nature ecology & evolution

ARTICLES

PUBLISHED: 10 APRIL 2017 | VOLUME: 1 | ARTICLE NUMBER: 0123

Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge

Dietrich J. Epp Schmidt[1], Richard Pouyat[2], Katalin Szlavecz[3], Heikki Setälä[4], D. Johan Kotze[4], Ian Yesilonis[5], Sarel Cilliers[6], Erzsébet Hornung[7], Miklós Dombos[8] and Stephanie A. Yarwood[1]*

Urbanization alters the physicochemical environment, introduces non-native species and causes ecosystem characteristics to converge. It has been speculated that these alterations contribute to loss of regional and global biodiversity, but so far most urban studies have assessed macro-organisms and reported mixed evidence for biodiversity loss. We studied five cities on three continents to assess the global convergence of urban soil microbial communities. We determined the extent to which communities of bacteria, archaea and fungi are geographically distributed, and to what extent urbanization acts as a filter on species diversity. We discovered that microbial communities in general converge, but the response differed among microbial domains; soil archaeal communities showed the strongest convergence, followed by fungi, while soil bacterial communities did not converge. Our data suggest that urban soil archaeal and bacterial communities are not vulnerable to biodiversity loss, whereas urbanization may be contributing to the global diversity loss of ectomycorrhizal fungi. Ectomycorrhizae decreased in both abundance and species richness under turf and ruderal land-uses. These data add to an emerging pattern of widespread suppression of ectomycorrhizal fungi by human land-uses that involve physical disruption of the soil, management of the plant community, or nutrient enrichment.

We are in an era of unprecedented human impact[1] as human modification of the landscape alters ecosystem function[2,3], shifting the local physical, chemical and biotic environments[4–6]. Urban ecosystems favour species that are well-adapted to a variety of anthropogenic processes such as frequent disturbance, increased nutrient inputs, landscape fragmentation and facilitated dispersal[7–9]. The impact of urbanization on global species distribution has been characterized as biotic homogenization[6,8,10]. It has been suggested that biotic homogenization is a major mechanism of global biodiversity loss[7]. However, there is conflict in the literature, as biotic homogenization is often interpreted to mean that particular species are expected to appear across all urban areas and exclude endemic populations[11]. Although it is possible that urbanization leads to the spread of cosmopolitan species, such as *Columba livia domestica* (pigeons) or *Festuca arundinacea* (tall fescue grass), it is also possible for different urban centres to contain different species, for example *F. arundinacea* and *Poa pratensis* (Kentucky blue grass), but converge in terms of functional guilds or traits[12]. Such convergence can lead to biodiversity loss if it results in the exclusion of one species or set of species in favour of another[9]. It is important to note that highly endemic species are more vulnerable to being driven extinct by local exclusion events[13].

Extinction is not measured directly[8] and thus must be inferred. When community data are examined using dissimilarity indices, extirpation is usually captured implicitly only when total richness decreases, resulting in a smaller denominator (typically similarity/dissimilarity indices place total diversity of combined sites in the denominator, and shared/unique species in the numerator) and thus a greater similarity (or convergence) among sites[8]. But convergence could be due to an increase in shared taxa without any loss in diversity across sites; to account for this possibility, one must also examine species richness to confirm that convergence was due to species loss at each site. Thus, it is not possible to infer global biodiversity loss using a single metric, and instead we must infer biodiversity loss based on a constellation of features of the global community. In the case of soil microbial communities, we considered global biodiversity loss to be likely only if they met the following conditions: first, there is a strong geographic pattern (representing a high 'average' degree of endemism and thus vulnerability to extinction); second, there is a convergence in community composition similarity (implying a decreased prevalence of unique taxa); and third, there is a decrease in abundance and species richness within each sample (demonstrating site-specific decreases in biodiversity)[8,14]. We hypothesized that urban land-use will structure the microbial community, causing a global convergence of community composition under urban land-use relative to reference ecosystems, and potentially lead to biodiversity loss. To test this, we compared the soil microbial communities from three different urban land-use categories and a reference site, in five cities, on three continents.

Soils were sampled from five cities: Baltimore, USA; Helsinki and Lahti, Finland; Budapest, Hungary; and Potchefstroom, South Africa (Fig. 1). These cities are all part of the Global Urban Soil

[1]Environmental Science and Technology, University of Maryland, College Park, Maryland 20742, USA. [2]USDA Forest Service, Research & Development, Washington, District of Columbia 20250, USA. [3]Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, Maryland 21218, USA. [4]Department of Environmental Sciences, University of Helsinki, FIN-15140, Lahti, Finland. [5]USDA Forest Service, c/o Baltimore Ecosystem Study, Baltimore, Maryland 21228, USA. [6]Unit of Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa. [7]Department of Ecology, University of Veterinary Science, Budapest Pf. 2, Hungary. [8]Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest Pf. 102, Hungary. *e-mail: syarwood@umd.edu

Ecology and Education Network[5] (GLUSEEN), a distributed experimental network where agreed upon protocols are used to investigate urban soils. The study design used in GLUSEEN constitutes previously defined land-use (habitat) categories that represent a range of disturbance and management impacts of the urban landscape (Table 1). In our definition, disturbance is an event that substantially physically disrupts the soil profile. We define the management category as habitat subjected to intentional and repeated alteration that maintains a particular state of the ecosystem. Four land-use categories were sampled: reference, remnant, turf and ruderal (Table 1). Reference sites were unmanaged, undisturbed soils located outside the urban matrix that represented native soil type and biome typical of the region[5]. A typical reference site is one that has been intentionally set aside for habitat conservation or preservation. Remnant sites are reflective of the reference habitat, but are embedded within the urban matrix (that is, are unused or undeveloped lots with similar vegetative cover to those found in reference sites). Turf sites included park, residential and municipal lawns maintained in a stable state due to mowing. Ruderal sites were chosen based on a recent history of substantial disturbance to the soil profile, typically associated with construction or demolition activity. Each land-use category was replicated five times within each city, meaning that 20 sites were sampled in each city and a total of 100 sites were sampled in the study.

Results

An important indicator of a community's vulnerability to biodiversity loss is the degree of endemism among its members[13]; cosmopolitan organisms are less vulnerable to being driven extinct by local exclusion events (either by habitat alteration or through competition) than are highly endemic species. We used the strength of geographic patterns in community composition among sites to indicate the 'average' degree of endemism. The abundance of major taxonomic groups differed across cities for all three domains.

