltimore had the highest woody plant species richness in the interstitial sites, in the sapling layer (9.4 ± 2.54). Boston had the highest woody species richness in the reference sites, also in the sapling layer (6.6 ± 1.12). Woody plant communities were most even (measured on a scale from 0 to 1, where 1 is the most even) in interstitial sites in Boston and Baltimore and in reference sites in Miami, Minneapolis-St. Paul, Los Angeles, and Phoenix (Table 1). Evenness was higher in the sapling layer of interstitial sites for most cities, except for Baltimore (0.88 vs. 0.56), but was higher in reference site canopy layers for most cities (Table 1).

Overall, there was a higher proportion of introduced species in interstitial sites than in natural reference sites, except in Boston (30% vs. 50%, respectively; Table 1). Overall, the sapling layer (DBH < 10 cm) contained a greater proportion of introduced species than the canopy layer (DBH > 10 cm), except in Minneapolis-St. Paul and Miami reference sites (7.14% and 9.60%, respectively). Introduced species were not found in any sites in Los Angeles and Phoenix (Table 1).

Across cities, species abundance and dominance differed between interstitial and reference sites, but some species were present in both site types in most cities (average of 37%; Table 2; Fig. S1; Fig. S2). The exceptions were Los Angeles and Phoenix, which had fewer total species (Table 1), and less compositional overlap (in Phoenix) between interstitial and reference sites (Table 2; Figs. S1; Figs. S2). In the majority of cities, native species were the most abundant and dominant in both interstitial and reference sites, with the exception of Miami and Minneapolis-St. Paul, where introduced species were more dominant in interstitial sites (Table 2; Figs. S1). Several native species were common in temperate cities, e.g., *Fraxinus pennsylvanica, Quercus velutina*, and *Quercus alba* (Table 2; Fig. S1; Fig. S2).

Non-metric multidimensional scaling (NMDS) showed differences in woody plant community composition between reference and interstitial sites in most cities, as shown by the lack or minimal overlap of group centroids amongst interstitial and reference sites (Fig. 1). However, there were strong, but not statistically significant compositional differences between interstitial and reference sites in Los Angeles ($r^2 = 0.47$, $p = 0.10$, respectively; Table S2). Moreover, dispersion from group centroids (homogeneity among group variances) amongst interstitial and reference sites were significant for Los Angeles and Phoenix ($F_{1,3} = 35.66$, $p = 0.008$ and $F_{1,6} = 8.20$, $p = 0.03$, respectively; Table S2). Native species were most important in determining variation in composition along both dimensions in the NMDS analysis (Table S3), except for Baltimore, Boston and Minneapolis-St. Paul (Figs. 1b, 1f). In Baltimore, the introduced species *Prunus avium* was significant ($p = 0.013$) at explaining variation in species composition in sites along MDS2. In Boston, species that were important at explaining variations in species composition in sites along MDS1 included *Acer ginnala* ($p = 0.011$), *Ailanthus altissima* ($p = 0.011$), *Lonicera tatarica* ($p = 0.011$), *Malus floribunda* ($p = 0.034$), and *Rhamnus cathartica* ($p = 0.011$), while the cultivated hybrid *Tilia x europaea* was significant ($p = 0.01$) at explaining variation in community composition in sites along MDS2 (Fig. 1b; Table S3). In Minneapolis-St. Paul, the introduced species *Rhamnus cathartica* and *Ulmus pumila* were significant ($p = 0.03$ and $p = 0.003$, respectively) at explaining variation in community composition in sites along MDS1 and MDS2, respectively (Fig. 1f; Table S3).

Question #2: Is woody plant community composition in reference and interstitial areas related to variation in soil C and N cycling processes?

Across all cities (Fig. 2), N cycling variables (inorganic N pools, microbial biomass N, potential net N mineralization and nitrification) and Carbon cycling variables (organic matter content, microbial biomass C, respiration) did not differ between reference and interstitial sites (Fig. 2). On a city-by-city basis, microbial biomass C was significantly higher in reference sites in Boston ($p < 0.05$; Fig. S4). There was marked variation in C and N cycle variables among sites in each city (Figs. S3-S8). There was no visual or taxonomic evidence of extensive human alteration of interstitial site soil profiles (Table S1), and there were no consistent differences in soil moisture or bulk density between interstitial and reference sites (data not presented).

Across cities, at least 61% of the total variance in soil parameters was explained by the first two components (Dim1 and Dim2) in a principal components analysis (Fig. 3; Table S4). On average, the first principal component (Dim1) explained 44.6% and the second principal component (Dim2) explained 26.3%. of the variation in interstitial and reference soils. Nitrogen cycle variables were the most strongly loaded on the two principal component axes, especially Dim1. NO_3^- and NH_4^+ strongly loaded on Dim1 and Dim2 in every city, except for Miami (Fig. 3; Table S4). Potential net nitrification and denitrification potential were strongly loaded on Dim1 or Dim2 in every city except for Los Angeles (Fig. 3; Table S4). Potential net nitrification, microbial biomass N and total inorganic N had strong loadings with both Dim1 and Dim2 in every city. Carbon cycle variables were rarely significantly loaded on either Dim1 or Dim2 although organic matter and bulk density were strongly loaded on Dim2 in Miami (Fig. 3; Table S4). Centroids of reference and interstitial sites did not overlap, except in Los Angeles and Miami. Variation among sites was noticeable in many cities, with some interstitial and reference sites having strong association with particular soil variables, while in other cities, site variation was not strongly associated with soil variables (Fig. 3).

Across cities, in a canonical correlation analysis (CCA) of woody plant community and soil variables, the proportion of variance explained by the first CCA axis was at least 12%, and the second axis explained at least 10% of the variation across both interstitial and reference sites (Fig. 4; Table S5). Variance explained was higher (> 30%) in the driest cities, Los Angeles and Phoenix, that had many fewer species present (Fig. 4; Table S5). The proportion of variance in woody plant community composition explained by soil variables was at least 62%, except in

Phoenix (22%; Fig. 4; Table S5). While the number of soil variables that influenced community composition varied per city, some variables were consistent across cities (Fig. 4; Table S5). For example, soil NO_3^-, $NH_4^{+,}$ and organic matter contents were related to woody plant community composition in both interstitial and reference sites in all cities except Phoenix (Fig. 4; Table S5). NO_3^- and community composition were significant in Boston ($p = 0.033$) and Miami ($p = 0.004$), while organic matter content was statistically significant in Baltimore ($p = 0.019$), and Miami ($p = 0.005$, Fig. 4; Table S5). Furthermore, the CCA models were only statistically significant in Baltimore ($p = 0.044$) and Miami ($p = 0.021$), and marginally significant in Boston ($p = 0.064$; Fig. 4; Table S5).

Discussion

In this study, we addressed the question if analysis of forests that have spontaneously assembled in in urban interstitial spaces provide insight into how global environmental change will affect the forests of the future. We hypothesized that the complex mix of anthropogenic factors affecting these spaces (altered climate atmospheric chemistry, disturbance regimes, and species pool) are analogous to factors playing out across the globe at lower intensity (McDonnell and Pickett 1988) and that the novel communities that assemble in these spaces provide a glimpse of the forests that may become widespread beyond urban areas. Our observations of marked differences in woody vegetation composition between interstitial and reference sites in six cities with very different climate across the U.S. support this idea and provide insight into the novel communities that may become common across the U.S. over the next 50 – 100 years. In the sections below, we first discuss these differences in plant communities and then discuss if local human alteration of soils has reduced the value of our sites as analogs for future environmental conditions. Finally, we discuss the effects of altered plant communities on soil processes and ecosystem services.

The differences in woody plant communities was most clearly shown by NMDS for Baltimore, Boston, Los Angeles, and Phoenix, where interstitial and reference sites separated along NMDS axes (Fig. 1; Table S3). Even in cities that did not show clear differences along the NMDS axes (e.g., Minneapolis-St. Paul), there was evidence for clustering among the interstitial sites indicating that interstitial sites were more similar to each other than reference sites. As we discuss below, the differences between reference and interstitial sites were likely the result of a greater proportion of introduced species and higher species richness in interstitial sites, especially in the sapling layers (Table 1).

Our careful selection of sites allowed us to assess how changes in plant community composition affect soil N cycling, which is important for a variety of ecosystem services (e.g., primary productivity). In our study sites, there were no noticeable anthropogenic effects on soils, e.g., compaction, profile disturbance, or fertilization. Therefore, we were able to examine how differences in plant community composition between interstitial and reference sites were associated with variation in N availability. This finding is shown by separation of interstitial and reference sites along PCA axes of soil characteristics in Baltimore, Boston, Los Angeles, Miami, and Minneapolis-St. Paul in the PCA, and by relationships between N pools (NO_3^-, NH_4^+, TIN, and microbial biomass N) and woody vegetation composition in the CCA. It is important to note that there were no systematic differences in N availability between interstitial and reference sites, and no evidence that interstitial sites had artificially elevated N availability based on soil taxonomy (Table S1). Closely matching the soil series allowed us to avoid differences in soil moisture retention and having the sites interspersed across the region avoid local pollution (e.g., atmospheric N deposition) gradients. Thus, careful selection of sites allows for examination of how natural variation in N availability across native reference and interstitial sites is a driver of woody plant community composition (Table S3).

As detailed below, the differences that we observed between interstitial and reference sites have implications for how forests are assessed and managed to provide ecosystem services related to native species diversity, carbon storage, and ecosystem resilience to global environmental change.

How does composition of the woody plant community differ between interstitial and natural reference sites?

The observed differences in woody plant community composition between native reference and interstitial sites are likely the result of the expanded species pool – native and introduced – in urban areas. While there might be multiple confounding factors (e.g., land-use history, plant physiology, abiotic effects), woody plant communities in the interstitial sites have developed adjacent to altered landscapes, such as residential areas with human-managed landscapes (yards and neighborhoods, transportation corridors, etc.). These areas are planted and managed with highly selected species (Padullés Cubino and others 2019b; Padullés Cubino and others 2020) that have the potential to establish in unmanaged areas such as our interstitial sites, and disperse beyond managed areas, such as our reference sites.

Woody plant canopies in interstitial sites across the six cities were dominated by native species, while sapling layers had greater percentages of introduced species (Table 2). While this could be a function of ecological time lags (e.g., phase in biological invasion; Blackburn et al. 2011), it could also be the result of invasive species cultivated in highly managed urban areas that have successfully dispersed to relatively unmanaged areas (e.g., Buckthorn; Heneghan 2004). However, the majority of saplings in the interstitial sites were still native (Table 1, Fig. S1). Despite native woody plant canopies, previous studies suggest that the presence of introduced species in the understory layer indicate that interstitial sites are likely to develop different trajectories than the reference natural areas over time (Kowarik et al. 2019; Trammell and Carreiro 2011; Trammell and others 2020b), especially if native progeny are outcompeted by introduced (exotic/invasive) species. Previous studies comparing urban forests (e.g., large parks) with natural reference areas have found that the presence of introduced species was greater in forests within the urban matrix, particularly in the understory layer (Templeton and others 2019). While different factors would contribute to the presence and persistence of introduced species in different cities (e.g. forest age, land-use type and mechanism of introduction; Schoenenberger and Conedera 2013; Trammell and others 2020b; Jiang and others 2022), interstitial sites – which are embedded within the urban matrix – are closer in proximity to residential areas than reference sites. Padullés Cubino and others (2020) found that residential yards had higher proportions of introduced species compared to reference natural areas in the cities we studied. It is possible that dispersal through residential yards may facilitate the spread of introduced species to interstitial sites (Vieira and others 2014). Moreover, invasive species are widely available through the horticultural industry (e.g., *Rhamnus cathartica*; Nóvoa and others 2015; Beaury and others 2021). In this study, only woody plant species were included, and the presence of introduced species in other herbaceous layers may provide additional support for this idea (Trammell and Carreiro 2011; Trammell and others 2020; Deljouei and others 2017; Fratarcangeli and others 2022).

In forest ecosystems, introduced woody species that are also invasive are especially concerning due to their potential to threaten recruitment of native species (e.g. shading out) and out-competing native understory over time (Reinhart and others 2006; Doroski and others 2018). For example, Templeton and others 2019 showed that urban forests tended to have greater abundance of invasive plants in both the canopy and understory layers. Other studies have found canopies dominated by native species and understories dominated by exotics (Trammell and Carreiro 2011; Pregitzer and others 2019; Trammell and others 2020), supporting the idea that exotics will become more important over time.

Among the invasive species that we observed, *Acer platanoides* and *Rhamnus cathartica* are of particular interest because they were both found in multiple cities, which suggests they have a wide-ranging distribution. *Rhamnus cathartica* has the ability to establish and persist in disturbed areas and proliferate in sites undergoing succession (Zouhar 2015), while *Acer platanoides'* strong shade-tolerance allows it to dominate in closed-canopies and suppress native understory seedlings (Munger 2003).

