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# Supplementary Materials for

## Global homogenization of the structure and function in the soil microbiome of urban greenspaces

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**The PDF file includes:**

Figs. S1 to S17
Tables S1 and S2
Legend for table S3

**Other Supplementary Material for this manuscript includes the following:**

(available at advances.sciencemag.org/cgi/content/full/7/28/eabg5809/DC1)

Table S3

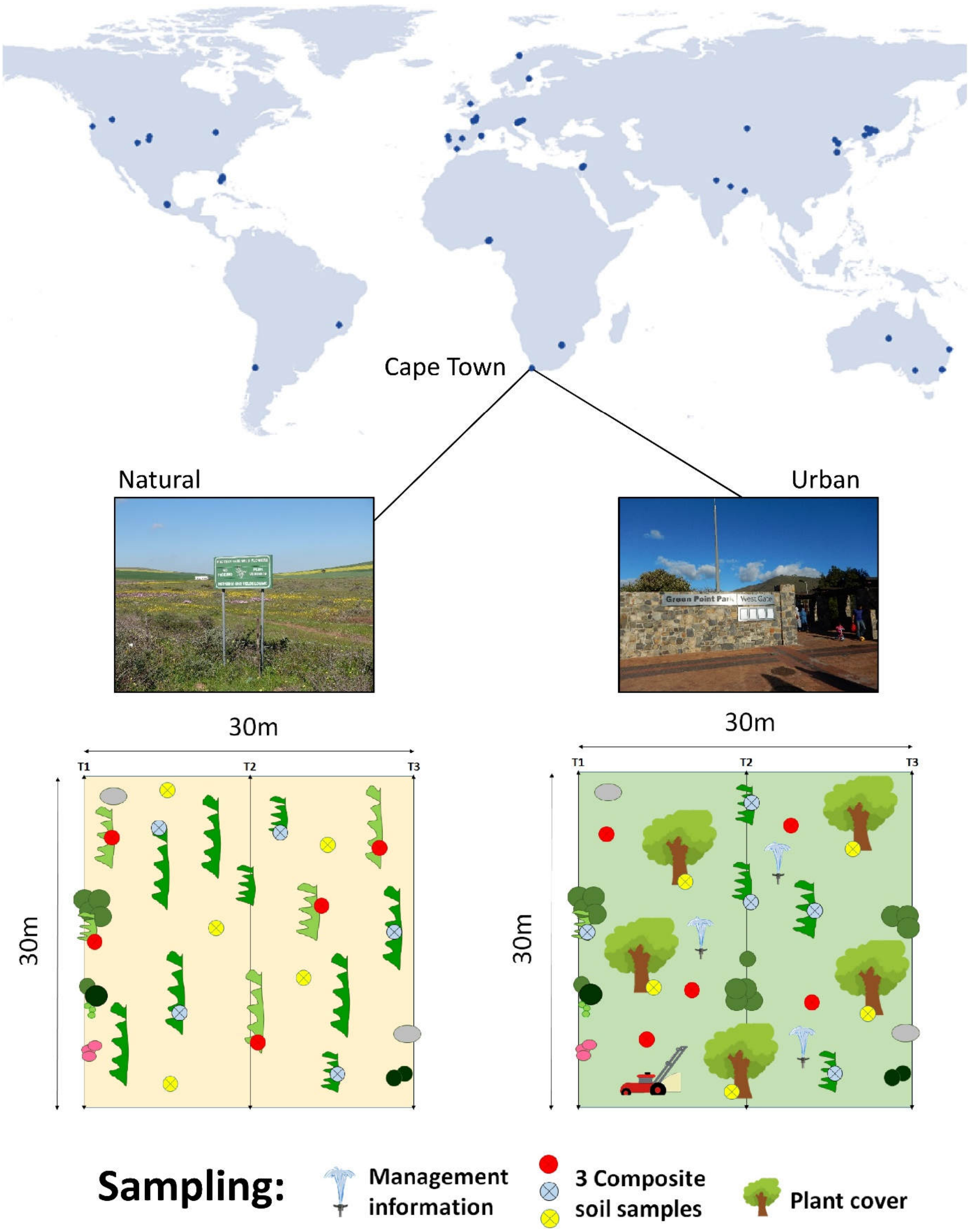


**Figure S1.** Summary of the sampling design in one of the 56 urban greenspaces and natural ecosystems included in this study. T = transect. Photo Credit: Thulani P. Makhalanyane, University of Pretoria.

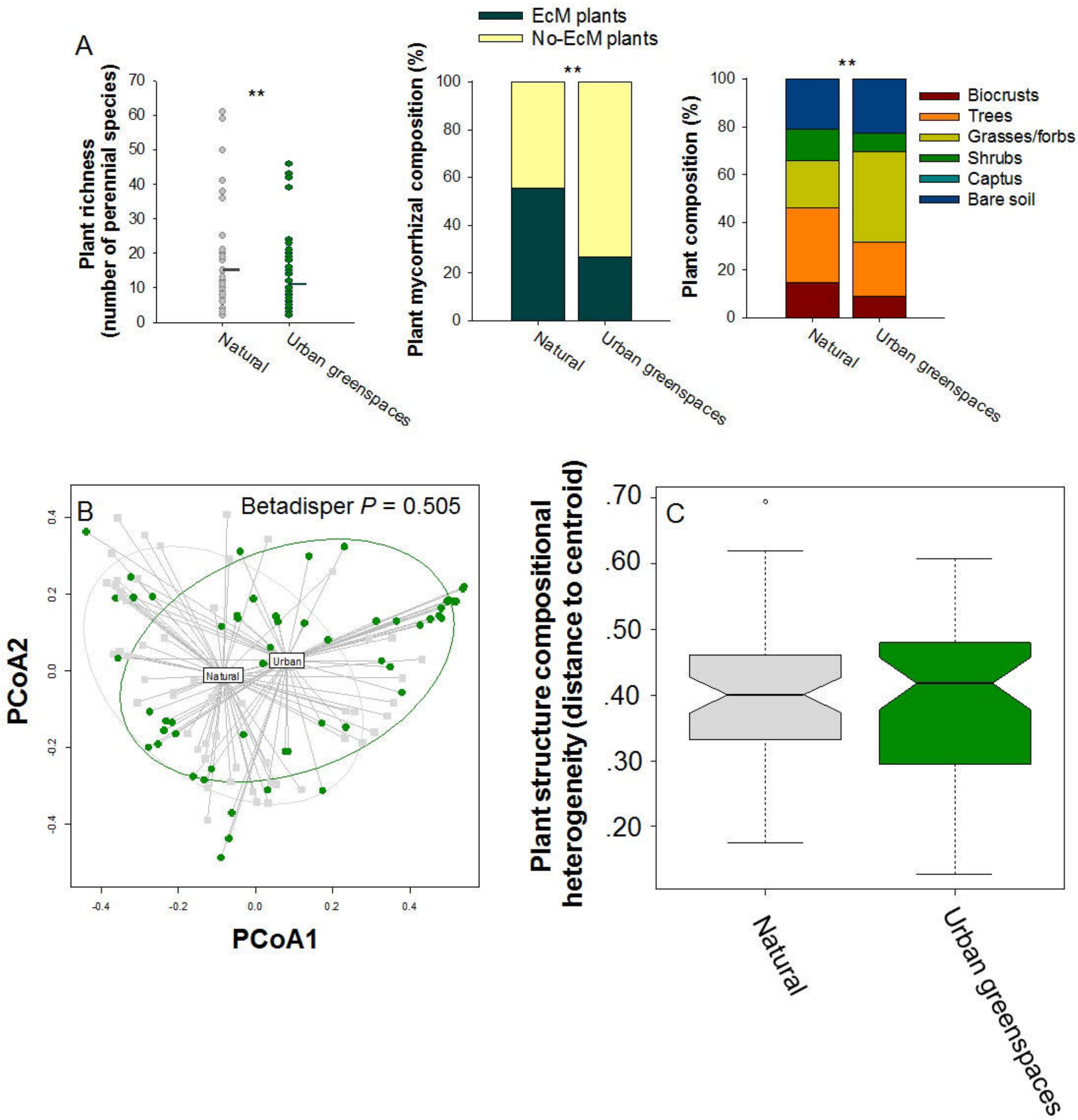


**Figure S2. Vegetation structure in natural ecosystems and urban greenspaces.** Panel (A) includes plant species richness (number of perennial plant species), plant cover (%) and proportion of natural ecosystems and urban greenspaces in which dominant plant species were ectomycorrhizal (EcM) plants. The solid lines show mean values (n = 112 natural and urban greenspaces). Asterisks indicate significant differences in Nested PERMANOVA analyses using a block design as described in the Method section. **P < 0.01; *P < 0.05. Panel B includes a PCoA showing variation in vegetation structure in natural ecosystems and urban greenspaces. Panel C boxes represent the median and 25th/75th percentile of the distances to the group centroid derived from betadisper (39). Asterisks indicate significant differences in compositional heterogeneity based on permutation test for homogeneity of multivariate dispersions (39).