The bacterial communities exhibited differentiation among cities and among land-use categories (nested two-level PERMANOVA of Bray–Curtis similarity by land-use categories nested within city: city: $n = 20$, $F = 16.06$, $R^2 = 0.31$, $P = 0.001$; land-use: $n = 5$, $F = 4.14$, $R^2 = 0.30$, $P = 0.001$). Thus bacteria were significantly structured by geography (Fig. 1a,b). Pairwise comparisons show that among the cities, Potchefstroom and Budapest exhibited the greatest differentiation (t-test: $n = 20$, $t = 9.96$, $P < 0.001$), wheras Baltimore bacterial communities had the highest evenness (two-tailed ANOVA of Peilou's J index by city: $n = 20$, $F = 47.92$, $P < 0.001$). A post-hoc Tukey analysis indicated that only Baltimore differed in evenness from other cities ($\alpha = 0.05$). Although some bacterial groups such as Planctomycetes, which averaged 17% of bacterial sequences in Baltimore soils but made up less than 7% of sequences in each of the other four cities (Fig. 1a,b), exhibited noticeable geographic patterns, no single taxon alone explained the pattern of geography. For example, 30 phyla were significant indicators of Baltimore, whereas no other city had more than 8 significant indicator phyla (Monte Carlo test of significance: 1,000 permutations, $\alpha = 0.05$; Supplementary Table 6); SIMPER[15] analysis revealed that many phyla drove differences among pairwise comparisons between cities, particularly for Baltimore (Supplementary Table 7); and in a generalized linear model regression of phyla versus city, roughly 50% of taxa were significant predictors of city category (Supplementary Table 8). Land-use also imposed a filter on the bacterial community; within each city, bacterial communities in the reference and remnant sites were significantly different from turf and ruderal sites. However, the turf and ruderal sites for the individual cities remained distinct, suggesting that although urbanization changes the bacterial community, geographic distribution is still a stronger organizing factor (Fig. 2a). Land-use category did not affect the taxonomic richness (that is, the number of operational taxonomic units (OTUs) per sample; Fig. 2c), which is consistent with recent data from urban parks in New York City[16]. Along with community composition, bacterial gene quantity was determined by multiplying quantitative polymerase chain reaction (Q-PCR)-determined 16S ribosomal RNA (rRNA) gene copy numbers to the percent abundance of sequences matching bacterial OTUs. This multiplication was necessary, because we used the same primers for both Q-PCR and sequencing (Supplementary Information), a primer set that simultaneously amplifies both archaea and bacteria (archaea discussed below). Bacterial gene copy numbers were not significantly different across all site categories (Fig. 2b; two-tailed ANOVA with land-use category blocked by city: $n = 25$, $F = 2.50$, $P = 0.064$).

Fungal communities, like the bacteria, were significantly structured by geography and land-use (PERMANOVA Bray–Curtis dissimilarity by land-use nested within city: city: $n = 20$, $F = 13.19$, $R^2 = 0.30$, $P = 0.001$; land-use: $n = 5$, $F = 3.51$, $R^2 = 0.30$, $P = 0.001$; Fig. 2d). However, unlike the bacteria, turf and ruderal sites contained significantly fewer fungal internal transcribed spacer (ITS) gene copy numbers than the reference and remnant sites (ANOVA with city as blocking factor for land-use category 'treatments': $n = 25$, $F = 4.05$, $P = 0.01$; Fig. 2e). Taxonomic richness did not change between the four site categories (Fig. 2f). The relative proportions of Basidiomycota were highest in Baltimore, then Finland, and decreased in the remaining three cities (Fig. 1a,b).

The archaeal community also exhibited a significant effect of geography and a significant effect of land-use category among archaeal OTUs (Fig. 2g; PERMANOVA of Bray–Curtis dissimilarity indices with land-use categories nested within city: city: $n = 20$, $F = 8.35$, $R^2 = 0.21$, $P = 0.001$; land-use: $n = 5$, $F = 2.92$, $R^2 = 0.28$, $P = 0.001$). More detailed abundance distribution data are provided in the Supplementary Information. Along with composition, archaeal gene quantity was determined by multiplying Q-PCR-determined 16S gene copy numbers to the percent abundance of sequences matching archaeal OTUs. Surprisingly, turf sites contained the most archaeal 16S rRNA gene copies (2.34×10^9 gene copies per gram of dry soil; ANOVA of gene copy number with city as blocking factor for land-use category 'treatments': $n = 25$, $F = 2.8$, $P = 0.044$), with fewer archaeal sequences detected in both the reference and remnant sites (Fig. 2h). Likewise, archaeal species richness was also higher in the turf sites (ANOVA of OTU richness with city as blocking factor for land-use category 'treatments': $n = 25$, $F = 16.53$, $P < 0.001$; Fig. 2i). These data suggest that management practices such as increased nutrients may favour a larger, more diverse archaeal community (see Supplementary Fig. 1 for more detail).

Microbial community composition was correlated with a number of soil physiochemical parameters measured and reported elsewhere[5], further elucidating distribution patterns. Global bacterial and fungal community compositions were highly correlated with soil pH, percent organic matter and organic C (Fig. 1c). Soil pH was the strongest predictor of Bray–Curtis distance in both the bacterial (PERMANOVA: $n = 94$, $R^2 = 0.31$, $F = 23.83$ $P = 0.001$; Fig. 1c) and fungal community (PERMANOVA: $n = 84$, $R^2 = 0.043$, $F = 6.77$, $P = 0.001$; Fig. 1c), a phenomena that has previously been reported[17–19]. The most significant covariate in the archaeal community was also pH (PERMANOVA: $n = 94$, $R^2 = 0.10$, $F = 15.16$, $P = 0.001$; Fig. 1c), followed by humus (PERMANOVA: $n = 94$, $R^2 = 0.024$, $F = 3.68$, $P = 0.003$). Humus was also the second most significant covariate in bacteria (PERMANOVA: $n = 94$, $R^2 = 0.025$, $F = 5.02$, $P = 0.001$), but not fungi, for which potassium was the second most significant covariate (PERMANOVA: $n = 84$, $R^2 = 0.023$, $F = 3.68$, $P = 0.001$; Fig. 1c). These data suggest that the archaeal distribution does not necessarily correspond to bacterial or fungal distributions.

Urban soil microbial community convergence. Convergence of microbial communities represents a decrease in dominance of