Amongst the six cities, introduced species were most common in Boston (Table 1) and it was the only city with a greater percentage of introduced species in reference sites than interstitial sites (Table 1). *Tilia x europaea* was both abundant and dominant in the canopy of one reference site, and it was also present in the sapling layer, along with *Rhamnus cathartica* and *Ailanthus altissima* (Table 1; Fig. S2). Previous studies of temperate forests in Massachusetts have found that heavily fragmented forest patches are more susceptible to invasion of introduced species (McDonald and others 2008). Temperate forests of New England have experienced increased fragmentation over recent decades resulting from increasing human population expansion, even at low densities (Vogelmann 1995). Therefore, not just proximity to the urban matrix, but also forest size, age and structure may play an important role in the susceptibility of forested ecosystems to plant species invasion (e.g., Trammell and others 2020b). The "edges" of these fragmented forests are highly dynamic, with notably high rates of C fixation and growth, that vary with species composition (Reinmann and Hutyra 2017; Morreale and others 2021). There is thus great interest in how woody plant species composition is changing in these increasingly common fragmented landscapes.

In Minneapolis-St. Paul, the relative openness of forest stands in a prairie landscape may facilitate the spread of invasive species. One of the reference sites in this city had high abundance of *Rhamnus cathartica,* but only in the sapling layer (Table 1; Fig. S2). This species is known to occur not only in disturbed areas but can also be found in open wildlands (Zouhar 2015). Given the openness of savanna landscapes, reference sites in the Minneapolis-St. Paul region may be especially susceptible to this species, which is readily dispersed by birds and tends to out-compete native understory plants by creating dark, dense thickets (Mascaro and Schnitzer 2007; Knight and others 2017). *Rhamnus cathartica* also has the advantage of "extending" the growing season through early leaf-out and delayed senescence, probably contributing to its success in the understory (Zouhar 2015). Interestingly, this reference site was the only one without any *Quercus* species (a shade-intolerant genus) and was the only one with *Ulmus pulima* in the canopy layer. This species is fast-growing, which allows it to develop rapidly once established, becoming highly invasive in prairie ecosystems (Gaskin and others 2020). The presence of this invasive species in the canopy layer may facilitate invasion by *Rhamnus cathartica* in the understory, which thrives in the shade of other trees. This species has also been found spontaneously growing in residential yards in Boston and Minneapolis-St. Paul

(Cubino and others 2019a), indicating its ability to spread and establish without human intervention.

Despite the abundance of introduced and invasive species in interstitial and some reference sites, native species dominated most sites across cities (Table 2; Fig. S1; Fig. S2). Still, there were important differences in native species dominance between interstitial and native sites. For example, reference areas in Baltimore were dominated by *Quercus* species (e.g., *Quercus michauxii*, *Quercus alba*, *Quercus velutina*) and co-dominated by *Liriodendron tulipifera* (Table 2; Fig. S2). However, *Quercus michauxii* was not present in any of the interstitial sites, and *Fraxinus Pennsylvanica* was co-dominant in two interstitial sites, but not present in any reference sites. *Quercus michauxii* is shade-intolerant and requires openings for establishment allowing it to survive in the understory, while *Fraxinus Pennsylvanica* is tolerant to shade and adaptable in many landscapes (Gucker 2005), including urban environments, where it is commonly planted as part of municipal planting campaigns (Doroski and others 2020) and in residential yards (Wheeler and others 2017). This indicates that there might be different ecological sorting processes occurring in some interstitial sites that allow for different species than those found in reference sites to establish and dominate stands. For example, Schurman and others (2012) found that environmental constraints, such as N availability and soil moisture regime influenced the species distribution of younger trees in temperate forests, but stand age was more likely to predict mature tree distribution. In addition, the loss of late successional species impacts the structure and functions of forested ecosystems such as microclimate, biomass, and chemical processes (Ellison and others 2005; Thompson and others 2013).

Comparison of interstitial and reference sites in our most arid cities (Phoenix and Los Angeles) produced an interesting contrast to mesic cities. Woody plant species richness was very low in these cities, with most sites in Phoenix dominated by a single species (Table 1; Fig. S1; Fig. S2). Additionally, all the species in both the reference and interstitial sites within these cities were native and dominated by the same species. For example, in Los Angeles, *Malosma laurina*, which was highly abundant in interstitial sites and one reference site in Los Angeles, is a successful colonizer following disturbance (e.g., fire; Howard 1992), making it a resilient species. These results suggest that forests developing on interstitial sites in Los Angeles may have similar trajectories to current native reference sites.

Is variation in woody plant community composition related to variation in soil C and N cycle processes?

A major objective of our study was to determine if community assembly processes in urban interstitial areas are leading to the development of novel ecosystems that have the potential to spread within and beyond urban areas. We found little evidence for unique urban effects on soils in the interstitial sites in this study. However, evaluation of plant-soil interactions in urban ecosystems is complicated by extensive alteration of soils by human activities. This alteration can limit the use of urban ecosystems as analogs of global environment change (McDonnell and Pickett 1990). For example, if soil profiles at a site are altered by the presence of human-altered or human-transported materials such as coal ash or municipal trash (Mejía and others 2022), they cannot be used to learn about the effects of interacting factors such as changes in climate, atmospheric chemistry, and local species pool. In this study, interstitial sites were selected to avoid areas with extensive alteration. Consistent with this effort, we did not see significant differences between interstitial and reference sites for any N cycling variable across cities, suggesting that these sites were suitable for studying the effects of altered urban climate, atmospheric chemistry, and species pools on community assembly (McDonnell and Pickett

1990). However, we did observe significant variation in soil processes within individual cities that sheds light on the causes of variation in N cycle processes such as the presence of introduced species, and the influence of these processes on woody plant dynamics (Fig. 3; Figs. S3-S8). In drier regions, these variations may be affected by local N cycling patterns (e.g., deposition), such as those observed in California (Fenn and others 1996, Fenn and others 2010).

Although we did not see significant differences between interstitial and reference sites for any N cycling variable across cities, city-by-city analysis of relationships between N dynamics and tree community composition supports the idea that there are relationships between these variables that cut across interstitial and reference sites. For example, in Baltimore, two interstitial sites located farther outside the confidence interval in the vegetation NMDS were the same sites outside the confidence intervals in the soil PCA. Similarly, in Boston, two reference sites clustered in the vegetation NMDS were also clustered in the soil PCA. In Los Angeles, one interstitial site was distinct on both vegetation (NMDS) and soil (PCA) analyses. In addition, these patterns were supported by the CCA that illuminated multiple soil variables (basal respiration, NO_3^-, total inorganic N, microbial biomass N, potential nitrification and N mineralization, denitrification potential, organic matter, bulk density) that had significant relationships with community composition across interstitial and reference sites. However, these dynamics varied by city, indicating that the mechanisms driving the variation in N dynamics, and the relationships with woody plant community composition are context-dependent. Factors such as soil texture, pH, and water holding capacity influence N availability in soils, and have been well studied in rural context (Pastor and others 1984; Pastor and Post 1986), but less so within urban areas (Groffman and others 2006). The spread of invasive species can also affect nutrient cycling (Ehrenfeld 2003; Mueller and others 2018). There is a clear need for further analysis of these uncertainties as the N cycle is affected by multiple components of environmental change that will need to be considered when evaluating future trajectories of urban (and beyond) forests (Mason and others 2022).

While the PCA analysis showed significant and coherent variation in N cycling among sites, variation in soil C cycle processes was less marked, i.e., there were few significant correlations between soil C cycle processes and the two PCA axes from soil characteristics. Moreover, we did not observe significant differences between interstitial and reference sites for any C cycling variables across cities. The lack of difference in C dynamics between interstitial and reference sites was surprising given their differing proximity to human-dominated landscapes and the effects of this proximity on woody plant communities and N cycling. In both site types, organic matter quality appears to be high enough to support high levels of microbial biomass and respiration, which are indices of the nature and extent of soil C cycling activity (Powlson and others 2017). These C dynamics are controlled to a large extent by abiotic factors, such as temperature and moisture, that vary geographically. Cities are also subject to biological factors that affect soil C dynamics, such as the presence of earthworms (Pouyat and others 2002). Our results support the idea that there is high variation in organic matter content within forests embedded in the urban matrix (Zhu and Carreiro 2004). Unraveling the controls of this variation will be important for understanding the composition and C sequestration capacity of future forests.

What are the implications of differences in woody plant community composition in the interstitial sites for the structure and function of future forests within and beyond urban areas?

Our results suggest that novel woody plant communities are assembling in interstitial areas in cities across the U.S. These communities may affect the ecosystem services provided by urban forests (e.g., wildlife habitat, water quality, climate regulation; Solomou and others 2019; Berglihn and Gómez-Beggethun 2021), and the resilience of these forests to environmental change. To the extent that environmental changes currently occurring in cities are harbingers of changes that will occur more broadly across the landscape (McDonnell and Pickett 1990), these communities may spread beyond urban areas and influence forest structure and function across large areas of North America. Johnson and Handel (2016) found that successional trajectories of urban forests have diverged between forests that underwent restoration (e.g., invasive species removal) and invaded forests that were not restored, indicating that novel communities that emerge in unmanaged areas can become dominant over time. Species invasions create temporal and ecological processes that differ from native communities (Blackburn and others 2011), which suggests that effective management of invasive species is critical to prevent and mitigate their spread within and beyond urban areas (Simberloff and others 2010; Dickie and others 2014; Krumm and Vítková 2016; Brundu and others 2020).

Our results also suggest that the assembly of novel communities in urban interstitial areas varies markedly in cities with different climates. For example, only native species (albeit very few) were found in interstitial sites in hot and dry climates of Los Angeles and Phoenix. In hot and wet Miami, a much larger pool of both native and introduced species were present. Pearse and others (2018) showed that species from both native and exotic pools were present in both cultivated and spontaneous communities in residential yards in the cities we studied, highlighting the dynamic interactions between direct and indirect human activities and a variety of natural biodiversity controls consistent with global trends in urban environments (Gaertner and others 2017).

The process of novel community assembly will also vary with land-use legacies and forest patch size which have a direct effect on a site’s susceptibility to species invasions and soil nutrients (Hall and others 2013; Ziter and Turner 2018; Ward and others 2020). Forests growing on land that was under previous land-use (e.g., agriculture or timber) have lower abundance of native woodland species when compared with reference forests (Peña and others 2016). These differences between land-use also point to the importance of soil conditions (e.g., nutrient content; Baeten and others 2010) as a regulator of community assembly. Young forest patches are more likely to have invasive plants and increased pH and Ca than older patches, regardless of land-use context (Trammell and others, 2020b, 2021). As noted above, forest fragmentation creates edge effects that expose tree communities to disturbances that may affect stand microclimate and species physiology (Reinmann and Hutyra 2017; Morreale and others 2021). Trammell and others (2022) showed that edge effects and other environmental factors had greater influence on species invasion than land-use context in forest patches. Garvey and others (2021) found that edge effects had a strong effect on soil respiration in both urban and rural forests. The interaction of these factors will have a great effect on future forest structure and function within and beyond urban areas. These effects are being experienced in forests around the globle (Essl and others 2020), especially from the spread of woody invasive species (Jäger and others 2007; Ayanu and others 2015; Le Maitre and others 2020).

Forests embedded in the urban matrix are exposed to stressors from the surrounding matrix that can produce effects that may be different from those in less disturbed landscapes (Trammell and others 2022). These effects can include altered soil chemistry, elevated temperatures, and light regimes, altered foliage (e.g., caterpillars) and soil fauna (e.g., earthworms), pollination and seed dispersal agents, and changes in the nature and frequency of disturbance (Pouyat and others 2007, 2010; Lerman and others 2021b). Further research in forests along urban to rural gradients is needed to assess these effects and their relevance to forests beyond urban areas.

Conclusions

Our results show that urban land-use change has resulted in alterations to natural ecosystems embedded in the urban matrix, creating conditions for novel woody plant assemblages to emerge with potentially altered functions that persist in the absence of human intervention. These results have implications for how we assess and manage the urban forests across the U.S. As novel communities emerge, there will be a need for assessments of how they function relative to demands for specific ecosystem services, and decisions about whether management actions can affect these outcomes will need to be made.

For example, urban forest restoration efforts in many cities focus on invasive species removal (Pregitzer and others 2019). Invasive species can change soil conditions and/or adapt to altered soils, but whether soil conditions drive community dynamics or vice versa is still poorly understood (Ward and others 2020). Our results support the idea that these community dynamics are associated with variation in local soil conditions, especially N availability (Smith and others

2020; Smith and others 2021, Mejía and others 2022). There is a clear need for further research on how soil conditions interact with changing climate, herbivory pressure (e.g., deer), and other environmental changes (Templeton and others 2019) to influence the trajectories, functions, and services of forest communities.