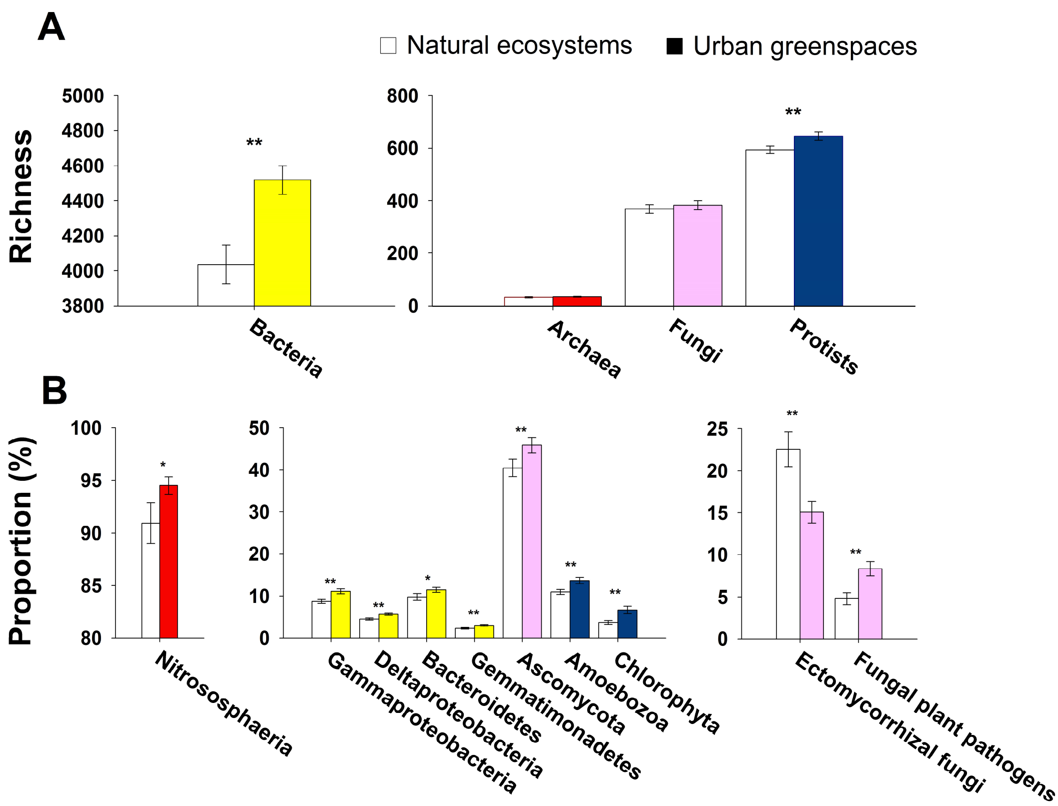


**Figure S3. The diversity and structure of the soil microbiome in urban greenspaces across the globe.** Mean values (±SE) for selected examples for the diversity and proportion of main groups of soil organisms influenced by urban greenspaces (n = 112 urban greenspaces and natural ecosystems). *P < 0.05; **P < 0.01. Empty bars (white) = natural ecosystems. Colored bars (blue = protists; pink = fungi; yellow = bacteria; red = archaea) = urban greenspaces.

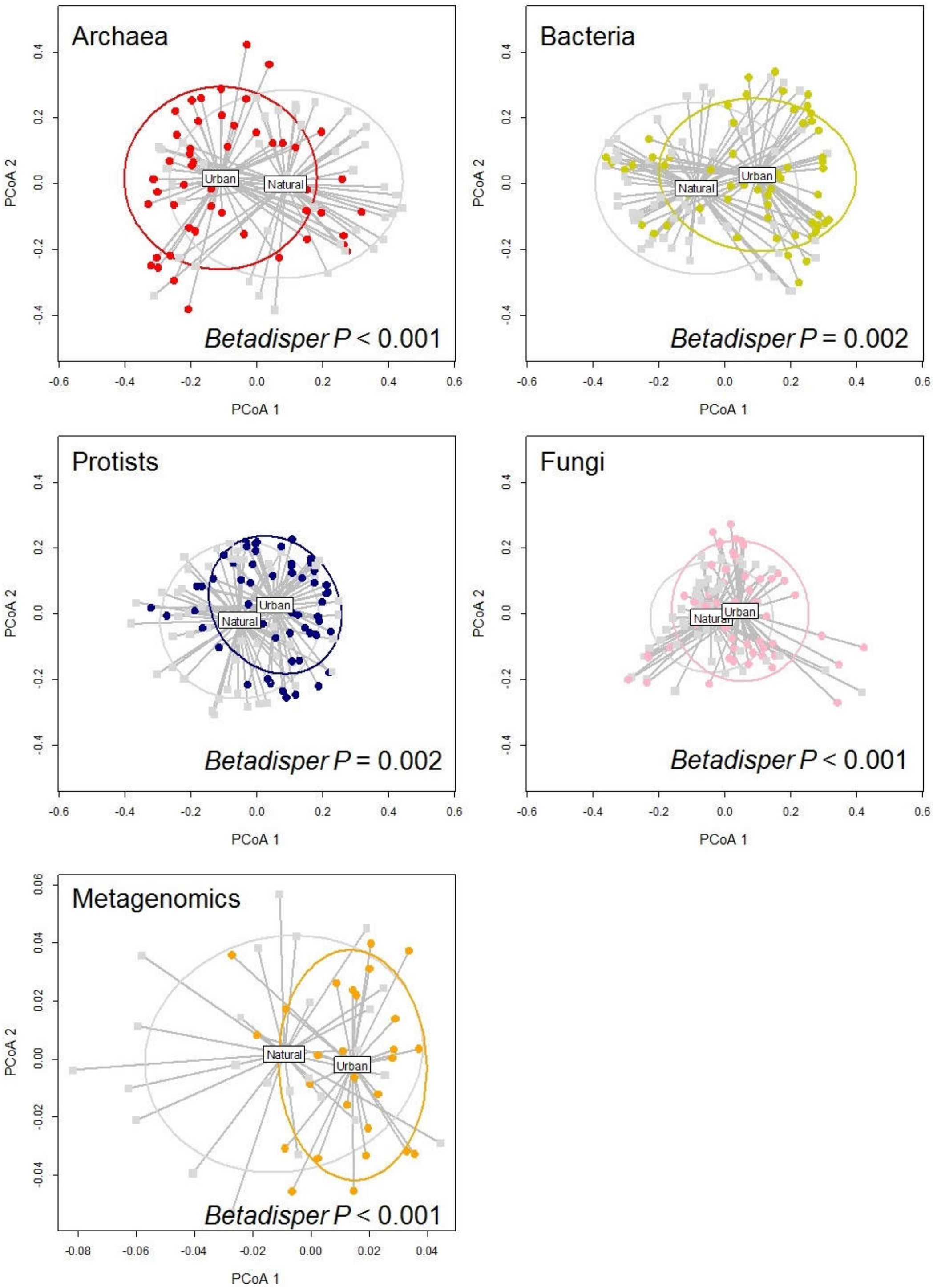


**Figure S4. Betadisper analyses showing the data variation of the soil microbiome within natural ecosystems and urban greenspaces (n = 112 urban greenspaces and natural ecosystems).** PCoA, based on Bray-Curtis matrices, summarizing the data variation in all soil phylotypes of archaea, bacteria, fungi and protists in our study. Gray colored circles = natural ecosystems. Colored circles (blue = protists; pink = fungi; yellow = bacteria; red = archaea) = urban greenspaces.