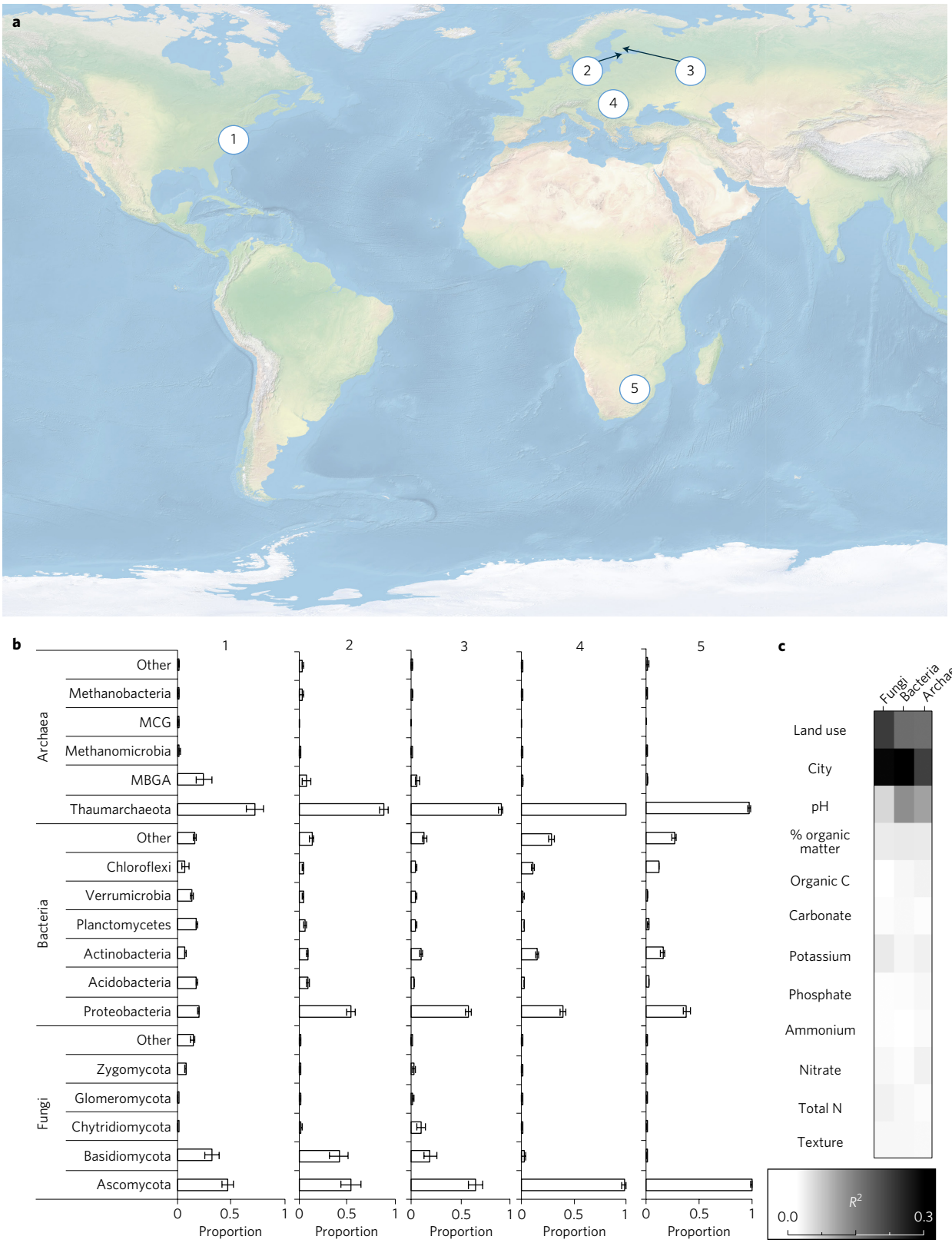

Figure 1 | Geographic distribution of cities sampled, edaphic co-factors and community composition. a,**b**, Geographic distribution of cities (**a**) and the mean ($n = 25$) relative abundance of the most common microbial taxa (**b**; from top to bottom: most abundant six archaeal classes; most abundant seven bacterial phyla; and most abundant six fungal classes). Each taxon is averaged within each of the five cities from left to right: (1) Baltimore, USA; (2) Helsinki, Finland; (3) Lahti, Finland; (4) Budapest, Hungary; (5) Potchefstroom, South Africa. Error bars represent standard error. White circles indicate the location of each city on the map, and the number corresponds to that city's community composition (bar charts 1–5 in **b**). **c**, Heat map representing the R^2 values (0.0–0.3) of correlations between edaphic factors and community composition of each community. Map made with Natural Earth (http://www.naturalearthdata.com/).

Table 1 | A summary of the four land-use categories defined along a disturbance–management spectrum.

Site	Within city	Disturbed soil	Unmanaged
Reference			X
Remnant	X		X
Turf	X	X	
Ruderal	X	X	X

site-specific species, and may result in biodiversity loss. We measured convergence as a reduction of between-site variance under the three categories of urban land-use, and regarded reference sites as a proxy for the historic state of the soil conditions for each city. We used Bray–Curtis dissimilarity index to compare site similarity. If microbial communities had converged, we expected to see a smaller multivariate dispersion (variance) of urban sites among cities compared with a larger multivariate dispersion of reference sites among cities.

We tested for convergence using the betadisper function in the vegan[15] statistical package in R (version 3.1.3; ref. 20), a multivariate analogue of the Levene's test of homogeneity of variances[21]. We analysed variances according to three site groupings (Fig. 3): first we compared the four land-use categories ($n = 25$); then we categorized sites into high or low management impact groups (discussed below; $n = 50$); and finally we categorized sites into groups based on whether they fall inside or outside the urban matrix (inside $n = 75$; outside $n = 25$). These groupings were applied to the global dataset (Fig. 3) and within each individual city (Supplementary Fig. 4) to elucidate potential drivers of convergence.

The archaeal communities showcased convergence particularly well; the archaeal community converged in turf and ruderal sites relative to reference and remnant sites (two-tailed ANOVA of multivariate dispersion by land-use: $n = 25$, $F = 19.78$, $P < 0.001$; Fig. 3a). The fungal community also converged (two-tailed ANOVA of multivariate dispersion by land-use: $n = 25$, $F = 2.73$, $P = 0.048$); ruderal sites were less variable relative to reference sites. The bacterial community differed in variance between site categories (two-tailed ANOVA of multivariate dispersion by group: $n = 25$, $F = 3.31$, $P = 0.023$); however, a post-hoc Tukey's HSD indicated that this was driven by a slight increase in variance among remnant sites and slight decrease in variance among turf sites relative to the reference. Thus, although the variance of remnant and turf sites was different, the variance of neither turf nor ruderal sites differed significantly from the reference (Fig. 3a). These data show that the three microbial domains each respond differently to urbanization, and that bacteria in particular did not converge.

Using the same approach, we explored whether soil microbial communities converge simply because they exist within the urban matrix, or whether direct management and disturbance lead to a greater degree of convergence. To do this, we conducted two tests. First, we grouped all reference and remnant sites together (low impact), and compared them with a grouping of all turf and ruderal (high impact; Fig. 3b). In this case, all three microbial communities converged (two-tailed ANOVA of multivariate dispersion by group: archaea: $n = 50$, $F = 19.31$, $P < 0.001$; bacteria: $n = 50$, $F = 9.82$, $P = 0.002$; fungi: $n = 50$, $F = 6.32$, $P = 0.014$). To test for differences due to being within the city, reference sites were compared with a grouping of remnant, turf and ruderal (Fig. 3c). Soil bacteria and fungi did not converge in cities, suggesting that human actions such as soil disturbance and direct management of the flora alter microbial communities more than other indirect effects such as increased air temperature or atmospheric nitrogen deposition within an urban centre. Thus, distinct human processes define each of the high impact sites, yet when grouped together they have lower variation than the reference and remnant sites across all three domains. This suggests that one or both of the following mechanisms is at play: a common set of organisms is able to exploit these altered environments; or, as has been reported in the biotic homogenization literature for other taxa, there is a consistent suppression of certain functional groups (guilds)[9].