Our results suggest that urban interstitial communities are a useful experimental venue for investigating the structure and function of future forests. These communities are highly variable and dynamic and illustrate a wide range of possible future forest trajectories that have the potential to spread beyond urban areas under changing environmental conditions. As human activities expand at the residential-wildland interface, unmanaged interstitial areas are likely to function as mediators of native, exotic, cultivated, and spontaneous species pools (Pearse and others 2018), potentially altering natural habitats on regional scales and influencing the structure and function of future forests well beyond urban areas.

Acknowledgements

This research was supported by the Macrosystems Biology program of the Biological Sciences Directorate at the National Science Foundation (NSF) through collaborative awards: DEB-1638648 (Baltimore), DEB-1638606 (Los Angeles), DEB-1638560 (Boston), DEB-1638725 (Phoenix), DEB-1638657 (Miami), DEB-1638519 (Minneapolis/St. Paul), DEB-1638690, DEB-

1836034, and DEB-1638676. The authors are appreciative of all the field coordinators and assistants that helped collect the data that made this project possible. In Boston, the authors thank the Massachusetts Department of Conservation and Recreation and Mass Audubon for providing permission to work in natural and interstitial sites. In Minneapolis-St. Paul, the authors thank Christopher Buyarski for helping to coordinate the field campaign and data management, and the Minnesota Department of Natural Resources, the Nature Conservancy, Three Rivers Park District, the cities of Brooklyn Park, Eden Prairie, Arden Hills, and Ramsey County Parks and Recreation for providing permission to work in natural and interstitial areas. In Baltimore, the authors thank Laura Templeton for coordinating the field campaign and data management, Ben Glass-Seigel, Dan Dillon, Juan Botero, Katherine Ralston, and Alyssa Wellman Houde for fieldwork and Baltimore City, Baltimore County, and the State of Maryland for providing permission to work in interstitial and natural sites. In Miami, the authors thank Martha Zapata, Sarah Nelson, Sebastian Ruiz for fieldwork and Miami-Dade County Parks, Florida State Parks and Pine Ridge Sanctuary for providing permission to work in natural and interstitial areas. In Los Angeles, the authors thank UCLA/La Kretz Center for California Conservation Science, National Park Service, Los Angeles City Department of Recreation and Parks, the Audubon Center, Mountains Recreation and Conservation Authority, Palos Verdes Peninsula Conservancy for providing permission to work in natural and interstitial sites. In Phoenix, the authors thank Laura Steger for coordinating the field campaign and data management, Alicia Flores, John Talarico, and Brittany Strobel for fieldwork and Maricopa County Parks and Recreation Department, City of Scottsdale, and City of Phoenix for providing permission to work in natural and interstitial sites. The authors are also thankful to the subject-matter editor, and anonymous reviewers for their thorough review, and constructive, thoughtful comments that greatly improved the manuscript.

Data availability

Data are available as Supporting Information. Data are also available in Environmental Data Initiative (EDI) Data Portal at https://doi.org/10.6073/pasta/8b29dc7fd536f4649f8cf6a536421fc9 (DOI) reference number edi.309.1, and https://doi.org/10.6073/pasta/c1f9302b4ccef46cbe003ac41f58ef4a (DOI) reference number edi.374.2.

Literature cited

Ayanu YZ, Jentsch A, Müller-Mahn D, Rettberg S, Romankiewicz C, Koellner T. 2014. Ecosystem engineer unleashed: Prosopis juliflora threatening ecosystem services? Regional Environmental Change 15:155–167.

Andrade R, Franklin J, Larson KL, Swan CM, Lerman, SB, Bateman HL, Warren PS, York A. 2021. Predicting the assembly of novel communities in urban ecosystems. Landscape Ecology 36:1–15.

Avolio ML, Pataki DE, Gillespie TW, Jenerette GD, McCarthy HR, Pincetl S, Clarke LW. 2015. Tree diversity in southern California's urban forest: the interacting roles of social and environmental variables. Frontiers in Ecology and Evolution 3:e0073.

Avolio ML, Swan C, Pataki DE, Jenerette GD. 2021. Incorporating human behaviors into theories of urban community assembly and species coexistence. Oikos 130:1849–1864.

Aronson MF, Nilon CH, Lepczyk CA, Parker TS, Warren PS, Cilliers SS, Goddard MA, Hahs AK, Herzog CP, Katti M, La Sorte FA, Williams NS, Zipperer WC. 2016. Hierarchical filters determine community assembly of urban species pools. Ecology 97:2952–2963.

Baeten L, Velghe D, Vanhellemont M, De Frenne P, Hermy M, Verheyen K. 2010. Early Trajectories of spontaneous vegetation recovery after intensive agricultural land use. Restoration Ecology 18: 379–386.

Beaury EM, Patrick M, Bradley BA. 2021. Invaders for sale: the ongoing spread of invasive species by the plant trade industry. Frontiers in Ecology and the Environment 19:550-556.

Berglihn EC, Gómez-Baggethun E. 2021. Ecosystem services from urban forests: The case of Oslomarka, Norway. Ecosystem Services 51:101358.

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JR, Richardson DM. 2011. A proposed unified framework for biological invasions. Trends in ecology & evolution 26:333–9.

Brown DG, Johnson SM, Loveland TR, Theobald DM. 2005. Rural land-use trends in the conterminous United States, 1950-2000. Ecological Applications 15:1851–1863.

Brown, J. Running NMDS using 'metaMDS' [Internet]. RPubs brought to you by RStudio; 2019 August 5 [Cited 2022 July 21]. Retrieved from: http://rpubs.com/CPEL/NMDS

Brundu G, Pauchard A, Pyšek P, Pergl J, Bindewald A, Brunori A, Canavan S, Campagnaro T, Celesti-Grapow L, Dechoum MD, Dufour-Dror J, Essl F, Flory SL, Genovesi P, Guarino F, Guang-zhe L, Hulme PE, Jäger H, Kettle CJ, Krumm F, Langdon B, Lapin K, Lozano V, Le Roux JJ, Nóvoa A, Nuñez MA, Porte AJ, Silva J, Schaffner U, Sitzia T, Tanner RA, Tshidada NJ, Vítková M, Westergren M, Wilson JR, Richardson DM. 2020. Global guidelines for the sustainable use of non-native trees to prevent tree invasions and mitigate their negative impacts. NeoBiota 61:65–116.

Blouin D, Pellerin S, Poulin M. 2019. Increase in non-native species richness leads to biotic homogenization in vacant lots of a highly urbanized landscape. Urban Ecosystems 22:1–14.

Carreiro MM, Tripler CE. 2005. Forest remnants along urban–rural gradients: examining their potential for global change research. Ecosystems 8:568–582.

Cavender-Bares J, Padullés Cubino J, Pearse WD, Hobbie SE, Lange AJ, Knapp S, Nelson KC. 2020. Horticultural availability and homeowner preferences drive plant diversity and composition in urban yards. Ecological Applications 30:e02082.

Curtis JT, McIntosh RP. 1951. An Upland Forest Continuum in the Prairie-Forest Border Region of Wisconsin. Ecology 32:476–496.

Deljouei A, Abdi E, Marcantonio M, Majnounian B, Amici V, Sohrabi H. 2017. The impact of forest roads on understory plant diversity in temperate hornbeam-beech forests of Northern Iran. Environmental Monitoring and Assessment 189:1–15.

Dickie IA, Bennett BM, Burrows L, Nuñez MA, Peltzer DA, Porte AJ, Richardson DM, Rejmánek M, Rundel PW, Wilgen BW. 2014. Conflicting values: ecosystem services and invasive tree management. Biological Invasions 16:705–719.

Doroski DA, Felson, AJ, Bradford MA, Ashton MP, Oldfield EE, Hallett RA, Kuebbing SE. 2018. Factors driving natural regeneration beneath a planted urban forest. Urban Forestry & Urban Greening 29:238–247.

Doroski DA, Ashton M, Duguid MC. 2020. The future urban forest – A survey of tree planting programs in the Northeastern United States. Urban Forestry & Urban Greening, 55:126816.

Ehrenfeld, JG. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503–523.

Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foste DR, Kloeppel BD, Knoepp JD, Lovett GM, Mohan J. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3:479–486.

Essl F, Lenzner B, Bacher S, Bailey SA, Capinha C, Daehler CC, Dullinger S, Genovesi P, Hui C, Hulme PE, Jeschke JM, Katsanevakis S, Kühn I, Leung B, Liebhold AM, Liu C, MacIsaac HJ, Meyerson LA, Nuñez MA, Pauchard A, Pyšek P, Rabitsch W, Richardson DM, Roy HE, Ruiz GM, Russell JC, Sanders NJ, Sax DF, Scalera R, Seebens H, Springborn MR, Turbelin AJ, van Kleunen M, von Holle B, Winter M, Zenni RD, Mattsson BJ, Roura-Pascual N. 2020. Drivers of future alien species impacts: An expert-based assessment. Global Change Biology 26:4880–4893.

Fenn M, Poth MA, Johnson DW. 1996. Evidence for nitrogen saturation in the San Bernardino Mountains in southern California. Forest Ecology and Management 82:211–230.

Fenn M, Allen E, Weiss S, Jovan SE, Geiser L, Tonnesen GS, Johnson RF, Rao LE, Gimeno BS, Yuan F, Meixner T, Bytnerowicz A. 2010. Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. Journal of environmental management 91:2404–23.

Fratarcangeli C, Fanelli G, Testolin R, Buffi F, Travaglini A. 2022. Floristic changes of vascular flora in the city of Rome through grid-cell census over 23 years. Urban Ecosystems 25:1851–1864

Frelich LE, Blossey B, Cameron EK, Dávalos A, Eisenhauer N, Fahey T, Ferlian O, Groffman P M, Larson E, Loss SR, Maerz JC, Nuzzo V, Yoo K, Reich PB. 2019. Side-swiped: ecological cascades emanating from earthworm invasions. Frontiers in Ecology and the Environment 1:502–510.

Gaertner M, Wilson JR, Cadotte MW, Macivor JS, Zenni RD, Richardson DM. 2017. Non-native species in urban environments: patterns, processes, impacts and challenges. Biological Invasions 19:3461–3469.

Gaskin JF, Espeland EK, Johnson C, Larson DL, Mangold JM, Mcgee R, Milner C, Paudel S, Pearson DE, Perkins LB, Prosser CW, Runyon JB, Sing SE, Sylvain ZA, Symstad AJ, Tekiela DR. 2020. Managing Invasive Plants on Great Plains Grasslands: A Discussion of Current Challenges. Rangeland Ecology and Management 78:235–249.

Garvey SM, Templer PH, Pierce EA, Reinmann AB, Hutyra LR. 2022. Diverging patterns at the forest edge: Soil respiration dynamics of fragmented forests in urban and rural areas. Global Change Biology 28:3094–3109.

Groffman PM, Holland EA, Myrold DD, Robertson GP, Zou X. 1999. Denitrification. Robertson GP, Bledsoe CS, Coleman DC, Sollin P, editors. Standard Soil Methods for Long Term Ecological Research. Oxford: Oxford University Press. p272-288.

Groffman PM, Pouyat RV, Cadenasso ML, Zipperer WC, Szlavecz K, Yesilonis ID, Band LE Brush GS. 2006. Land Use Context and Natural Soil Controls on Plant Community Composition and Soil Nitrogen and Carbon Dynamics in Urban and Rural Forests. Forest Ecology and Management 246:296–7.

Groffman PM, Cavender-Bares JM, Bettez ND, Grove JM, Hall SJ, Heffernan JB, Hobbie SE, Larson KL, Morse JL, Neill C, Nelson KC, O'Neil-Dunne JP, Ogden LA, Pataki DE, Polsky C, Chowdhury RR, Steele MK. 2014. Ecological Homogenization of Urban USA. Frontiers in Ecology and the Environment 12:74–81.

Groffman PM, Avolio ML, Cavender-Bares JM, Bettez ND, Grove JM, Hall SJ, Hobbie SE, Larson KL, Lerman SB, Locke DH, Heffernan JB, Morse JL, Neill C, Nelson KC, O'Neil-Dunne J, Pataki DE, Polsky C, Chowdhury RR, Trammell TL. 2017. Ecological homogenization of residential macrosystems. Nature Ecology & Evolution 1: 0191.

Gucker CL. Fire Effects Information System [internet]. Missoula (MT): U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula Fire Sciences Laboratory; c2023. Fraxinus pennsylvanica; 2005 [cited 2022 April 30]. Available from: https://www.fs.fed.us /database/feis/plants/tree/frapen/all.html

Hall SJ, Trujillo J, Nakase D, Strawhacker C, Kruse-Peeples M, Schaafsma H, Briggs J. 2013. Legacies of Prehistoric Agricultural Practices Within Plant and Soil Properties Across an Arid Ecosystem. Ecosystems 16:1273–1293.