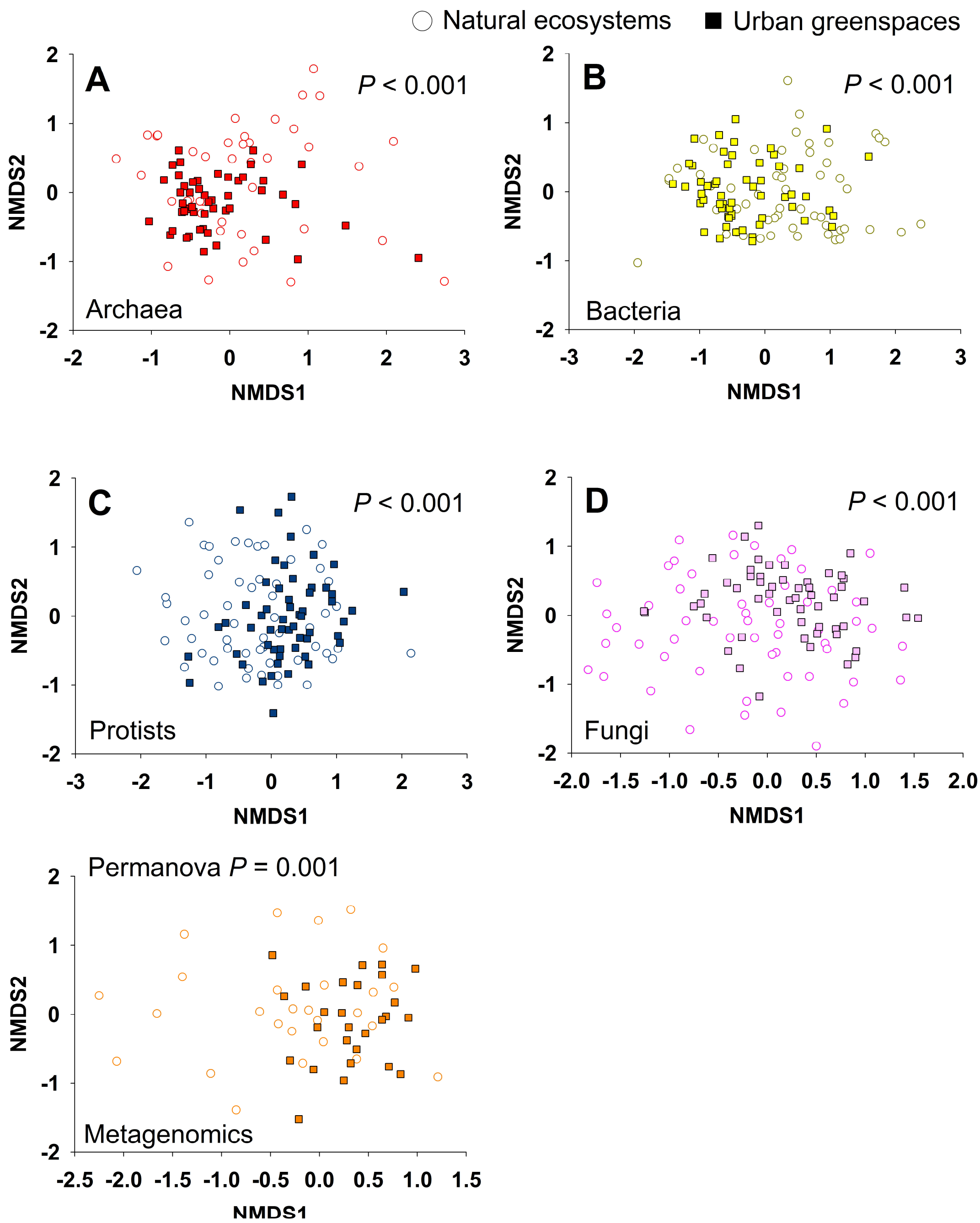


**Figure S5. Community composition of the soil microbiome in natural ecosystems and urban greenspaces (n = 112 urban greenspaces and natural ecosystems).** NMDS, based on Bray-Curtis matrices, summarizing the proportion of all soil phylotypes of archaea, bacteria, fungi and protists in our study. P-values are based on Nested PERMANOVA analyses using a block design comparing natural with urban greenspaces as described in the Method section. Empty circles (white) = natural ecosystems. Colored circles (blue = protists; pink = fungi; yellow = bacteria; red = archaea) = urban greenspaces.

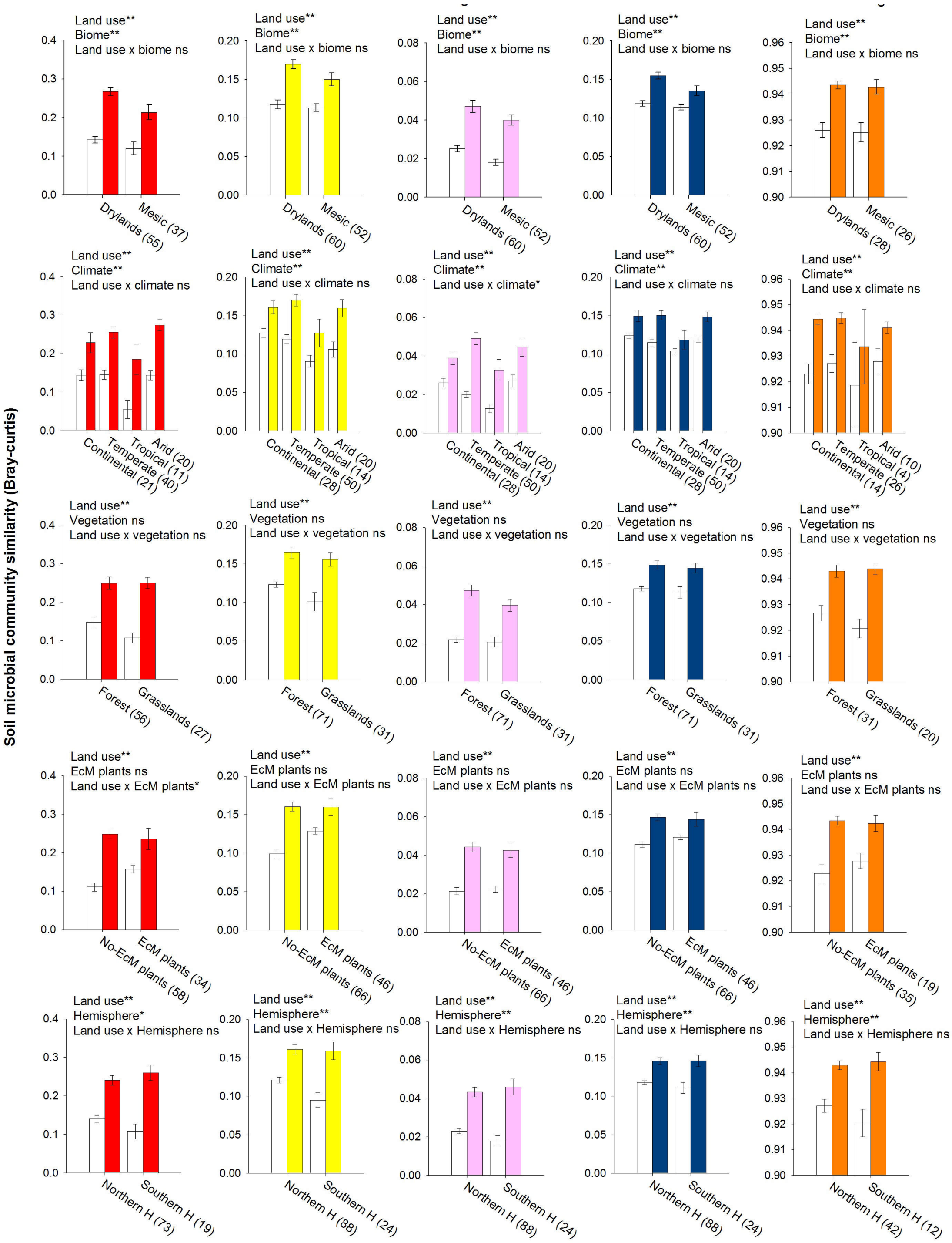


**Figure S6. Within-group (urban or natural) community similarity for archaea, bacteria, fungi, protists and metagenomics (mean ± SE).** These panels always show a greater similarity in the community composition of archaea, bacteria, fungi, protists and functional genes across globally distributed urban greenspaces than across the corresponding natural ecosystems. Asterisks indicate significant differences in Nested PERMANOVA analyses using a block design as described in the Method section (n in brackets). ns = no significant. *P < 0.05; **P < 0.01. EcM = Ectomycorrhizal.