To answer whether urban land-use suppresses organisms of certain metabolic strategies (or functions), we assigned functional guilds to our identified OTUs, using publicly available databases. Attempts to characterize bacterial and archaeal functional differences were hampered due to database limitations. Functional guild assignment by FunGUILDs pointed to a loss of ectomycorrhizal fungi (ECM) as the main driver for convergence and differences between land-uses in the fungal community. When ECM sequences were examined at the OTU level, there were significant effects of land-use nested within city (Fig. 4a; two-tailed PERMANOVA of Bray–Curtis distance by land-use nested within city: city: $n = 12–20$, $F = 2.32$, $P = 0.001$; land-use: $n = 3–5$, $F = 1.35$, $R^2 = 0.23$, $P = 0.001$). Turf and ruderal sites converged relative to reference and remnant sites at the family level (ANOVA of multivariate dispersion by group: $n = 12–20$, $F = 6.06$, $P = 0.016$; Fig. 4d). There was also a significant decrease in ECM abundance (ANOVA of OTU sequence calls by land-use: $n = 25$, $F = 5.25$, $P = 0.003$; Fig. 4b) and OTU richness of ECM within the turf and ruderal sites (ANOVA of OTU richness by land-use: $n = 25$, $F = 8.63$, $P < 0.001$; Fig. 4c). These analyses underestimate the degree of ECM diversity loss because there were 14 sites omitted due to a lack of ECM sequences (one reference site, two remnant sites, four turf sites and seven ruderal sites); five of the omitted sites, including the reference, remnant and three of the ruderal sites, were from Potchefstroom, which is situated in a grassland biome that typically has few ECM hosts. Our ECM data support previous research that reported lower ECM colonization in disturbed urban soils[22]. As we targeted DNA rather than mRNA, these data also suggest that if a tree is planted in these soils in the future, there will not be ECM present to colonize the new vegetation.

Discussion

A major concern of urbanization is that biotic homogenization will lead to global biodiversity loss. However, the pattern of convergence among urban biotic communities does not inherently imply a loss in biodiversity[8]. Our fungal, archaeal and bacterial datasets demonstrate how biotic homogenization may be associated with biodiversity loss in some communities, but not others. Our most compelling evidence for biodiversity loss was found in the fungal dataset, where we found evidence of endemism and an urban filter effect; the convergence of ruderal sites relative to the reference shows that this filter is consistent across cities globally. Many factors probably contribute to this observed change, including soil factors such as pH and nutrient concentration, but also the presence of particular host plants[23]. We found that the convergence of urban sites was associated with a precipitous decline in abundance and richness of ECM in the four northern cities. There is a caveat, however; of all the land-use categories in Potchefstroom (South Africa), turf sites exhibited the highest abundance of ECM, with a similar average abundance to those found in other turf sites in the other cities (representing a true convergence of ECM abundance under turf management). It is important that we investigate more cities that are situated in diverse biomes, outside temperate or boreal forest ecosystems, to confirm this result. It is likely that ECM are suppressed in northern cities due to shifts in vegetation; because ECMs are mutualists, they rely on the colonization of woody plants, and when these plants are removed this functional group declines. Our results support previous research that human management and disturbance decreases ECM diversity, particularly in temperate and boreal biomes where they are most diverse[24]. Our data also support previous studies suggesting that loss of diversity may be an important

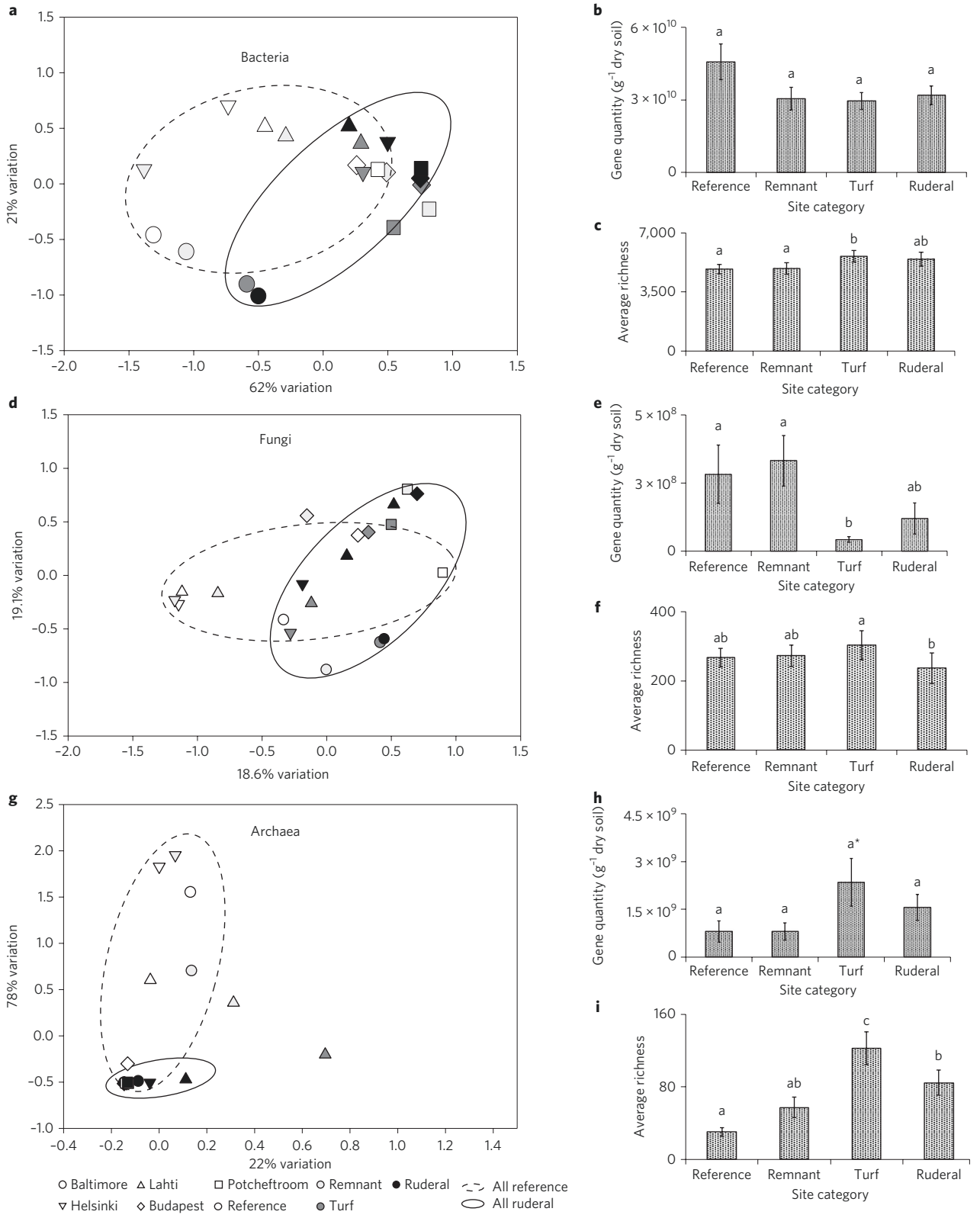

Figure 2 | Effect of land-use on beta diversity, abundance and alpha diversity of microbial domains. a–c, NMS ordination (**a**; final stress value 9.44), gene quantities per gram of dry soil under each land-use (**b**) and average species richness (**c**) for bacteria. **d–f**, NMS ordination (**e**; final stress value 11.84), gene quantities per gram of dry soil under each land-use (**e**) and average species richness under each land-use (**f**) for fungi. **g–i**, NMS ordination. (**g**; final stress value 3.46), gene quantities per gram of dry soil under each land-use (**h**) and average species richness under each land-use (**i**) for archaea. Each point in each ordination represents the mean Euclidian position ($n = 5$) for each land-use category in each city. Gene quantities per gram of dry soil represent the mean value under each land-use ($n = 25$). In all cases, error bars represent standard error and letters indicate post-hoc Tukey's HSD significance at $\alpha = 0.05$. The asterisk indicates an instance where the ANOVA found a significant effect of land-use (two-tailed ANOVA of archaeal abundance by land-use: $F = 2.81$, $P = 0.044$,), but a Tukey's HSD did not identify any pairwise comparisons that met $\alpha = 0.05$; reference and remnant sites each differed from turf at $\alpha = 0.07$.