Hallett LM, Jones SK, MacDonald AA, Jones MB, Flynn DF, Ripplinger J, Slaughter P, Gries C, Collins SL. 2016. Codyn: An r package of community dynamics metrics. Methods in Ecology and Evolution 7:1146–1151.

Johnson LR, Handel SN. 2016. Restoration treatments in urban park forests drive long-term changes in vegetation trajectories. Ecological Applications 26:940–956.

Heneghan L, Rauschenberg CD, Fatemi FR, Workman M. 2004. European Buckthorn *(Rhamnus cathartica*) and its Effects on Some Ecosystem Properties in an Urban Woodland. Ecological Restoration 22:275–280.

Herrmann DL, Schifman LA, Shuster WD. 2020. Urbanization drives convergence in soil profile texture and carbon content. Environmental Research Letters 15:114001.

Hobbs RJ, Aricò S, Aronson J, Baron JS, Bridgewater P, Cramer VA, Epstein P, Ewel JJ, Klink CA, Lugo AE, Norton DA, Ojima DS, Richardson DM, Sanderson EW, Valladares F, Vilà M, Zamora R, and Zobel M. 2006. Novel ecosystems: theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography 15:1–7.

Hobbs RJ, Higgs E, Harris JA. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution 24:599-605.

Howard JL. Fire Effects Information System [internet]. Missoula (MT): U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula Fire Sciences Laboratory; c2023. *Malosma laurina*; 1992 [cited 2022 April 30]. Available from: https://www.fs.fed.us/database/feis/plants/shrub/mallau/all.html

Jäger H, Tye A, Kowarik I. 2007. Tree invasion in naturally treeless environments: impacts of quinine (*Cinchona pubescens*) trees on native vegetation in Galápagos. Biological Conservation 140:297–307.

Jenkinson DS, Powlson DS. 1976. The Effects of Biocidal Treatments on Metabolism in Soil, V. A Method for Measuring Soil Biomass. Soil Biology and Biochemistry 8:209–13.

Jiang S, Sonti NF, Avolio ML. 2022. Tree communities in Baltimore differ by land use type, but change little over time. Ecosphere 13:e4054.

Johnson LR, Handel SN. 2016. Restoration treatments in urban park forests drive long-term changes in vegetation trajectories. Ecological Applications 26:940–956.

Kaye JP, Groffman PM, Grimm NB, Baker LA, Pouyat RV. 2016. A distinct urban biogeochemistry? Trends Ecology & Evolution 21:195–199.

Knapp S, Dinsmore LA, Fissore C, Hobbie SE, Jakobsdottir I, Kattge J, King JY, Klotz S, McFadden JP, Cavender-Bares JM. 2012. Phylogenetic and functional characteristics of household yard floras and their changes along an urbanization gradient. Ecology 93:S83–S98.

Knight KS, Kurylo JS, Endress AG, Stewart JR Reich PB. 2007. Ecology and ecosystem impacts of common buckthorn (*Rhamnus cathartica*): A review. Biological Invasion 9:925–937.

Kowarik I, Hiller A, Planchuelo G, Seitz B, von der Lippe M, Buchholz S. 2019. Emerging Urban Forests: Opportunities for Promoting the Wild Side of the Urban Green Infrastructure. Sustainability 11:6318.

Krumm F, Vítková, L, editors. 2016. Introduced tree species in European forests: opportunities and challenges. European Forest Institute. p423.

Lê S, Josse J, Husson F. 2008. *FactoMineR*: A Package for Multivariate Analysis. Journal of Statistical Software 25:1–18.

Lerman SB, Narango DL, Avolio ML, Bratt AR, Engebretson JM, Groffman PM, Hall SJ, Heffernan JB, Hobbie SE, Larson KL, Locke DH, Neill C, Nelson KC, Padullés Cubino J, Trammell TLE. 2021a. Residential yard management and landscape cover affect urban bird community diversity across the continental USA. Ecological Applications 31:e02455.

Lerman SB, Narango DL, Andrade R, Warren PS, Grade AM, Straley K. 2021b. Wildlife in the city: Human drivers and human consequences. Barbosa P, editor. Urban ecology: its nature and challenges. Wallingford UK: CABI Publishing. p37-66

Locke DH, Polsky C, Grove JM, Groffman PM, Nelson KC, Larson KL, Cavender-Bares J, Heffernan JB, Chowdhury RR, Hobbie SE, Bettez ND, Hall SJ, Neill C, Ogden L, O'Neil-Dunne J. 2019. Residential household yard care practices along urban-exurban gradients in six climatically-diverse U.S. metropolitan areas. PLOS One 14:e0222630.

Legendre P, Legendre LF. 2012. Numerical Ecology. New York: Springer.

Lopez BE, Urban D, White PS. 2018. Testing the effects of four urbanization filters on forest plant taxonomic, functional, and phylogenetic diversity. Ecological Applications 28:2197–2205.

Nóvoa A, Le Roux JJ, Robertson MP, Wilson JR, Richardson DM. 2015. Introduced and invasive cactus species: a global review. AoB Plants 7:plu078.

Mascaro J, Schnitzer SA. 2007. *Rhamnus cathartica* L. (common buckthorn) as an ecosystem dominant in southern Wisconsin forests. Northeastern Naturalist 14:387–402.

McDonald RI, Motzkin G, Foster DR. 2008. Assessing the influence of historical factors, contemporary processes, and environmental conditions on the distribution of invasive species1. The Journal of the Torrey Botanical Society 135:260–271.

Mcdonnell MJ, Pickett ST. 1990. Ecosystem Structure and Function along Urban-Rural Gradients: An Unexploited Opportunity for Ecology. Ecology 71:1232–1237.

Le Maitre DC, Versfeld DB, Chapman RA. 2000. The impact of invading alien plants on surface water resources in South Africa: A preliminary assessment. Water SA 26:397–408.

Mason RE, Craine JM, Lany NK, Jonard M, Ollinger SV, Groffman PM, Fulweiler RW, Angerer J, Read QD, Reich PB, Templer PH, Elmore AJ. 2022. Evidence, causes, and consequences of declining nitrogen availability in terrestrial ecosystems. Science 376:eabh3767.

Mejía GA, Groffman PM, Downey AE, Cook EM, Sritrairat S, Karty R, Palmer MI, McPhearson T. 2022. Nitrogen cycling and urban afforestation success in New York City. Ecological applications 32:e2535.

Morreale LL, Thompson JR, Tang X, Reinmann AB, Hutyra LR. 2021. Elevated growth and biomass along temperate forest edges. Nature Communications 12:7181.

Mueller KE, Lodge AG, Roth AM, Whitfeld TJ, Hobbie SE, Reich PB. 2018. A tale of two studies: Detection and attribution of the impacts of invasive plants in observational surveys. Journal of Applied Ecology 55:1780–1789.

Munger GT. Fire Effects Information System [internet]. Missoula (MT): U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula Fire Sciences Laboratory; c2023. Acer platanoides; 2003 [cited 2022 April 30]. Available from: https://www.fs.usda.gov /database/feis/plants/tree/acepla/all.html

Narango DL, Douglas WT, Marra PP. 2018. Nonnative plant reduce population growth of an insectivorous bird. Proceedings of the National Academy of Sciences, 115:11549–11554.

Nowacki GJ, Abrams MD. (2008). The demise of fire and “mesophication” of forests in the Eastern United States. BioScience 58:123–138.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J. Vegan: community ecology package [Internet]; c2022. R package version 2.6-2. Available from: http://CRAN.R-project.org/package=vegan

Padullés Cubino J, Cavender-Bares J, Groffman PM, Avolio ML, Bratt AR, Hall SJ, Larson KL, Lerman SB, Narango DL, Neill C, Trammell TLE, Wheeler MM, Hobbie SE. 2020. Taxonomic, phylogenetic, and functional composition and homogenization of residential yard vegetation with contrasting management. Landscape and Urban Planning 202:103877.

Padullés Cubino JP, Cavender-Bares J, Hobbie SE, Hall SJ, Trammell TLE, Neill C, Avolio ML, Darling LE, Groffman PM. 2019a. Contribution of non-native plants to the phylogenetic homogenization of U.S. yard floras. Ecosphere 10:e02638.

Padullés Cubino JP, Cavender-Bares J, Hobbie SE, Pataki DE, Avolio ML, Darling LE, Larson KL, Hall SJ, Groffman PM, Trammell TLE, Steele MK, Grove JM, Neill C. 2019b. Drivers of plant species richness and phylogenetic composition in urban yards at the continental scale. Landscape Ecology 34:63–77.

Pastor J, Aber JD, McClaugherty CA, Melillo JM. 1984. Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. Ecology 65:256–268.

Pastor J, Post WM. 1986. Influence of Climate, Soil Mois- ture, and Succession on Forest Carbon and Nitrogen Cycles. Biogeochemistry 2:3–27.

Pearse WD, Cavender-Bares JM, Hobbie SE, Avolio ML, Bettez ND, Chowdhury RR, Darling LE, Groffman PM, Grove JM, Hall SJ, Heffernan JB, Learned J, Neill C, Nelson KC, Pataki DE, Ruddell BL, Steele MK, Trammell TLE. 2018. Homogenization of plant diversity, composition, and structure in North American urban yards. Ecosphere 9:1.

Peña ED, Baeten L, Steel H, Viaene N, Sutter ND, Schrijver AD, Verheyen K 2016. Beyond plant–soil feedbacks: mechanisms driving plant community shifts due to land-use legacies in post-agricultural forests. Functional Ecology 30:1073–1085.

Polsky C, Grove JM, Knudson C, Groffman PM, Bettez ND, Cavender-Bares JM, Hall SJ, Heffernan JB, Hobbie SE, Larson KL, Morse JL, Neill C, Nelson KC, Ogden LA, O'Neil-Dunne JP, Pataki DE, Chowdhury RR, Steele MK. 2014. Assessing the homogenization of urban land management with an application to US residential lawn care. Proceedings of the National Academy of Sciences 111:4432–4437.

Pouyat RV, Groffman PM, Yesilonis I, Hernández, L. 2002. Soil carbon pools and fluxes in urban ecosystems. Environmental pollution 11:107–118.

Pouyat RV, Yesilonis ID, Russell-Anelli J, Neerchal NK. 2007. Soil chemical and physical properties that differentiate urban land-use and cover. Soil Science Society of America 71:1010–1019.

Pouyat RV, Szlavecz K, Yesilonis ID, Groffman PM, Schwarz K. 2010. Chemical, physical and biological characteristics of urban soils. Aitkenhead-Peterson J, Volder A, editors. Urban Ecosystem Ecology. Agronomy Monograh 55. Madison: American Society of Agronomy. p119–152.

Powlson D, Xu J, Brookes P. 2017. Through the Eye of the Needle — The Story of the Soil Microbial Biomass. KR Tate, editor. Microbial Biomass: A Paradigm Shift in Terrestrial Biogeochemistry. London: World Scientific. p1-40.

Pregitzer CC, Charlop-Powers S, Bibbo S, Forgione HM, Gunther B, Hallett RA, Bradford MA. 2019. A city-scale assessment reveals that native forest types and overstory species dominate New York City forests. Ecological Applications 29:e1819.

Raciti SM, Groffman PM, Jenkins JC, Pouyat RV, Fahey TJ, Pickett STA, Cadenasso ML. 2011. Nitrate production and availability in residential soils. Ecological Applications 21:2357–2366.

R Core Team. R: a language and environment for statistical computing [Internet]. Vienna (Austria): R Foundation for Statistical Computing, c2020. Available from: https://www.R-project.org/

Reinhart KO, Gurnee J, Tirado R, Callaway RM. 2006. Invasion through quantitative effects: intense shade drives native decline and invasive success. Ecological applications 16:821–31.

Reinmann AB, Hutyra LR. 2017. Edge effects enhance carbon uptake and its vulnerability to climate change in temperate broadleaf forests. Proceedings of the National Academy of Sciences 114:107–112.

Richardson DM, van Wilgen BW. 2004. Invasive alien plants in South Africa: how well do we understand the ecological impacts? South African Journal of Science 100:45–52.

Ryan CD, Groffman, PM, Grove JM, Hall SJ, Heffernan JB, Hobbie SE, Locke DH, Morse JL, Neill C, Nelson KC, O'Neil-Dunne JP, Chowdhury RR, Steele MK, Trammell TLE.

2022. Ecological homogenization of soil properties in the American residential macrosystem. Ecosphere 13:e4208.

Schurman JS, Baltzer JL. 2012. Environmental correlates of tree species distributions vary among age classes in a northern temperate forest. Plant Ecology 213:1621–1632.

Schoenenberger N, Conedera M. 2013. Invasive neobiota in forest ecosystems: opportunity or threat? D. Kraus D, Krumm D, editors. Integrative approaches as an opportunity for the conservation of forest biodiversity. European Forest Institute. p224-231.