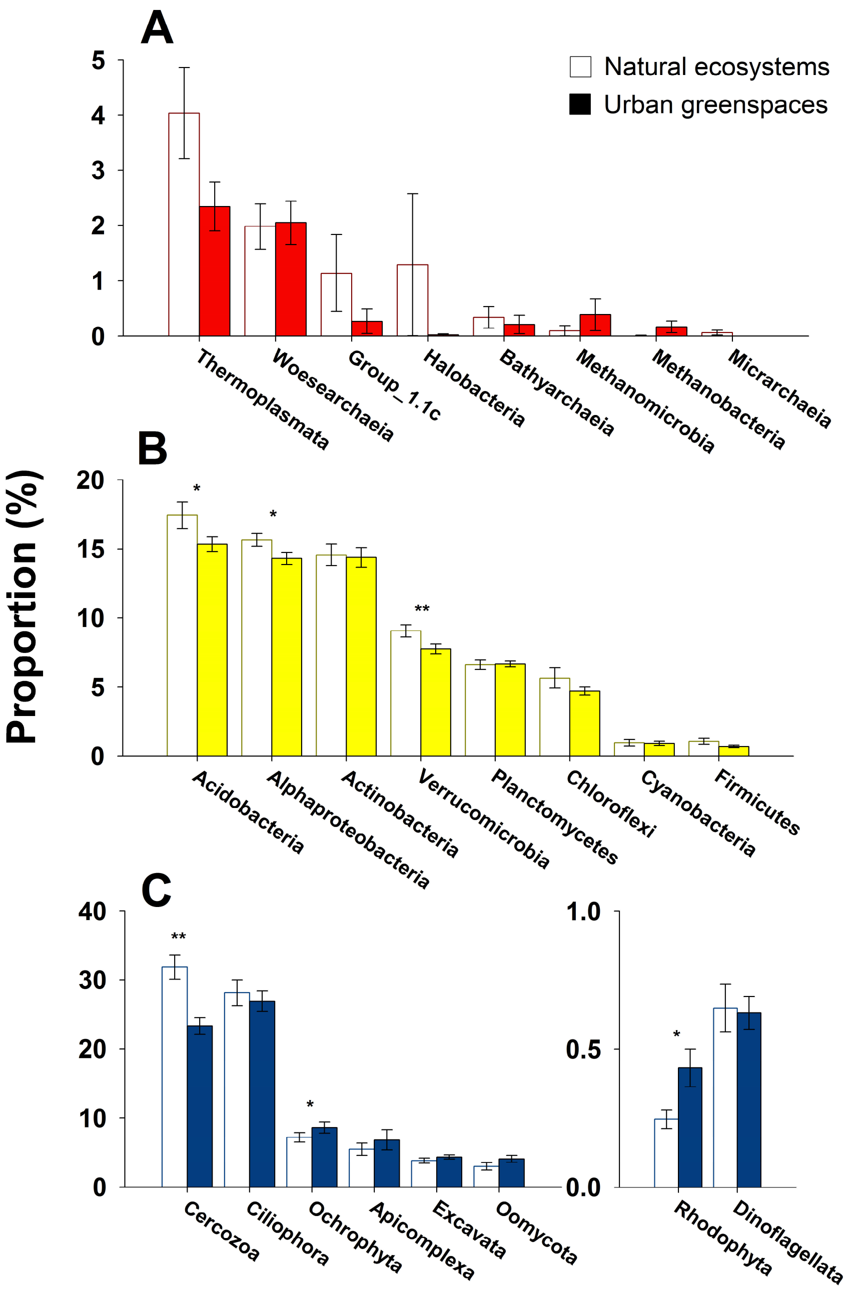


**Figure S7. Proportion (mean ± SE) of main groups of soil archaea, bacteria, and protists in urban greenspaces and natural ecosystems (n = 112 urban greenspaces and natural ecosystems).** *P < 0.05; **P < 0.01. P-values are based on Nested PERMANOVA analyses using a block design as described in the Method section. Empty bars (white) = natural ecosystems. Colored bars (blue = protists; yellow = bacteria; red = archaea) = urban greenspaces.

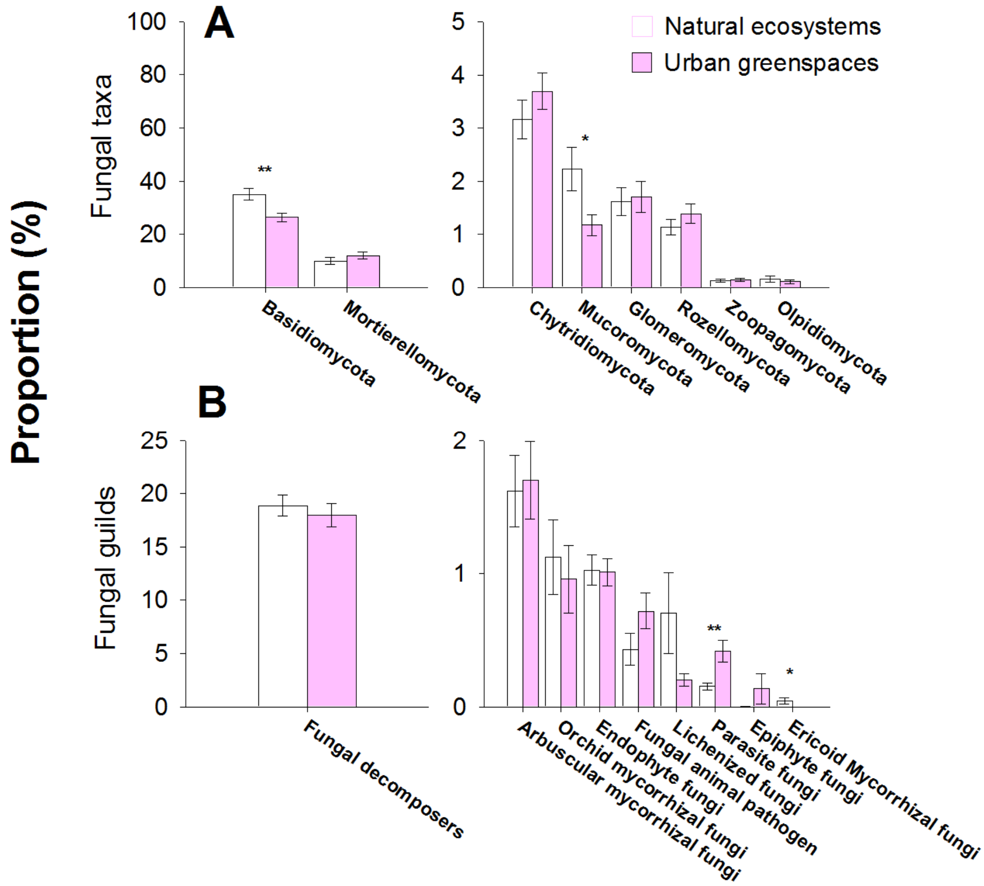


**Figure S8. Proportion (mean ± SE) of main groups of soil fungal taxonomic and functional groups in urban greenspaces and natural ecosystems (n = 112 urban greenspaces and natural ecosystems).** *P < 0.05; **P < 0.01. P-values are based on Nested PERMANOVA analyses using a block design as described in the Method section.