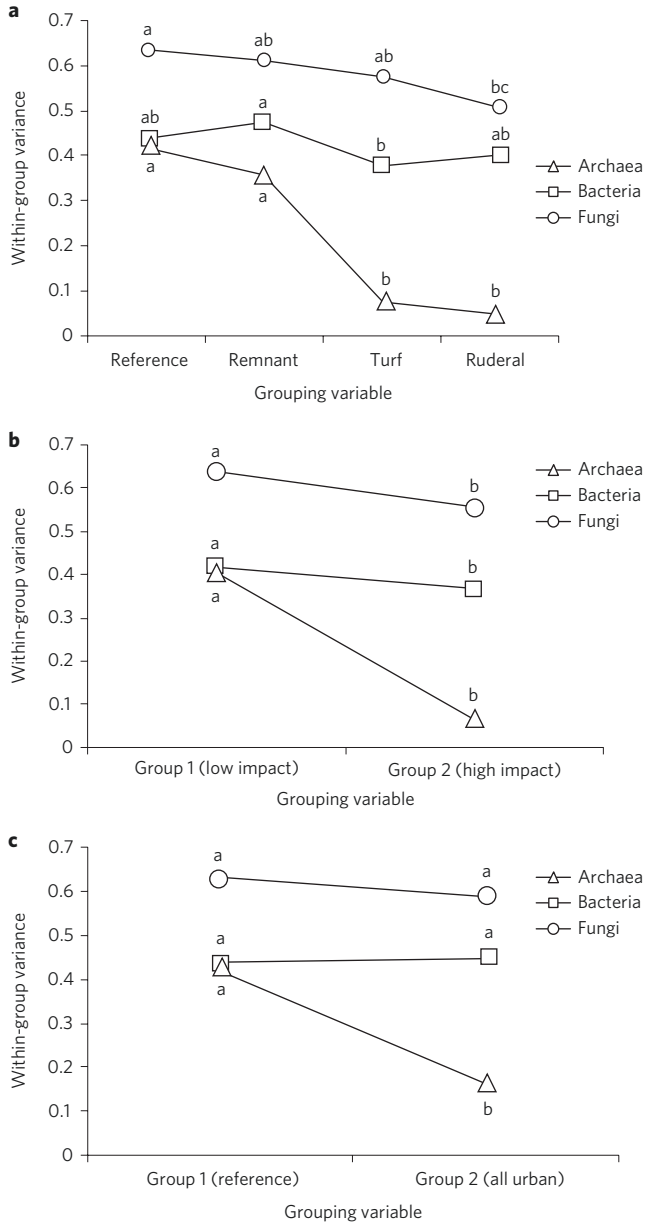

Figure 3 | Within-group multivariate dispersal by grouping variable in each domain. Multivariate dispersion, representing within-group variance, among communities at the OTU level. Points represent the total variance of all cities. **a**, Group 1 is reference, group 2 is remnant, group 3 is turf, group 4 is ruderal (each with an $n = 25$). Archaea ($F = 19.78$, $P < 0.001$), bacteria ($F = 3.31$, $P = 0.023$) and fungi ($F = 2.73$, $P = 0.048$) have unequal variances. **b**, Group 1 is reference and remnant sites, and group 2 is turf and ruderal (each with an $n = 50$). Archaea ($F = 19.31$, $P < 0.001$), bacteria ($F = 9.82$, $P = 0.002$) and fungi ($F = 6.32$, $P = 0.014$) have unequal variance among groups. **c**, Group 1 is reference sites and group 2 is all other sites ($n = 25$ for reference and $n = 75$ for all others). Only archaea had unequal variances among groups ($F = 16.64$, $P < 0.001$). Lowercase letters indicate significant difference between groups, determined using a multivariate version of the Levene's test for equality of variances.

factor driving convergence[8,9], and provide the first microbial evidence supporting the connection between biotic homogenization and global biodiversity loss[7].

In contrast, convergence of the archaeal communities provides a scenario in which communities may converge without losing biodiversity. Archaeal communities converged in turf and ruderal sites, suggesting that urbanization is an environmental filter that increases community similarity. But there was little evidence that endemic species were being excluded; archaeal species richness and abundance actually increased in turf and ruderal sites, suggesting that new archaea may not have been competing with endemic diversity for resources. The bacterial community presents yet a third response. Even though the bacterial communities did exhibit geographic distribution patterns and urbanization did seem to impact bacterial abundance, urban land-use does not seem to impact bacterial diversity *per se*; there was little evidence for convergence of the community composition and no change in species richness.

Our study shows a similar geographic structuring of the microbial community to that found in atmospheric dust distributed across the USA[25]; although we found no evidence of convergence among cities and little evidence of convergence within cities relative to the soil community reference sites, a previous study[25] found that the urban atmospheric microbial community was enriched with soil-associated taxa, and homogenized within the city relative to more rural locals. In cases where some bacterial lineages seem to be extirpated locally, the rapid rate of bacterial evolution[26,27] and horizontal transfer of genetic materials[28] may have allowed bacterial diversity and function to remain intact. Given the numerous differences between soil archaea and bacteria, we cannot be sure exactly why they responded differently. There is evidence, however, that each domain may inhabit different niches. For example, researchers studying bacterial and archaeal ammonia oxidizers have shown that ammonia-oxidizing archaea are able to use lower concentrations of ammonia[29] and increase in abundance with lower pH[30], suggesting that both metabolic strategy and the soil environment may be a factor in the response of bacteria and archaea to urbanization[31,32].

Although several previous studies have examined soil microbial communities within a single city[22,33], those studies could not infer global diversity loss, because they did not examine multiple regions. Our regional comparisons have shown that although urbanization leads to changes in community composition across a wide range of microbes, communities from each of the three microbial domains had specific response patterns to the different land-use categories. We found that ECM seemed to be the only group that experienced significant diversity loss due to urban land-uses. Our findings on the loss of ECM are consistent with the literature documenting ECM suppression by management[24] and nitrogen enrichment[34], and suggest that loss of host species through land conversion (to agriculture and otherwise) may be significant factors in ECM loss. This is consistent with studies showing that habitat loss is perhaps the most significant driver of global biodiversity loss[35]. Moreover, ECM are important members of the community because they increase the efficiency of plant nutrient capture, and increase carbon storage in temperate and boreal soils[36]. To mitigate the loss of these important organisms, we recommend that future urban planning should do the following: include remnants of native plant communities that will support these microbes; landscape using native host species; and reduce nutrient loading for turf sites in particular.