Scott DF, Versfeld DB, Lesch W. 1998. Erosion and sediment yield in relation to afforestation and fire in the mountains of the western cape province, South Africa. South African Geographical Journal 80:52–59.

Simberloff D, Nuñez MA, Ledgard NJ, Pauchard A, Richardson DM, Sarasola M, van Wilgen BW, Zalba SM, Zenni RD, Bustamante RO, Peña EA, Ziller SR, Blanca B. 2010. Spread and impact of introduced conifers in South America: Lessons from other southern hemisphere regions. Austral Ecology 35:489–504.

Smith J, Hallett R, Groffman PM. 2020. The state factor model and urban forest restoration. Journal of Urban Ecology 6:1018.

Smith J, Hallett RA, Deeb M, Groffman PM. 2021. Fine-scale soil heterogeneity at an urban site: Implications for forest restoration. Restoration Ecology 29:e13409.

Smith MS, Tiedje JM. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biology & Biochemistry 11:262-267.

Solomou AD, Topalidou ET, Germani R, Argiri A, Karetsos G. 2019. Importance, Utilization and Health of Urban Forests: A Review. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 47:10–16.

Tallamy DW, Narango DL, Mitchell AB. 2020. Do non-native plants contribute to insect declines? Ecological Entomology 46:729-742.

Templeton LK, Neel MC, Groffman PM, Cadenasso ML, Sullivan, JH. 2019. Changes in vegetation structure and composition of urban and rural forest patches in Baltimore from 1998 to 2015. Forest Ecology and Management 454:117665.

Thompson JR, Carpenter DN, Cogbill CV, Foster DR. 2013. Four centuries of change in northeastern United States forests. PloS one 8:72540.

Tirmenstein DA. Fire Effects Information System [internet]. Missoula (MT): U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula Fire Sciences Laboratory; c2023. Acer rubrum; 1991 [cited 2022 April 30]. Available from: https://www.fs.fed.us/database/feis/plants/tree/acerub/all.html

Trammell TLE, Carreiro MM. 2011. Vegetation composition and structure of woody plant communities along urban interstate corridors in Louisville, KY, USA. Urban Ecosystems 14:501–524.

Trammell TLE., Pataki DE, Cavender-Bares JM, Groffman PM, Hall SJ, Heffernan, JB, Hobbie SE, Morse JL, Neill C, Nelson KC. 2016. Plant nitrogen concentration and isotopic composition in residential lawns across seven US cities. Oecologia 181:271–285.

Trammell TLE, Pataki DE, Pouyat RV, Groffman PM, Rosier C, Bettez ND, Cavender-Bares JM, Grove M, Hall SJ, Heffernan JB, Hobbie SE, Morse JL, Neill C, and Steele MK. 2020a. Urban soil carbon and nitrogen converge at a continental scale. Ecological Monographs 90:e01401.

Trammell TLE., D'amico V, Avolio L, Mitchell JC, Moore ER. 2020b.

Temperate deciduous forests embedded across developed landscapes: Younger forests harbour invasive plants and urban forests maintain native plants. Journal of Ecology 108: 2366–2375.

Trammell TLE, Pouyat RV, D'amico V. 2021. Soil chemical properties in forest patches across multiple spatiotemporal scales in mid-Atlantic U.S. metropolitan areas. Urban Ecosystems 24:1085–1100.

Trammell TLE, Pouyat RV, D 'Amico V. 2022. Heterogeneity in soil chemistry relates to urbanization while soil homogeneity relates to plant invasion in small temperate deciduous forests. Landscape Ecology 37:1417–1429.

Uchytil RJ. Fire Effects Information System [internet]. Missoula (MT): U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula Fire Sciences Laboratory; c2023. Prunus serotina; 1991 [cited 2022 April 30]. Available from: https://www.fs.fed.us/database/feis/plants/tree/pruser/all.html

USDA, NRCS. 2023. The PLANTS Database National Plant Data Team, Greensboro, NC, USA; 2023. [cited 06/12/2023]. Available from: http://plants.usda.gov.

van Wilgen BW, Reyers B, Le Maitre DC, Richardson DM, Schonegevel L. 2008. A biome-scale assessment of the impact of invasive alien plants on ecosystem services in South Africa. Journal of environmental management 4:336–49.

Vieira R, Finn JT, Bradley BA. 2014. How does the landscape context of occurrence data influence models of invasion risk? A comparison of independent datasets in Massachusetts, USA. Landscape Ecology 29:1601–1612.

Vogelmann JE. 1995. Assessment of forest fragmentation in southern New England using remote sensing and geographic information systems technology. Conservation Biology 9:439–449.

Ward EB, Pregitzer CC, Kuebbing SE, Bradford MA. 2020. Invasive lianas are drivers of and passengers to altered soil nutrient availability in urban forests. Biological Invasions 22:935–955.

Wheeler MM, Neill C, Groffman PM, Avolio ML, Bettez ND, Cavender-Bares JM, Chowdhury RR, Darling LE, Grove JM, Hall SJ, Heffernan JB, Hobbie SE, Larson KL, Morse JL, Nelson KC, Ogden LA, O'Neil-Dunne J, Pataki DE, Polsky C, Steele MK, Trammell TLE. 2017. Continental-scale homogenization of residential lawn plant communities. Landscape and Urban Planning 165:54–63.

Ziter C, Turner MG. 2018. Current and historical land use influence soil-based ecosystem services in an urban landscape. Ecological Applications 28:643–654

Zouhar K. Fire Effects Information System [internet]. Missoula (MT): U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula Fire Sciences Laboratory; c2023. Rhamnus cathartica; 2011 [cited 2022 April 30]. Available from: https://www.fs.usda.gov/database/feis/plants/shrub/rhaspp/all.html

Zhu WX, Carreiro MM. 2004. Temporal and Spatial Variations in Nitrogen Transformations in Deciduous Forest Ecosystems along an Urban-Rural Gradient. *Soil* Biology and Biochemistry 36:267–278.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. Mixed effects models and extensions in ecology with R. New York: Springer.

Tables

Table 1. Average number of woody plant stems per plot (mean ± SE), species richness (mean ± SE), and evenness by forest layer (canopy and sapling) in interstitial and reference sites in each city (*n* = 6).

		Interstitial				*Reference*			
Forest layer	**City**	**No. of stems**	**Introduced species (%)**	**Richness**	**Evenness**	**No. of stems**	**Introduced species (%)**	**Richness**	**Evenness**
Canopy (>10 DBH)	Baltimore	7.4 ± 0.93	0	4.2 ± 0.37	0.88	11 ± 0.84	0	4.2 ± 0.58	0.80
	Boston	30.4 ± 4.63	30	5.2 ± 0.66	0.59	27 ± 5.94	50	5.8 ± 2.15	0.64
	Los Angeles	4 ± 1.53	0	3 ± 1.15	0.89	3 ± 0	0	3 ± 0	1.00
	Miami	28.5 ± 7.35	43	5.25 ± 1.65	0.66	34.75 ± 10.26	10	5.25 ± 1.49	0.68
	Minneapolis-St. Paul	44 ± 14.35	13	5.5 ± 0.76	0.65	27.13 ± 12.15	7	3.375 ± 0.73	0.68
	Phoenix	1.5 ± 0.5	0	1.5 ± 0.5	1.00	1.5 ± 0.5	0	1 ± 0.00	1.00
Sapling (<10 DBH)	Baltimore	26.6 ± 6.58	16	9.4 ± 2.54	0.56	46.4 ± 4.73	5	7.4 ± 0.87	0.44
	Boston	24.6 ± 2.34	34	4.8 ± 1.2	0.60	23.2 ± 5.42	53	6.6 ± 1.12	0.59
	Los Angeles	11.67 ± 2.03	0	2.33 ± 0.67	0.81	2.5 ± 0.5	0	2 ± 0.00	0.95
	Miami	26 ± 15.64	54	4 ± 0.71	0.71	49.25 ± 20.8	6	4.5 ± 1.19	0.66
	Minneapolis-St. Paul	39.67 ± 14.14	48	3.83 ± 0.87	0.71	32.5 ± 13.77	0	2.5 ± 0.67	0.69
	Phoenix	2.5 ± 0.5	0	1 ± 0.00	1.00	2 ± 1.00	0	1 ± 0.00	1.00

Table 2. Percent of the number species in interstitial sites, reference sites, and shared between interstitial and reference sites in each city (*n* = 6).

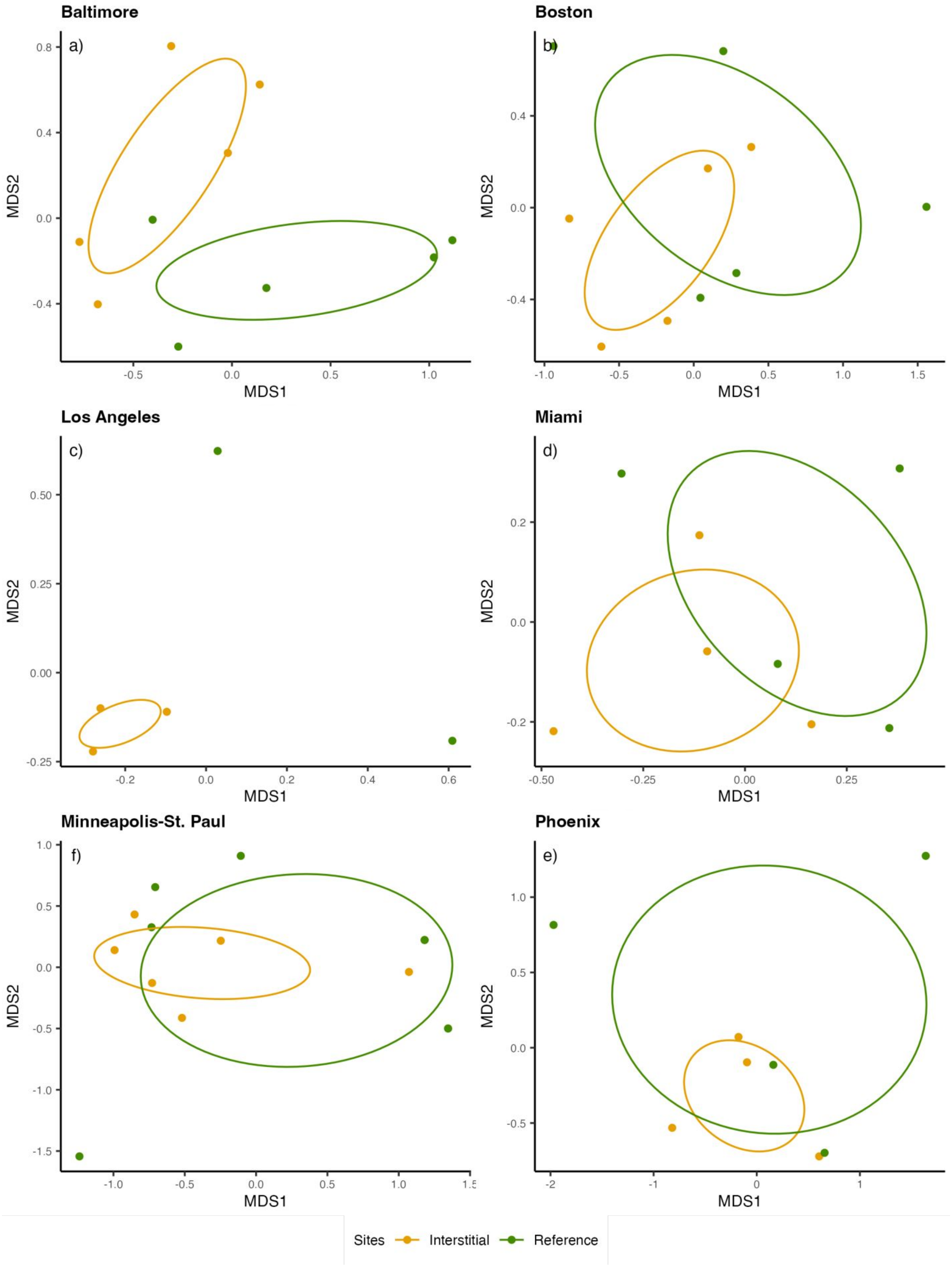
Cities	**Interstitial (%)**	**Reference (%)**	**Interstitial and reference (%)**
Baltimore	54	46	32
Boston	64	36	26
Los Angeles	57	43	64
Miami	51	49	18
Minneapolis-St. Paul	50	50	20
Phoenix	40	60	20

Figure 1. Interstitial and reference areas have distinct woody species compositions. Non-metric multidimensional scaling (NMDS) of woody community composition in interstitial and reference sites in (a) Baltimore (b) Boston (c) Los Angeles (d) Miami (e) Minneapolis-St. Paul (f) Phoenix. Plot points are based on Bray-Curtis distances of relative abundance data. Distance between points represents compositional similarity, with closer points being more similar than points further apart. Stress values for a) 0.091 b) 0.076 c) 0 d) 0.064 e) 0.048 f) 0.