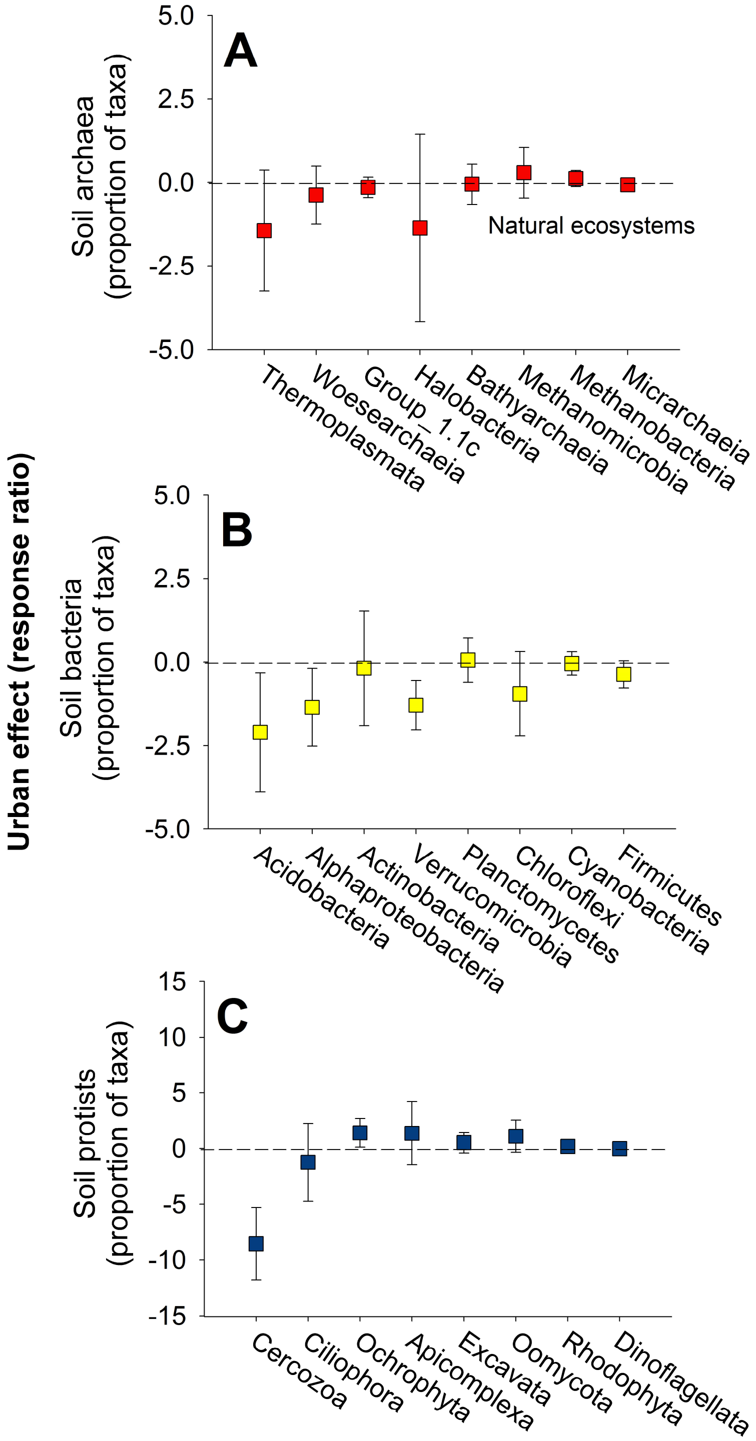


**Figure S9. Changes (site-level response ratios) in the proportion (mean ± CI95%) of main groups of soil archaea, bacteria and protists from natural to urban ecosystems (n = 56 response ratios).** The dashed line represents natural ecosystems with points above the dashed line indicating a positive response ratio, i.e. taxa are relatively more abundant in urban greenspace soils compared to the corresponding 'natural' sites.

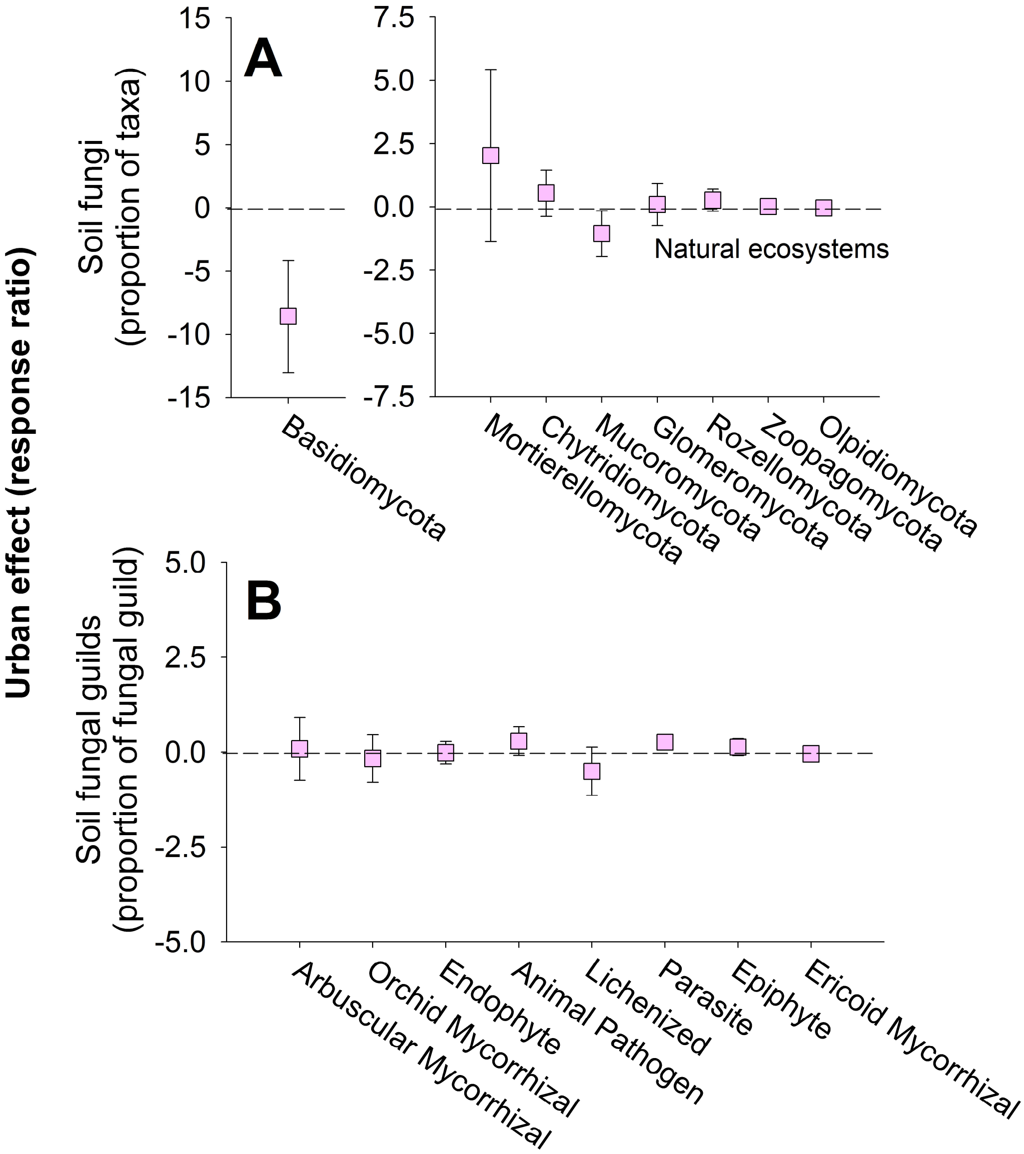


**Figure S10. Changes (site-level response ratios) in the proportion (mean ± CI95%) of main groups of soil fungal taxonomic and functional groups from natural to urban ecosystems (n = 56 response ratios).** The dashed line represents natural ecosystems with points above the dashed line indicating a positive response ratio, i.e. taxa are relatively more abundant in urban greenspace soils compared to the corresponding 'natural' sites

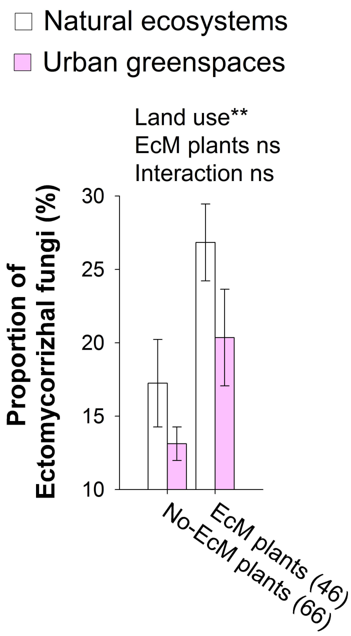


**Figure S11.** Changes in the proportion of ectomycorrhizal fungi in samples collected under ectomycorrhizal (EcM) and no- EcM dominant plants. ns = no significant. *P < 0.05; **P < 0.01. n in brackets.