Methods

Soil sampling. In each city, we took special care to ensure that soil type was consistent across all selected site categories (Table 1). In particular, we favoured well-drained upland soils, avoiding riparian zones and soils that are often saturated. Each sample consisted of five 2.5 cm diameter × 10 cm deep soil cores (excluding the Oi (L) and Oe (F) horizons), which were homogenized in a sterile plastic bag using a sterile scoopula. A soil sample from each homogenized bag, weighing ~2 g, was preserved in 4 ml Lifeguard preservation solution (MoBio). This procedure was repeated at 5 locations per land-use category in each of the five cities, resulting in 100 samples in total ($n = 5$). Physical and chemical soil properties of the sampled soils have been published previously[5].

Illumina amplicon sequencing returned 12.5 million archaeal and bacterial 16S rRNA sequences, and 6.2 million fungal ITS sequences. By comparing 16S rRNA sequences to the Greengenes database at 97% similarity, 270,000 OTUs were identified across all samples. This included 3,700 archaeal OTUs, 255,481

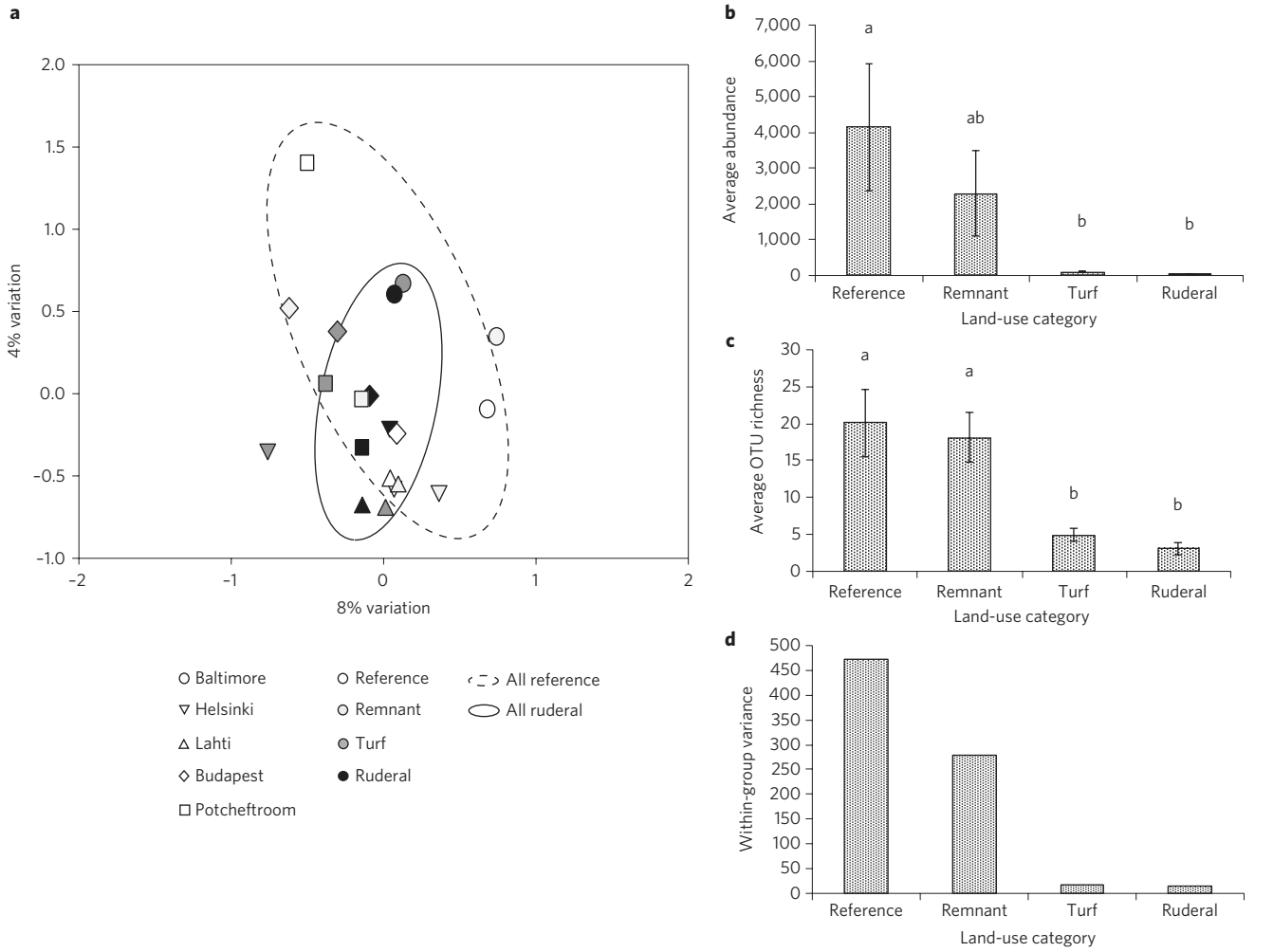

Figure 4 | Relationship bewtween land-use, and ECM diversity and abundance. a, NMS ordination of the ectomycorrhizal community (final stress value: 15.37) showing both land-use and city effects (two-tailed PERMANOVA of Bray–Curtis distance by land-use nested within city: land-use: $F = 2.55$, $P = 0.002$; city: $F = 5.46$, $P = 0.001$). Symbols represent the centroid of each land-use category in each city ($n = 3$–5; some sites were omitted from the analysis because they lacked any ECM). **b**, The average sequence abundance within each land-use category, measured after rarefying the fungal community to 20,000 OTUs and correcting the sequence abundance for differences in ITS gene abundance across sites. **c**, Average OTU richness of the ectomycorrhizal community, grouped according to land-use category. Error bars represent standard error. **d**, Within-group variance, using groups defined by land-use categories, of the OTU richness.

bacterial OTUs and 17,439 unassigned OTUs. Rarefaction analysis did not result in plateaued curves, suggesting additional sequencing would yield even more OTUs. On average, 62,132 sequences per sample were generated for the fungi and a total of 12,620 OTUs were identified by matching to the UNITE database[37]. Rarefaction curves of the fungal sequences indicated that this sequencing was sufficient to accurately sample fungal diversity. Rarefaction curves and sequences for each sample can be found in the Supplementary Information.

DNA extraction and preparation. Soils were extracted following the MOBIO Laboratories Powerlyzer Powersoils DNA isolation kit protocol (MoBio), with two exceptions. First, instead of adding 0.25 g of dry soil to the bead beating tubes in step two, 750 µl of soil suspended in Lifeguard solution was added to each tube. Second, a Fastprep-24 set to 3.5 m s^{-1} for 45 s was used in cell lysis (MP Biomedical). The exact weight of each soil sample used for extraction was calculated based on the measured weight of a 0.5 ml aliquot of soil slurry dried at 60 °C for one week. Extracted DNA was quantified using a QuBit 2.0 Fluorometer (Invitrogen). DNA extracts were diluted to 1.25 ng μl^{-1} for sequencing amplification and Q-PCR. DNA samples were stored at −20 °C between each analysis.