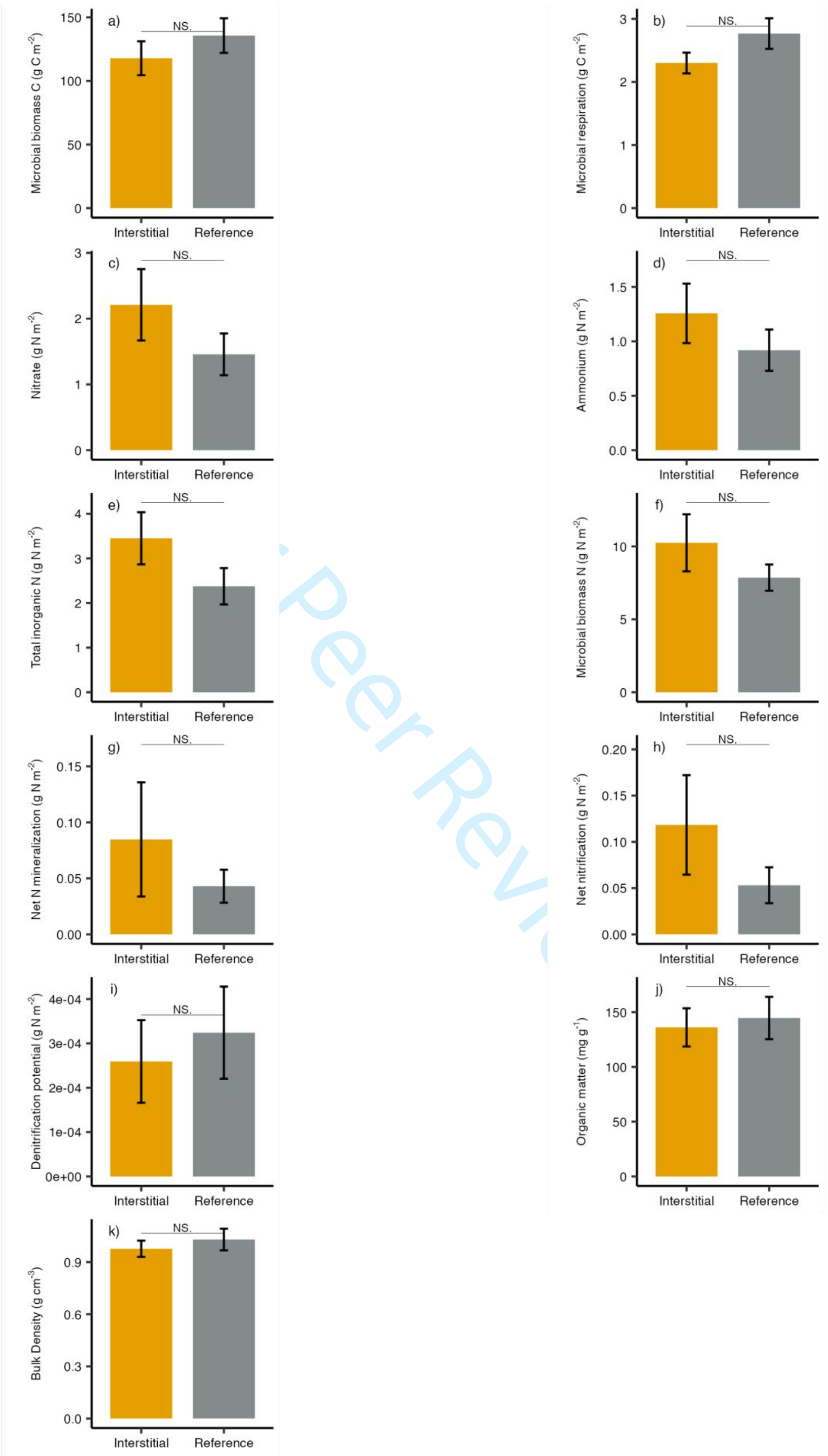
Figure 2. Mean values of soil (0 – 30 cm depth) parameters in interstitial and reference sites over all cities. Error bars represent +/- SE. Bars with asterisks are significantly different: $*p < 0.05$. Bars with NS = not significant.

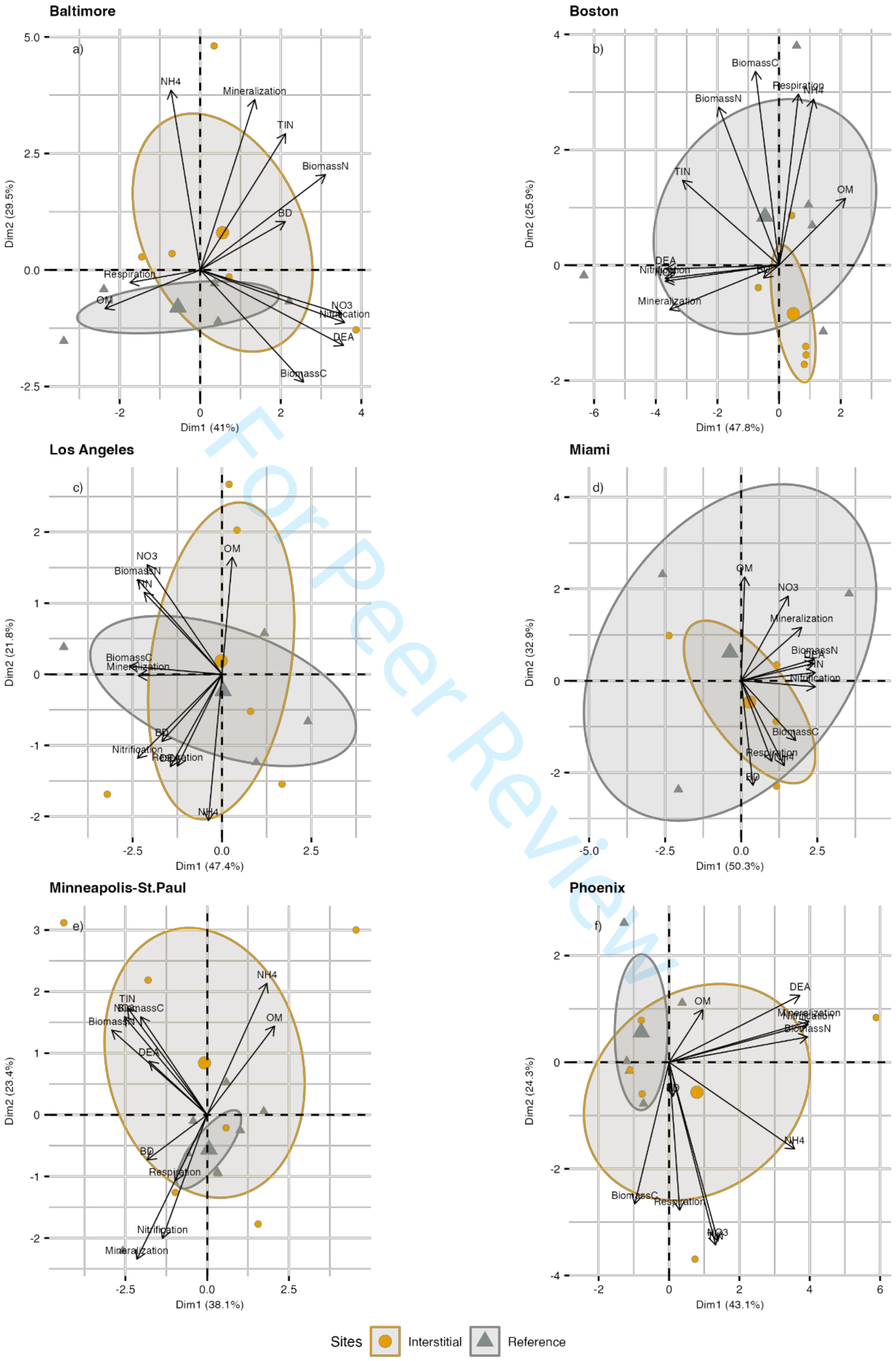
Figure 3. Principal components analysis (PCA) showing soil parameters across interstitial and reference sites in a) Baltimore b) Boston c) Los Angeles d) Miami e) Minneapolis-St. Paul f) Phoenix. Soil parameter codes: Microbial biomass C (BiomassC), microbial biomass N (BiomassN), basal respiration (Respiration), NO_3^- (NO3), NH_4^+ (NH4), total inorganic N (TIN), potential net N mineralization (Mineralization), potential net nitrification (Nitrification), denitrification potential (DEA), organic matter content (OM), and bulk density (BD).

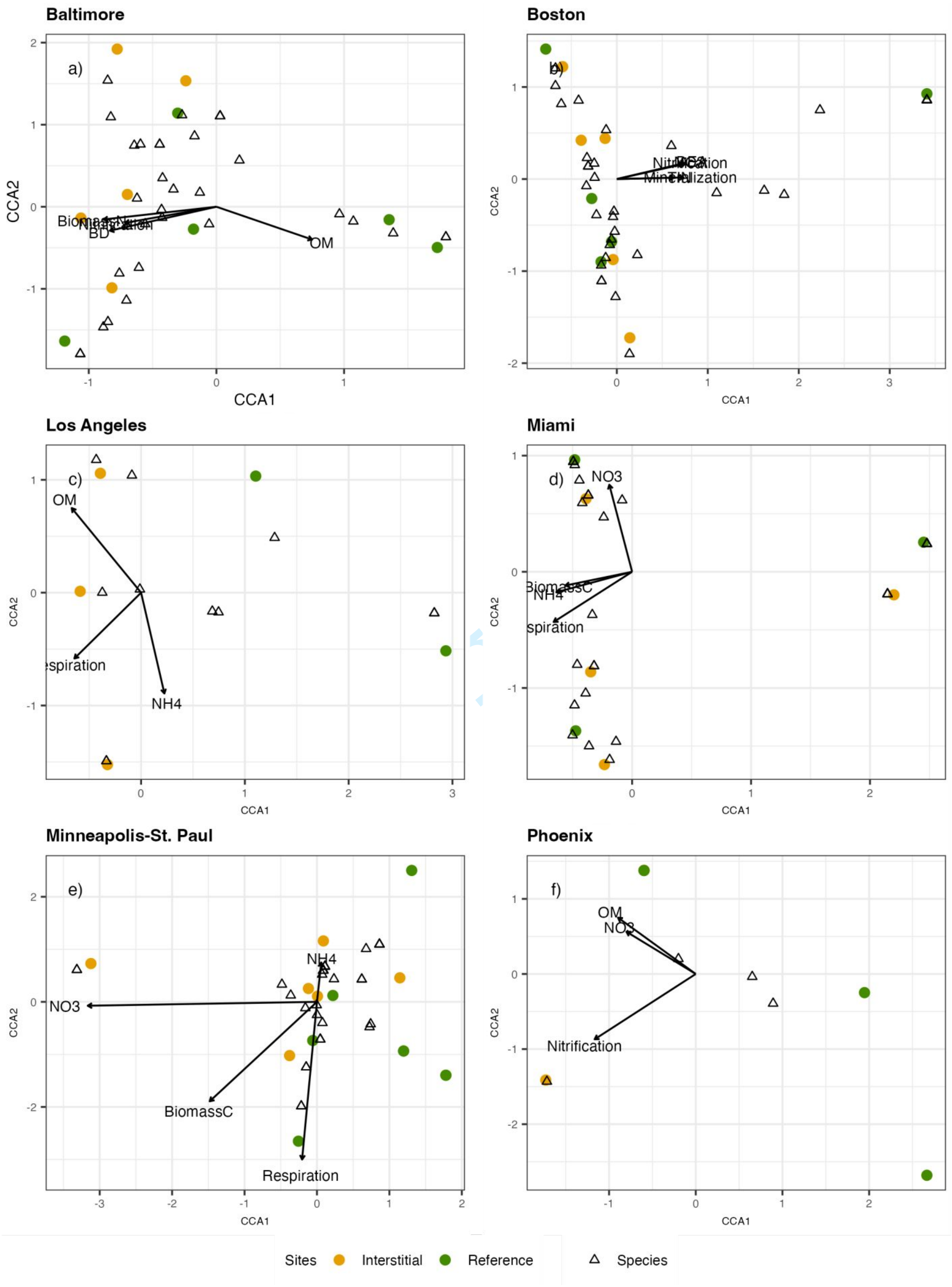
Figure 4. Canonical correlation analysis (CCA) showing similarity of woody species community composition in relation to soil parameters amongst interstitial and reference sites in a) Baltimore b) Boston c) Los Angeles d) Miami e) Minneapolis-St. Paul f) Phoenix. Sites (green and yellow dots), tree species (open triangles), soil parameters (black arrows): Microbial biomass C (BiomassC), microbial biomass N (BiomassN), basal respiration (Respiration), NO_3^- (NO3), NH_4^+ (NH4), total inorganic N (TIN), potential net N mineralization (Mineralization), potential net nitrification (Nitrification), denitrification potential (DEA), organic matter content (OM), and bulk density (BD). Distance between plot symbols indicates similarity of species composition and abundance. The proportion of variance explained by CCA1-CCA2 in each city a) 17%-10% b) 17%-10% c) 35% d) 14%-10% e) 16%-14% f) 5%-3%.