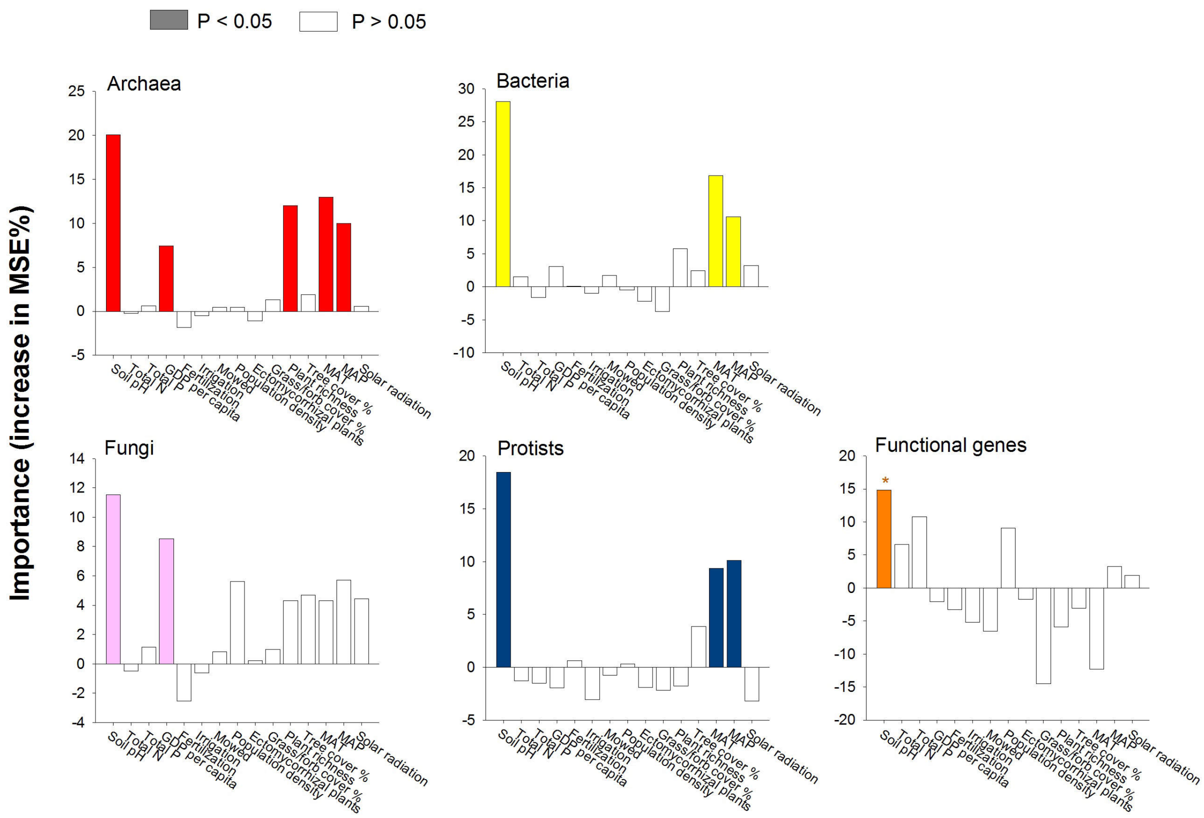


**Figure S12. Random Forest model aiming to identify the most important socio-economic, park management practices, and environmental drivers of the community similarity of urban greenspaces (n = 56 urban greenspaces).** MSE = Mean square error. Total P and N = Soil total P and N. Statistically non-significant correlations (P > 0.05) are shown in white.

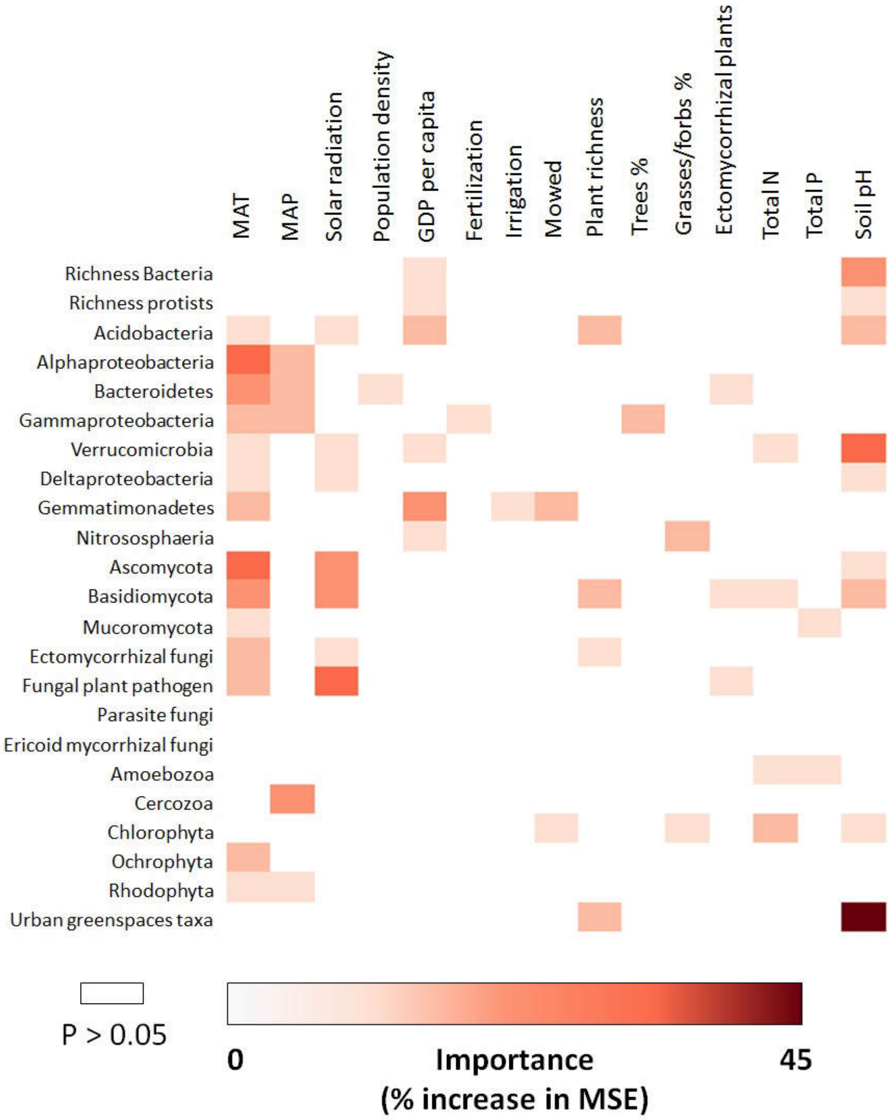


**Figure S13. Random Forest model aiming to identify the most important socio-economic, park management practices, and environmental drivers of the soil microbiome of urban greenspaces (n = 56 urban greenspaces).** MSE = Mean square error. Total P and N = Soil total P and N. Statistically non-significant correlations ($P > 0.05$) are shown in white.

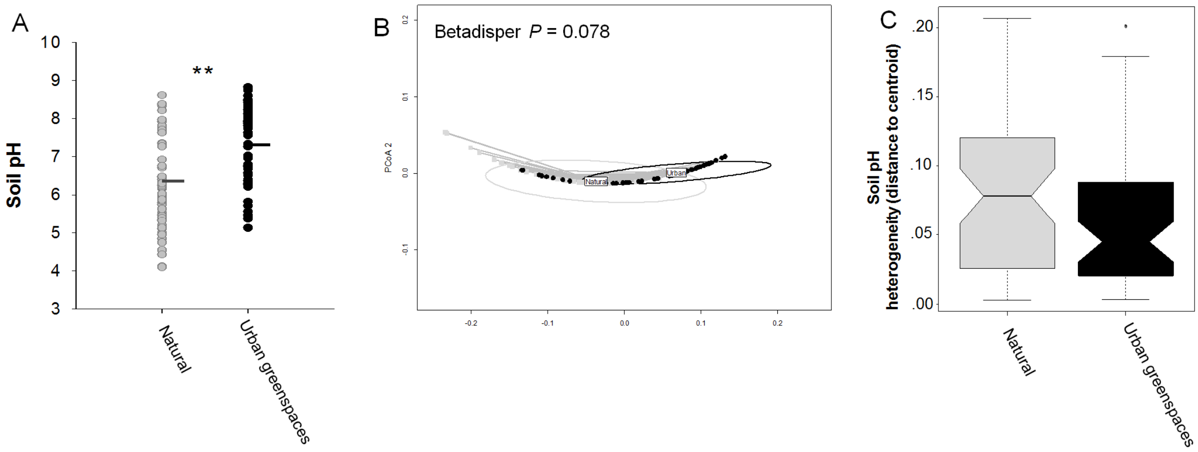


**Figure S14. Soil pH in natural ecosystems and urban greenspaces.** Panel (A) includes the soil pH in natural ecosystems and urban greenspaces. The solid lines show mean values (n = 112 natural ecosystems and urban greenspaces). Panel B boxes represent the median and 25th/75th percentile of the distances to the group centroid derived from betadisper (39). The P value in this panel is based on a permutation test for homogeneity of multivariate dispersions (39).