Microbial community analysis. Q-PCR for the combined bacterial and archaeal community was performed using the primers F515 and 806R of the V4 region[38] (Supplementary Table 2). Fungal community was quantified by targeting the ITS region using ITS1F and 5.8S (ref. 39; Supplementary Table 2). The master mix for each Q-PCR reaction was as follows: 16 µl of Kicqstart Sybr Green Readymix with Rox (Sigma), 1.0 µl of 10 µM forward and reverse primers, and 2 µl DNA diluted to 1.25 ng ml^{-1}. The standard curve was a 1:10 serial dilution of 2.5 µg ml^{-1} DNA and concentrations are calculated based on the size of the plasmid containing an insert. An inhibition correction was applied using the following strategy: 2 µl of each sample was pooled and quantified using the fluorometer; a soil standard curve consisted of 1:10 serial dilutions. We calculated a soil efficiency standard correction according to previously reported protocol[40], but rejected this correction because it applied a more significant correction to samples with higher gene counts rather than lower gene counts. There was a total of ten Q-PCR plates, five for 16S quantification and 5 for ITS quantification. Each contained 20 samples; one from each land-use category in each city.

Amplicon sequencing targeted a section of the 16S rRNA using primers 515F + adapter and 806R + adapter (Supplementary Table 2), and the ITS1 region in fungi using primers ITS1F + adapter and ITS2 + adapter (Supplementary Table 2). Adapters were ligated to the forward and reverse strands, and the libraries were indexed using the Nextera XT 96 index kit (Illumina). Samples were sequenced using an Illumina MiSeq 2000 at the Oregon State University Center for Genome Research and Biocomputing. The sequencing kit produced 250 base pairs, paired-end read and was run with a 10% Phix.

Bioinformatics and statistical analysis. The QIIME pipeline[41] was used in processing sequence libraries, with a few variations between the 16S and ITS sequence analysis. For ITS samples, the number of allowable homopolymer repeats in the split-libraries was increased from six to eight, because at the default of six, 80% of the sequences were eliminated. For both gene loci, we conducted

open-reference OTU picking. The Ribosomal Database Project (RDP)[42] classifier was used to assign taxonomy of representative fungal sequences against the UNITE database[37]; we used uclust to cluster the 16s OTUs and blasted representative sequences against the Greengenes database[43]. Both datasets were clustered at 97% similarity. Analyses at lower taxonomic resolutions were clustered according to putative taxonomy (that is, based on the Greengenes database). The reverse primers were removed in the split-libraries step and the minimum overlap in joining the paired-end reads (ea-utils[44], fastq-join.py) was increased from 6 to 50 bases in both amplicon libraries. All other functions in the QIIME pipeline were run on default settings. Our QIIME pipeline workflow went as follows: fastq-join.py; convert_fastqual_Fastq.py; split-libraries.py; cat; pick-otus.py; pick_rep_set.py; assign_taxonomy.py; make_otu_table.py; biom convert; and summarize_taxa.py. We used outputs from the make_otu_table and summarize_taxa functions in our downstream data analysis in R and PC-ORD. Full scripts can be found in the Supplementary Information.

The relative abundance of putatively defined archaea, bacteria and fungi were calculated separately for each domain by dividing the number of sequence calls for a given taxa in each sample by the total number of sequences in any given sample. Land-use categories within each city were averaged. The abundance of major taxonomic groups differed across cities for all three domains. Richness was calculated as the total OTUs at each site; 16S sequences were rarefied to 70,000 and ITS sequences were rarefied to 20,000 to ensure comparability of results across sites. Shannon–Weiner and Pielou's *J* diversity indices were also calculated and are available in the Supplementary Information. Both R and PC-ORD platforms were used to construct Bray–Curtis distance matrices. For all statistical analyses, we considered $\alpha = 0.05$ as statistically significant; PC-ORD[45] was used to compare differences in community composition and was also used to calculate indicator values[46] for each taxa, and subsequently employed a Monte Carlo test of significance on indicator values. The betadisper and adonis functions (of the vegan package[15]) in R (ref. 20; version 3.1.3) were used to test for difference in within group variance among site categories and to run nested PERMANOVA, respectively. We also used the vegan package to run blocked two-way ANOVAs and Tukey post-hoc analyses for differences in ITS and 16S gene abundances between site categories, using city as the blocking factor. For all statistical analyses, we applied $\alpha = 0.05$. All R scripts can be found in the Supplementary Information.

For all ECM analysis, each sample was rarefied to 20,000 sequences to standardize sampling effort. R scripts for rarefaction and downstream statistics are available in the Supplementary Information. We used the R statistical packages vegan, stats[20] and lawstat[47] (scripts in Supplementary Information). Ten sites falling under 20,000 sequences were omitted from all statistical analyses, while an additional 14 sites were omitted from our ECM community composition analysis because they lacked any ECM. The fungal database program FUNGuilds was used to assign putative function to identified OTUs[48]. As we rarefied the community to equal sampling depth, we treat sequence abundance as accurately representing the proportion or relative abundance of ECM that make up each community. We standardized sequence abundance across samples using a correction factor calculated by dividing the number of ITS genes in that sample (determined by Q-PCR[49]) by the average number of ITS genes across all samples. Richness values were calculated as the total number of distinct OTUs at each site. Convergence was identified by comparing within-group variances among land-use categories and tested using Levene's test of equality of variances. The non-metric multidimensional scaling (NMS) ordination was constructed in PC-ORD[45], with Beals smoothing applied to the distance matrix, accounting for the high incidence of zeroes within the ECM community.

Code availability. R scripts and data files necessary to replicate this research are publicly available on the Github repository (https://github.com/Djeppschmidt/GLUSEEN).

Data availability. Our sequence data are available on NCBI under project number PRJNA339869.