Baltimore
a)
Boston
b)
Los Angeles
c)
Miami
d)
Minneapolis-St. Paul
f)
Phoenix
e)
MDS1
MDS2
Sites
Interstitial
Reference

1222


a)
NS.
Microbial biomass C (g C m^{-2})
0
50
100
150
Interstitial
Reference
b)
NS.
Microbial respiration (g C m^{-2})
0
1
2
3
Interstitial
Reference
c)
NS.
Nitrate (g N m^{-2})
0
1
2
3
Interstitial
Reference
d)
NS.
Ammonium (g N m^{-2})
0.0
0.5
1.0
1.5
Interstitial
Reference
e)
NS.
Total inorganic N (g N m^{-2})
0
1
2
3
4
Interstitial
Reference
f)
NS.
Microbial biomass N (g N m^{-2})
0
5
10
Interstitial
Reference
g)
NS.
Net N mineralization (g N m^{-2})
0.00
0.05
0.10
0.15
Interstitial
Reference
h)
NS.
Net nitrification (g N m^{-2})
0.00
0.05
0.10
0.15
0.20
Interstitial
Reference
i)
NS.
Denitrification potential (g N m^{-2})
0e+00
1e-04
2e-04
3e-04
4e-04
Interstitial
Reference
j)
NS.
Organic matter (mg g^{-1})
0
50
100
150
Interstitial
Reference
k)
NS.
Bulk Density (g cm^{-3})
0.0
0.3
0.6
0.9
Interstitial
Reference


Baltimore
a)
NH4
Mineralization
TIN
BiomassN
BD
Respiration
OM
NO3
Nitrification
DEA
BiomassC
Dim1 (41%)
Dim2 (29.5%)
Boston
b)
BiomassC
Respiration
NH4
BiomassN
TIN
OM
DEA
Nitrification
Mineralization
BD
Dim1 (47.8%)
Dim2 (25.9%)
Los Angeles
c)
NO3
BiomassN
TIN
OM
BiomassC
Mineralization
BD
Nitrification
Respiration
NH4
Dim1 (47.4%)
Dim2 (21.8%)
Miami
d)
OM
NO3
Mineralization
BiomassN
DEA
TIN
Nitrification
BiomassC
Respiration
NH4
BD
Dim1 (50.3%)
Dim2 (32.9%)
Minneapolis-St.Paul
e)
NH4
TIN
NO3
BiomassC
BiomassN
OM
DEA
BD
Respiration
Nitrification
Mineralization
Dim1 (38.1%)
Dim2 (23.4%)
Phoenix
f)
DEA
OM
Mineralization
Nitrification
BiomassN
BD
NH4
BiomassC
Respiration
NO3
TIN
Dim1 (43.1%)
Dim2 (24.3%)
Sites
Interstitial
Reference


Baltimore
a)
OM
BD
CCA1
CCA2
Boston
b)
Nitrification
Mineralization
Los Angeles
c)
OM
NH4
espiration
Miami
d)
NO3
BiomassC
NH4
spiration
Minneapolis-St. Paul
e)
NH4
NO3
BiomassC
Respiration
Phoenix
f)
OM
NO3
Nitrification
Sites
Interstitial
Reference
Species

Response to Reviewer Comments: Woody plant-soil relationships in urban interstitial spaces have implications for future forests within and beyond urban areas (ECOSYSTEMS MS# ECO-23-0052)

(Reviewer comments are repeated below, *responses are in red italics*)

Subject-Matter Editor, Bürgi, Matthias

Comments to the Author:

Thank you for submitting your paper "Woody plant-soil relationships in urban interstitial spaces have implications for future forests within and beyond urban areas" to Ecosystems. We now received two reviews and both reviewers see the relevance and the potential in the study conducted.

However, both reviewers also raise a series of concerns, and based on my own reading, I must add another one: In the title, the highlights and throughout the manuscript you put a lot of weight on the relevance of your results for forests within and beyond urban areas. However, I do not see how your specific results contribute to this finding. The existing literature alone allows to draw these conclusions, but to which specific aspect do your results contribute? I strongly suggest being more precise and specific regarding the implications you refer to throughout the text.

We have addressed the concerns regarding the relevance of our results to forests within and beyond urban areas. At the beginning of the manuscript, we clarify that in this study, we asked the question "if analysis of forests that have spontaneously assembled in urban interstitial spaces provide insight into how global environmental change will affect the forests of the future." We ask if the complex mix of anthropogenic factors affecting these spaces (altered climate and atmospheric chemistry, altered disturbance regimes, altered species pool) are analogous to factors playing out across the globe at lower intensity. If so, the novel communities that assemble in these spaces may provide a glimpse of the forests that may become widespread across the world. The fact that we observed the formation of novel communities in these spaces in six cities across a very broad range of climates, suggests that our results do have relevance for forests beyond urban areas.

We note that Reviewer #2 appreciates this relevance, "it is of high relevance to better understand novel ecosystems as they develop in described urban and peri-urban areas. We will face novel ecosystems across the globe and therefore it is important to learn on how and where to manage in order to maintain ecosystem services, biodiversity and climate resilience."

On some occasions, there seems to be a tension between the desire to draw more general conclusion and the heterogeneity of site conditions, landscape level context, land use legacies etc. in your sites selected. At least this is my impression when I read for example on Line 576 about a lack of difference in C dynamics between interstitial and reference sites, but just five lines further down that your results support the idea that the quantity of C in urban ecosystems can be significant compared to native ecosystems. Reviewer 1 similarly refers to aspects where a more precise wording might help to avoid such misunderstandings. The reader has to be very clear regarding which insights arise from your results and what statements are based on the literature – this does not seem to be always the case now.

As described in detail below, we have clarified the specific places where this tension arises in our revised manuscript. We have eliminated the sentence that created confusion in (former) Line 576.

I do think that the topic addressed is indeed of high relevance and also of high interest to the readership of our journal. However, in its present form, the manuscript lacks specificity regarding the insights generated based on results and how these insights specifically translate into what kind of implications for future forest management. I therefore suggest that you revise and resubmit your manuscript based on the recommendations of the reviewers and the suggestions regarding sharpening the message outlined above.

Sincerely yours Matthias Bürgi

Thank you for considering our manuscript. We are glad to hear the topic is relevant and useful to readers of ECOSYSTEMS. We have revised the manuscript according to the comments suggested, and more specifically addressed the implications for future forest management.

Reviewer 1

General comments:

The study by Mejia et al examines woody plant species composition and richness with respect to soil C and N properties across six contrasting urban areas in the United States. These comparative types of studies are very important as they highlight where ecological theory becomes limited to different ecosystems. I think the data sets are robust and should absolutely be published.

Thanks for these positive comments and for your helpful and constructive review!

However, the content of the study needs revision as outlined in my following general and specific comments:

The introduction needs to be revised. First, the authors need to emphasize how this is novel with respect to their previous works, in particular, the "Trammell, T.L., Pataki, D.E., Pouyat, R.V., Groffman, P.M., Rosier, C., Bettez, N., Cavender-Bares, J., Grove, M.J., Hall, S.J., Heffernan, J. and Hobbie, S.E., 2020. Urban soil carbon and nitrogen converge at a continental scale. Ecological Monographs, 90(2), p.e01401." has very similar conclusions using much of the same data. Moreover, the Trammell paper was not cited in the introduction and should be as it is incorporating many of the same themes and concepts. Second, there has been extensive work on C and N cycling in urban systems and this work does not approach it in a quantitative fashion. I understand the need to advance theory, but there needs to be quantitative data to describe sizes of storage and fluxes and the current introduction is a disservice to those authors and to readers.

A major focus of our revision has been to clarify that the soil data were used for two very different purposes than the analyses in Trammell and others (2020) and Ryan and others (2022). Given that the focus of our study was what we can learn from the vegetation communities that spontaneously assemble in urban interstitial spaces, our soils analysis was focused on 1) ensuring that there were no major anthropogenic differences between reference and interstitial sites such as compaction, profile disturbance, or fertilization, and 2) to explore effects of novel plant communities on soil processes. A major focus of our revision was to clarify these objectives. We have also included references to the Trammell and others (2020) and Ryan and others (2022) papers and clarified that these were focused on evaluating homogenization and changes in soil pools and processes rather than relationships with vegetation communities.

The methods were written very well. I only had a specific comment on nondimensionalizing the data used in the PCA.

Thank you, we have clarified that the soil data was standardized prior to conducting the PCA (line 615).

My first issue with the content of the manuscript is what seems to be either an inconsistency or cherry-picking regarding composition vs richness. For example, the abstract states "We observed marked differences in woody plant community composition between interstitial and reference sites in most metropolitan regions." but the first line of the results state "Across cities, there was no consistent difference in mean woody plant species richness between reference and interstitial sites (Table 1)." This seems contradictory to the Cubino et al 2018 study which species richness is held in equal regard as composition and touted as an important response variable but here in the results and discussion it seems to have been forgotten/buried.

We have clarified in the Methods section (lines 600 – 602) that species composition is "the identity of species present in a community." We had already defined species richness as "the overall number of species," in a community (lines 344-345). In the Cubino and others (2019) study the focus was on the role of non-native species in biotic homogenization in residential yards and addressed both species richness and composition. Our focus here was not on homogenization, but rather to explore effects of novel plant communities (composition) in interstitial (i.e., unmanaged) spaces.

My second issue is that soil C and N are discussed and framed irrespective to soil moisture and pollution. Soil moisture controls the growth of woody plants in southern California (shrubs and invasive grasses dominate the dry areas). Soil moisture retention data should be available from NRCS to compare soil data within each city. Also, that could help eliminate any potential aquic conditions creating outliers in temperate forests. N pollution sin several of the cities are dominant factors, particularly LA which has had massive N fluxes of N deposition exceeds 10 kg ha−1 yr−1 well into the 1990s (N pollution during acid rain peaked around 12 kg ha-1 yr-1 in NY for example). These are two very important processes not covered in the manuscript as needed.

We have clarified in the methods section (lines 501 – 509) that the selection criteria for the interstitial sites "included sites with natural soil profiles similar in texture and landscape position to those in the reference areas, without signs of anthropogenic soil disturbance. In some cities, e.g., Minneapolis St.-Paul this required locating sites on different soil parent materials. Unmanaged patches that fit these criteria were located within the same region as the refence sites, either on the edge of the city, at the interface with suburban residential land, or within public parklands or woodlands (for more detailed description, see Padullés Cubino and others 2020; Lerman and others 2021). Soil taxonomy was identified using USDA Natural Resource Conservation Service (NRCS) maps for each native reference and interstitial site in each city (Table S1)." Closely matching the soil series allowed us to avoid differences in soil moisture retention and having the sites interspersed across the region avoid local pollution gradients. However, we have highlighted the importance of these gradients, especially in Los Angeles (lines 804 – 806).

Specific comments:

The title should be revised. Having "urban interstitial spaces" and "urban areas" in the title seems redundant.

We have revised to: "Woody plant-soil relationships in interstitial spaces have implications for future forests within and beyond urban areas."

For author contributions, authors should use CRediT to really specify their intellectual contributions. The second batch of authors contributed equally through what kind of comments? Making sure their names are spelled correctly?

We have extensively revised the author statement to more clearly specify the contributions that people made based on CRediT descriptions and the journal guidelines. This paper is the product of a research group that has been working together since 2010 so there are people who contributed to the study and experimental design as well as relative newcomers who were more involved in interpretation of this specific data. This is an active group; there were multiple iterations of comments and suggested edits on the early drafts.

Highlights:

Bullet 2: either 'N' or 'nitrogen'.

Thank you, this has been revised to Nitrogen (N).

Abstract:

The authors should consider being more quantitative, as the only number in their abstract is 'six' for the number of sites.

While we have extensively revised the Abstract to clarify several key points (as described above), we have not made it more quantitative as suggested here. Most of our results are from multivariate analyses that are not particularly conducive to simple quantitative statements.

Results:

Line 341: One of my least favorite aspects of ecology, p values. Please remember it is significant or it is not. Marginally significant defeats the purpose of a priori statistical tests and shows post-hoc significance hunting. Instead, focus on the R2 explanatory power which is huge for Los Angeles.