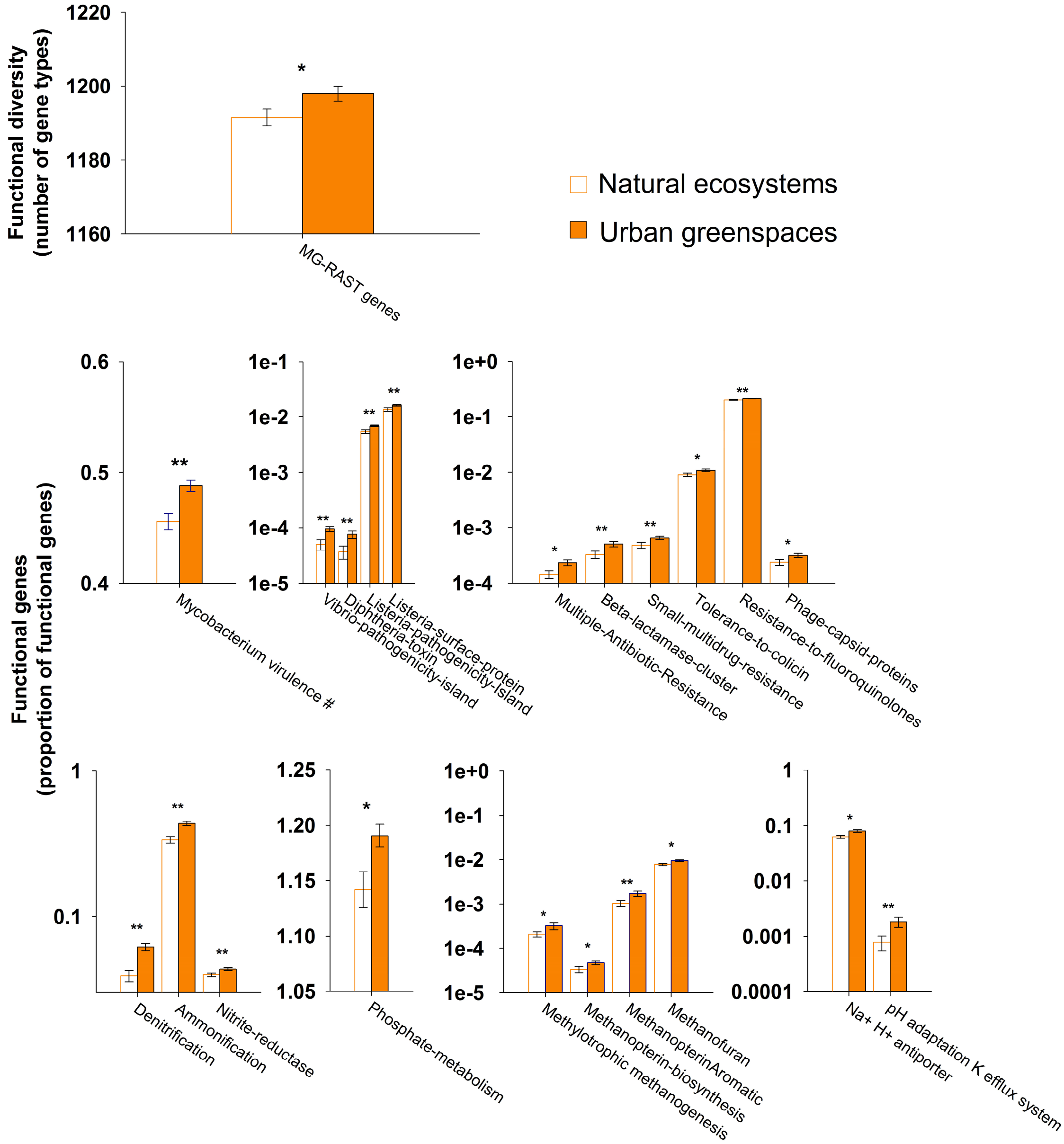


**Figure S15. The functional attributes of the soil microbiome in urban greenspaces across the globe.** Proportion and diversity (mean ± SE) of functional gene in natural and urban ecosystems (mean ± SE; n = 112 urban greenspaces and natural ecosystems). *P < 0.05; **P < 0.01. P-values are based on Nested PERMANOVA analyses using a block design as described in the Method section. Empty bars (white) = natural ecosystems.

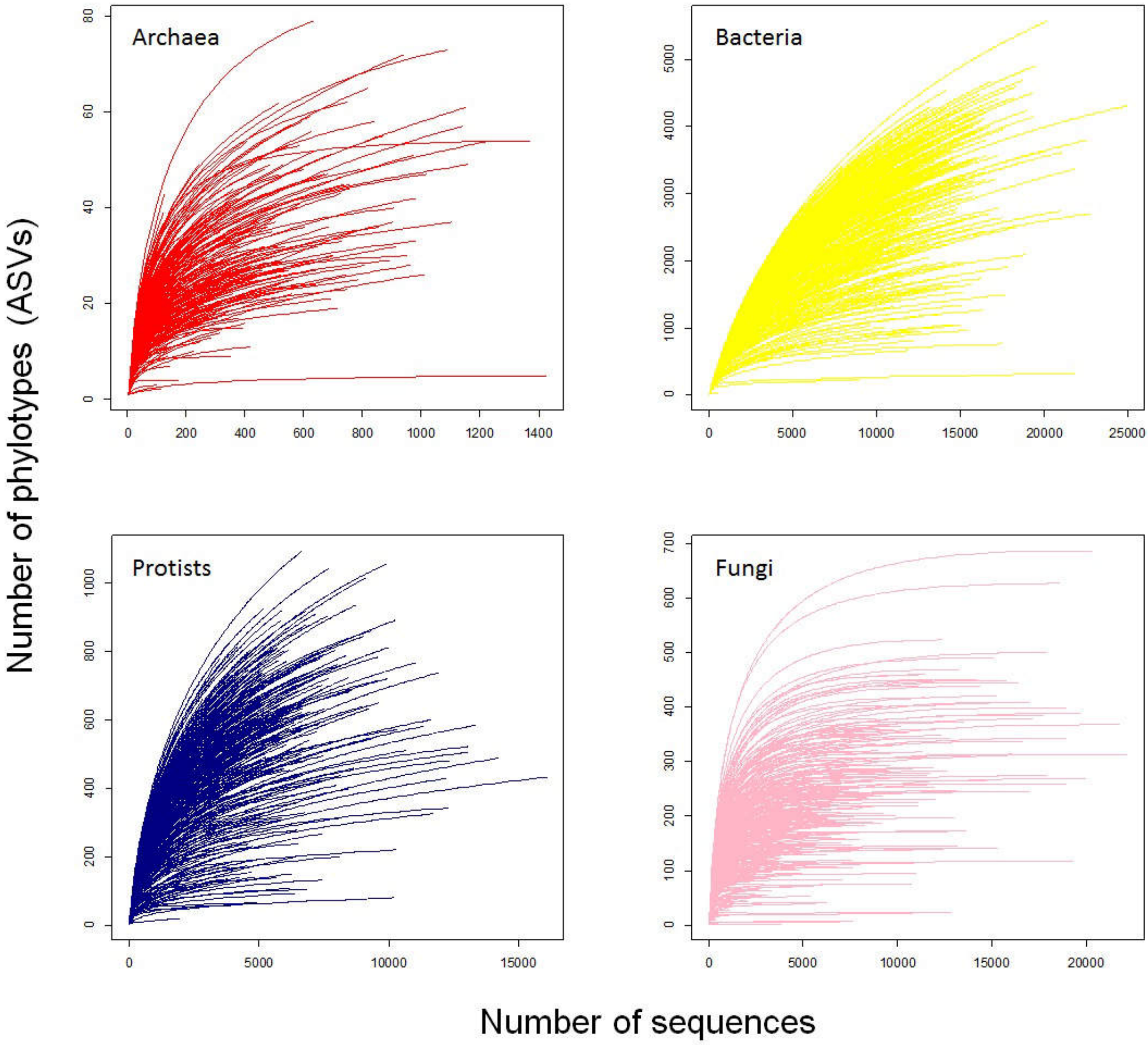


**Figure S16. Rarefaction curves for archaea, bacteria, fungi and protists in all sequenced soil samples.** ASVs = Amplicon sequence variants.