Received 30 August 2016; accepted 23 February 2017; published 10 April 2017

References

1. Ellis, E. C. & Ramankutty, N. Putting people in the map: anthropogenic biomes of the world. *Front. Ecol. Environ.* **6,** 439–447 (2007).
2. Foster, D. *et al.* The importance of land-use legacies to ecology and conservation. *Bioscience* **53,** 77–88 (2003).
3. Rothwell, A., Ridoutt, B., Page, G. & Bellotti, W. Feeding and housing the urban population: environmental impacts at the peri-urban interface under different land-use scenarios. *Land Use Policy* **48,** 377–388 (2015).
4. Scheffer, M., Carpenter, S., Foley, J. A., Folke, C. & Walker, B. Catastrophic shifts in ecosystems. *Nature* **413,** 591–596 (2001).
5. Pouyat, R. V. *et al.* A global comparison of surface soil characteristics across five cities: a test of the urban ecosystem convergence hypothesis. *Soil Sci.* **180,** 136–145 (2015).
6. Groffman, P. M. *et al.* Ecological homogenization of urban USA. *Front. Ecol. Environ.* **12,** 74–81 (2014).
7. McKinney, M. L. & Lockwood, J. L. Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends Ecol. Evol.* **14,** 450–453 (1999).
8. Baiser, B., Olden, J. D., Record, S., Lockwood, J. L. & McKinney, M. L. Pattern and process of biotic homogenization in the New Pangaea. *Proc. R. Soc. B* **279,** 4772–4777 (2012).
9. Tabarelli, M., Peres, C. A. & Melo, F. P. L. The 'few winners and many losers' paradigm revisited: emerging prospects for tropical forest biodiversity. *Biol. Conserv.* **155,** 136–140 (2012).
10. McKinney, M. L. Urbanization as a major cause of biotic homogenization. *Biol. Conserv.* **127,** 247–260 (2006).
11. Olden, J. D. & Rooney, T. P. On defining and quantifying biotic homogenization. *Glob. Ecol. Biogeogr.* **15,** 113–120 (2006).
12. Fukami, T., Martijn Bezemer, T., Mortimer, S. R. & van der Putten, W. H. Species divergence and trait convergence in experimental plant community assembly. *Ecol. Lett.* **8,** 1283–1290 (2005).
13. Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. Biodiversity hotspots for conservation priorities. *Nature* **403,** 853–858 (2000).
14. Boyero, L. *et al.* Global patterns of stream detritivore distribution: implications for biodiversity loss in changing climates. *Glob. Ecol. Biogeogr.* **21,** 134–141 (2012).
15. Oksanen, J. *et al.* Vegan: Community Ecology Package. R package version 2.0-4 (R Foundation for Statistical Computing, 2016).
16. Reese, A. T. *et al.* Urban stress is associated with variation in microbial species composition—but not richness—in Manhattan. *ISME J.* **10,** 751–760 (2016).
17. Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A. & Cleveland, C. C. Global patterns in belowground communities. *Ecol. Lett.* **12,** 1238–1249 (2009).
18. Rousk, J. *et al.* Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* **4,** 1340–1351 (2010).
19. Bru, D. *et al.* Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. *ISME J.* **5,** 532–542 (2011).
20. R Core Team *A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2015).
21. Anderson, M. J. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **62,** 245–253 (2006).
22. Karpati, A. S., Handel, S. N., Dighton, J. & Horton, T. R. *Quercus rubra*-associated ectomycorrhizal fungal communities of disturbed urban sites and mature forests. *Mycorrhiza* **21,** 537–547 (2011).
23. Jumpponen, A. & Egerton-Warburton, L. M. in *The Fungal Community: Its Organization and Role in the Ecosystem* 3rd edn (eds Dighton, J., White, J. F. & Oudemans, P.) Ch. **7,** 130–164 (Mycology Series Vol. 23, 2005).
24. Tedersoo, L. *et al.* Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Mol. Ecol.* **21,** 4160–4170 (2012).
25. Barberán, A. *et al.* Continental-scale distributions of dust-associated bacteria and fungi. *Proc. Natl Acad. Sci. USA* **112,** 5756–5761 (2015).
26. Taddei, F. *et al.* Role of mutator alleles in adaptive evolution. *Nature* **387,** 700–702 (1997).
27. Tenaillon, O., Toupance, B., Nagard, H. L., Taddei, F. & Godelle, B. Mutators, population size, adaptive landscape and the adaptation of asexual populations of bacteria. *Genetics* **152,** 485–493 (1999).
28. Ochman, H., Lawrence, J. G. & Groisman, E. A. Lateral gene transfer and the nature of bacterial innovation. *Nature* **405,** 299–304 (2000).
29. Verhamme, D. T., Prosser, J. I. & Nicol, G. W. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *ISME J.* **5,** 1067–1071 (2011).
30. Nicol, G. W., Leininger, S., Schleper, C. & Prosser, J. I. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ. Microbiol.* **10,** 2966–2978 (2008).
31. Hanson, C. A., Fuhrman, J. A., Horner-Devine, M. C. & Martiny, J. B. H. Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.* **10,** 497–506 (2012).
32. Nemergut, D. R. *et al.* Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. Rev.* **77,** 342–356 (2013).
33. Ramirez, K. S. *et al.* Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. *Proc. R. Soc. B* **281,** 20141988 (2014).
34. Högberg, M. N., Yarwood, S. A. & Myrold, D. D. Fungal but not bacterial soil communities recover after termination of decadal nitrogen additions to boreal forest. *Soil Biol. Biochem.* **72,** 35–43 (2014).
35. Newbold, T. *et al.* Global effects of land-use on local terrestrial biodiversity. *Nature* **520,** 45–50 (2015).
36. Averill, C. & Hawkes, C. V. Ectomycorrhizal fungi slow soil carbon cycling. *Ecol. Lett.* **19,** 937–947 (2016).

37. Abarenkov, K. *et al.* The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytol.* **186,** 281–285 (2010).
38. Caporaso, J. G. *et al.* Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **6,** 1621–1624 (2012).
39. Fierer, N., Jackson, J. A., Vilgalys, R. & Jackson, R. B. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl. Environ. Microbiol.* **71,** 4117–4120 (2005).
40. Hargreaves, S. K., Roberto, A. A. & Hofmockel, K. S. Reaction- and sample-specific inhibition affect standardization of Q-PCR assays of soil bacterial communities. *Soil Biol. Biochem.* **59,** 89–97 (2013).
41. Edgar, R. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26,** 2460–2461 (2010).
42. Wang, Q., Garrity, G., Tiedje, J. & Cole, J. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73,** 5261–5267 (2007).
43. DeSantis, T. *et al.* Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **72,** 5069–5072 (2006).
44. Aronesty, E. ea-utils (2011); https://expressionanalysis.github.io/ea-utils/
45. McCune, B. & Mefford, M. *PC-ORD: Multivariate Analysis of Ecological Data* (MjM Software, 2011).
46. Dufrêne, M. & Legendre, P. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.* **67,** 345–366 (1997).
47. Gastrwirth, J. *et al.* lawstat v. 3.0 (2015); https://cran.r-project.org/web/packages/lawstat/index.html
48. Nguyen, N. H. *et al.* FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **20,** 241–248 (2016).
49. Dlott, G., Maul, J. E., Buyer, J. & Yarwood, S. Microbial rRNA:rDNA gene ratios may be unexpectedly low due to extracellular DNA preservation in soils. *J. Microbiol. Methods* **115,** 112–120 (2015).

Acknowledgements

This research was funded through NSF ACI 1244820; soil chemical analysis was funded through SZIE-ÁOTK: KK-UK-12007. We acknowledge G. Dlott for technical support in QIIME, and R. E. Draskovits, S. Molnar, E. Powell, M. Bernard, Z. Toth and S. Mishra assisted with field sampling.

Author contributions

D.J.E.S. constructed the DNA and sequencing libraries, and conducted Q-PCR, bioinformatics processing and statistical analyses. K.S. is the PI of the grant, designed the study and selected the sites in Baltimore. R.V.P., H.S., D.J.K., E.H., S.C. and I.Y. designed the study, selected the sites and participated in soil sampling. M.D. participated in soil sampling and provided nutrient data on soils; S.A.Y. designed the study, and oversaw all of the lab work, bioinformatics and data analysis. All authors discussed results and commented on the manuscript.

Additional information

Supplementary information is available for this paper.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to S.A.Y.

How to cite this article: Epp Schmidt, D. J. *et al.* Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. *Nat. Ecol. Evol.* **1,** 0123 (2017).

Competing interests

The authors declare no competing financial interests.