We have clarified (lines 680 – 681) that 'there were strong, but not statistically significant compositional differences between interstitial and reference sites in Los Angeles (r2 = 0.47, p = 0.10, respectively; Table S2)."

Line 370 and 371: Is "Strongly loaded" the correct terminology? From the methods it is unclear if the data were normalized/nondimensionalized to their standard deviation and thus would have an oversized effect on the eigenvalues and eigenvectors. Please either clarify the methods or re-do the PCA with nondimensionalized data.

We clarified in the methods sections (lines 627 – 628) that the data was normalized/nondimensionalized to their standard deviation before conducting the PCA. This enabled us to use the loadings to determine the importance of the factors (i.e., strong or weak contribution to the components).

Line 384: The high variability seems like soil macro- and micro-topography were not properly controlled during sampling in LA and PHX. Such is the way of field sampling.

We have clarified (line 515) that "two soil cores up to 30 cm depth were collected at random locations along transects at each site." Indeed, some of the variability that we observed may be due to soil macro- and micro-topography as the reviewer suggests.

Line 399: Why not mention the lack of differences in richness?

We have clarified (lines 481 – 497) in the discussion that the focus of our analysis is species composition, which we define as "the identify of species present in a community." We do talk about species diversity (richness), later in the discussion, but our focus is composition.

Discussion:

Line 410: The authors should really consider nitrogen in the context of precipitation limitations and N pollution in their overview as it is misleading to draw a link directly between plants and soil N without those two major factors being stated immediately.

We have extensively revised this "overview" section as well as the description of the objectives and experimental approaches to our soil and nitrogen work. Of particular importance here is that having the sites interspersed across the region avoids local pollution gradients.

Line 476: I believe the major deforestation of New England in the 1800s-1900s was far more fragmentation than current parcels. Just look at the stone walls!

We have clarified (lines 922 – 924) that "temperate forests of New England have experienced increased fragmentation over recent decades" to avoid confusion with earlier periods of more intensive fragmentation.

Line 517: Finally soil moisture constraints on N are mentioned. Soil moisture constraints need to be a more prominent feature in describing the C and N data as soils in LA and PHX are moisture constrained for most of the year while C and N data were determined using incubated soils which are artificial conditions in the dry areas for most of the year.

We have a paragraph (lines 974 – 977) that discusses how "comparison of interstitial and reference sites in our most arid cities (Phoenix and Los Angeles) produced an interesting contrast to mesic cities." We note in the methods (lines 712 –714) that this comparison is not affected by differences in soil moisture between the interstitial and reference sites, none of which received water additions. We also note that there were no water additions to our incubations for C and N cycle processes.

Line 536: Soils and their moisture retention control plants success outside of the udic soil moisture regime (and even to an extent in udic regimes where aquic conditions dominate). This basic feature of soil moisture controlling plant richness and composition seems lost on the authors' discussion.

As noted above, we do spend some time talking about the importance of soil moisture effects on plants and soils, and how this varies between the mesic and arid cities. However, these effects do not affect our comparison of interstitial and reference sites, which were chosen to avoid differences in inherent soil characteristics. Detailed soil classifications are presented in Table S1.

Line 546: Finally, acknowledgement of N pollution. There have been tons of great papers examining this such as:

Fenn, M.E., Poth, M.A. and D.W., 1996. for in the San Bernardino Mountains in *Forest Ecology and*, *82*(1-3),

Fenn, M.E., Allen, E.B., Weiss, S.B., Jovan, S., L.H., Tonnesen, G.S., R.F., Rao, L.E., B.S., Yuan, F. and T., 2010. critical loads and for in *of* ,*91*(12), Line 578: Was organic matter quality measured? If not, this sentence is a stretch.

Thank you so much for the suggestions, we have added these references and clarified that "in drier regions, these variations may be affected by local N cycling patterns, such as those observed in California." Please note that our reference and interstitial sites should be equally affected by local N cycling patterns.

We have clarified that measurements of microbial biomass carbon and respiration are indices of organic matter quality (lines 1040 – 1043).

Appendix Table S1

The Taxonomy shown as "Soil Series" is actually the only the soil family with which the soil series belongs. In addition, the complexes shown you only include one of the soil family data but they are likely dissimilar units not belonging to the same family which very different soil properties. Such is the problem with the soil maps of the US :/

We have changed the column heading in Table S1 from "Soil Series" to "Soil Family"

Reviewer 2:

General comments:

This topic is extremely important for the provisioning of future ecosystem services. It is of high relevance to better understand novel ecosystems as they develop in described urban and peri-urban areas. The study is well presented and touches an important interface of forest dynamics considering soil dynamics. We will face novel ecosystems across the globe and therefore it is important to learn on how and where to manage in order to maintain ecosystem services, biodiversity and climate resilience. I do support the publication of this paper. However, it would be beneficial to increase the perspective a bit as there is quite a bit of experience across the world. There are some basic papers that might improve the manuscript especially in the light of a legal framework on introducing tree species. In the light of controlling and managing ecosystems in the future. e.g. Brundu et al. 2020 in Neobiota Global guidelines for the sustainable use of non-native trees to prevent tree invasions and mitigate their negative impacts.

Thank you for feedback and recognition of the contribution of our manuscript to understanding novel ecosystems. We agree that broadening our references would strengthen the paper and make the study more relevant to urban and peri-urban forests outside the U.S. We have added 23 additional sources, including Brundu et al. 2020.

What I miss and think it should be discussed is a stronger link to ecosystem services. Especially the provision of clean and filtered water in such environments seems to be a real challenge for now and the future. There are experiences with invasive woody species with regards to water consumption of introduced species from e.g. South Africa. There is lots of literature available for the Center of Invasion Biology in Stellenbosch (e.g. van Wilgen or Richardson). Also Australia has a broad expertise on this topic. The same accounts for biodiversity and the competition of non-native to native species. This is also only touched very briefly and might be expanded in a few sentences.

We expanded our discussion of ecosystem services, specifically the effects of invasive species on water consumption and soil conservation. We have also included the references recommended. Many thanks for these specific suggestions!

In general, the reference list is quite focussed on US literature. This certainly makes sense as the study is tailored for US environments, however, a view across borders is reasonable as in other countries where non-natives (in the US) are native, management experiences for certain species might be available. This also accounts for invading processes. Also here, countries as New Zealand, Australia, South Africa and many countries in South America have vast experiences with such developments. There are also strong pubications from author groups from across the globe that might be considered as refs. (e.g. Brundu et al, 2020 Neobiota Global guidelines for the sustainable use of non-native trees to prevent tree invasions and mitigate their negative impacts; or Krumm and Vitkova, Introduced tree species - opportunities and challenges, EFI 2016. Also the groups of Pysek, Essl or Blackburn have important publications on basic principles on invasive processes.

We have included the references suggested and expanded our discussion of management of non-native vegetation from other non-US references.

As the definition of crucial terms, such as exotic, native, non-native is interpreted and perceived in different ways, it would be helpful to include a definition in the manuscript. It usually confuses readers as there are also varying definitions around.

We have clarified in the introduction and methods our definition of introduced (non-native) species based on the USDA definition and offered more details on these definitions in the methods section. According to the USDA: Introduced species reproduce spontaneously in the wild without human help and tend to persist. Invasive species are (1) non-native (or alien) species to the ecosystem under consideration and

(2) a species whose introduction causes or is likely to cause economic harm, environmental harm, or harm to human health.

Specific comments:

line 200: What does shrub mean? Any threshold on size

We clarified in the methods that the threshold size for all vegetation measured was 1 cm diameter (line 514).

Discussion:

In my view, the first section "overview" would not be neccessary. It even confuses a bit and does not really support the readibility of the discussion. I would prefer to start right away with discussing the results and relationships. Adding a few sentsences on impacts on forest ecosystems would be valuable here.
Lines 425ff Any other potential reasons for the observed differences? Or why are the differences that pronounced? Climatic changes? Land use changes? Invading processes, e.g. missing Mycorrhiza, missing antagonists etc?

We have eliminated the “overview” section and re-organized the Discussion section as suggested here. We now start by reminded the reader that our overarching question is “if analysis of forests that have spontaneously assembled in urban interstitial spaces provide insight into how global environmental change will affect the forests of the future.” We then go on to discuss differences in plant communities and then discuss if local human alteration of soils has reduced the value of our sites as analogs for future environmental conditions. We then go on to discuss the effects of altered plant communities on soil processes and ecosystem services.

Line 435: The ref of Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., Wilson, J.R.U. and Richardson, D.M. 2011. A proposed unified framework for biological invasions. Trends in Ecology and Evolution. 26(7): 333-339. doi:10.1016/j.tree.2011.03.023 would fit well here.
Line 436: Another interesting ref might be from Kowarik et al about urban wilderness in Berlin.

Thank you for the suggestions, we have included these two references throughout the discussion (line 834, 848, and 1074).

Lines 437: Would it be an option that seeds are still rare from non-natives related to natives? This might then change soon? At least it could be a question of time and it might change. You mention this as a potential ecological time lag....there is this phase model from Blackburn et al. that might fit well. After the establishment phase of non-native species the bum and burst phase might follow.

We have revised this sentence to incorporate that introduced species may be going through some phase change as explained by the model developed by Blackburn and others.

Line 449 ff: This seems quite clear and there are refs around, see e.g. Conedera and Schoenenberger in studies of Ticino in Switzerland.
Line 456ff: There are many refs from across the globe that support your statement here. I would suggest to refer a bit broader.

We have revised this paragraph to include the works of Conedera and Schoenenberger, as well as other non-US based references.

Line 612: and probably also different soil conditions unnaturally enriched with nutrients?

This sentence has been revised (lines 1097 – 1099) to clarify that soil conditions is also an important factor to consider in lands previously used for agriculture.

For the discussion, it might be worth discussing also management objectives of urban forest or tree managers. This might have a strong influence and must be considered anyway. Objectives to make cities greener must include non-native species that might become invasive with a time lag. The potential list that must consider urban environmental condidtions, narrow the list of species quite a bit.

This is an interesting topic that is the focus of another paper in review by our research group. However, the focus of this paper is on what we can learn from spontaneously developing novel communities so we have not added discussion of management objectives for urban forests here.

August 11, 2023

Dear Editors-in-Chief Monica G. Turner and Stephen R. Carpenter,

Please consider the revised and resubmitted manuscript titled “Woody plant-soil relationships in interstitial spaces have implications for future forests within and beyond urban areas,” by Gisselle A. Mejía, Peter M. Groffman, Meghan L. Avolio, Anika R. Bratt, Jeannine Cavender-Bares, Noortje Grijseels, Sharon J. Hall, James Heffernan, Sarah E. Hobbie, Susannah B. Lerman, Jennifer L Morse, Desiree L. Narango, Christopher Niell, Josep Padullés Cubino, Tara L.E. Trammell as a resubmission for publication in *Ecosystems*.

We have extensively revised the manuscript (previously ECOSYSTEMS MS# ECO-23-0052) in response to the thoughtful reviewer comments and hope and feel that we have produced a substantially changed and improved manuscript. We would like to request that this revised version of the manuscript be handled by the same subject matter editor (Matthias Bürgi) and the same reviewers. The comments from the editor and the reviewers were extremely useful in the revision process. The anonymous reviewers acknowledged that the topic is extremely important and of high relevance to better understand novel ecosystems and provisioning of future ecosystem services in urban areas and beyond.

In relatively unmanaged interstitial spaces (i.e., spontaneously forested areas surrounded by residential development), native and non-native vegetation have the potential to mix and assemble into new or novel communities. Our study examined differences in woody plant community composition between interstitial areas - at the residential-wildland interface - and natural reference areas in six cities in the continental U.S. (Baltimore, MD; Boston, MA; Los Angeles, CA; Miami, FL; Minneapolis- St. Paul, MN; and Phoenix, AZ). We also examined whether these differences in woody plant community composition in interstitial and reference areas are related to variation in soil C and N cycling processes. We found that there are marked differences in woody vegetation composition between interstitial and reference areas in six cities across the U.S. These differences are likely the result of a greater proportion of introduced species in interstitial sites, and variation in inherent N availability. These results increase our basic understanding of novel ecosystems that have emerged from transportation and spread of introduced species and have assembled without human intervention. The potential effects of these novel ecosystems are largely unknown, but they are of great concern due to growing urban expansion and land-use change. I hope that this topic is of interest to readers of *Ecosystems*.

This manuscript has not been previously published and is not currently under consideration by another journal. There are no known conflicts of interest associated with this publication, and financial support provided for this study did not influence the results of the research. As the corresponding author, I confirm that all authors have approved of and have agreed to submit the manuscript to *Ecosystems*.

I hope that this manuscript can be considered for publication in the journal, and I look forward to your response.

Sincerely,

gisselle Mejía

Gisselle A. Mejía