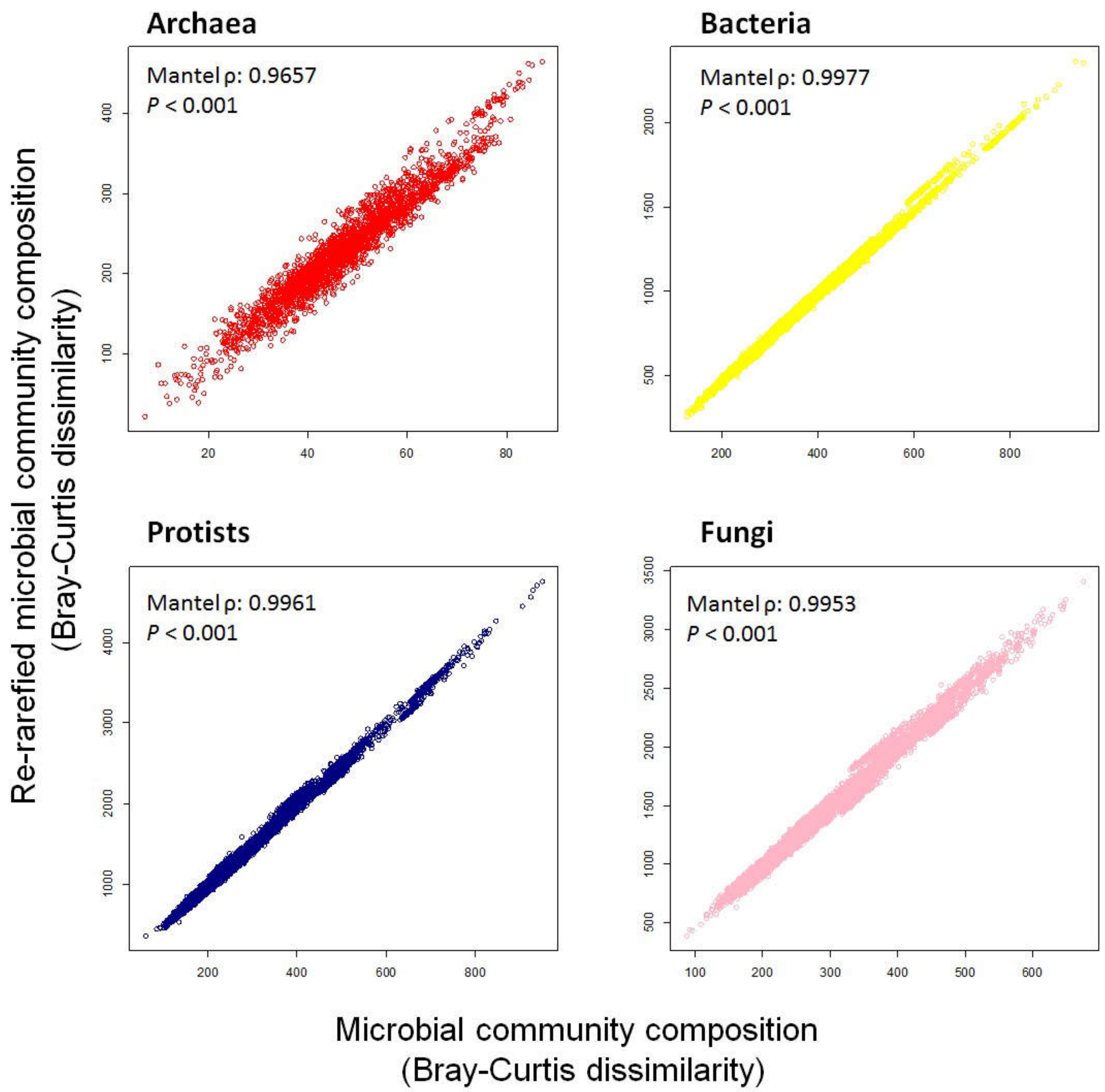


**Figure S17.** Relationships between diversity (number of phylotypes) of archaea (rarefied at 100 vs. 500 sequences/sample), bacteria (rarefied at 5000 vs. 12500 sequences/sample), fungi (rarefied at 1000 vs. 5000 sequences/sample for fungi) and protists (rarefied at 1000 vs. 5000 sequences/sample) in sequenced soil samples.

**Table S1.** Information associated with the municipalities investigated in this study. *indicates urban areas including shotgun metagenomic data.

| Site | City | Irrigated | Fertilized | Mowed | Population | Urban vegetation type | Aridity biome | Climate |
|---|---|---|---|---|---|---|---|---|
| 1 | Tonghua City, Jilin, China | No | No | No | 440000 | Forest | Mesic | Continental |
| 2 | Baishan City, Jilin, China* | No | No | No | 590000 | Grassland | Mesic | Continental |
| 3 | Yanji City, Jilin, China | No | No | No | 520000 | Forest | Dryland | Continental |
| 4 | Dunhua City, Jilin, China | No | No | No | 460000 | Forest | Dryland | Continental |
| 5 | Jilin City, Jilin, China* | No | No | No | 1810000 | Shrubland | Dryland | Continental |
| 6 | Santiago, Santiago Metropolitan Region, Chile* | No | No | No | 7112808 | Forest | Dryland | Temperate |
| 7 | Belo Horizonte, Minas Gerais State, Brazil* | Yes | No | Yes | 2502557 | Grassland | Mesic | Tropical |
| 8 | Contagem, Minas Gerais State, Brazil | Yes | No | Yes | 659070 | Forest | Mesic | Tropical |
| 9 | Betim, Minas Gerais State, Brazil | No | No | No | 408448 | Grassland | Mesic | Tropical |
| 10 | Longmont, CO, USA | Yes | Yes | Yes | 96577 | Grassland | Dryland | Arid |
| 11 | Grand Junction, CO, USA | No | No | Yes | 63374 | Grassland | Dryland | Arid |
| 12 | Cheyenne, WY, USA* | Yes | Yes | Yes | 63957 | Grassland | Dryland | Arid |
| 13 | South Lyon, MI, USA* | Yes | Yes | Yes | 11831 | Grassland | Mesic | Continental |
| 14 | Oxford, England, UK* | No | No | Yes | 154600 | Grassland | Mesic | Temperate |
| 15 | Bodø, Norway* | Yes | Yes | Yes | 52357 | Grassland | Mesic | Continental |
| 16 | Uppsala, Sweden* | Yes | Yes | Yes | 168096 | Grassland | Mesic | Continental |
| 17 | Poitiers, France* | Yes | Yes | Yes | 88291 | Forest | Mesic | Temperate |
| 18 | Niort, France | No | Yes | No | 58707 | Forest | Mesic | Temperate |
| 19 | Tours, France | No | No | No | 135787 | Forest | Dryland | Temperate |
| 20 | Ljubljana, Slovenia* | No | No | Yes | 292988 | Forest | Mesic | Continental |
| 21 | Koper, Slovenia | Yes | No | Yes | 25611 | Forest | Mesic | Temperate |
| 22 | Maribor, Slovenia | Yes | No | Yes | 95767 | Forest | Mesic | Continental |
| 23 | Pretoria, South Africa* | Yes | No | Yes | 2472612 | Forest | Dryland | Temperate |
| 24 | Germiston, South Africa* | No | No | No | 255863 | Grassland | Dryland | Temperate |
| 25 | Cape Town, South Africa | Yes | Yes | Yes | 3776000 | Forest | Dryland | Temperate |
| 26 | Durgapur, West Bengal, India | Yes | No | Yes | 580990 | Forest | Mesic | Tropical |
| 27 | Mirzapur, Uttar Pradesh, India | Yes | No | Yes | 233691 | Grassland | Dryland | Temperate |
| 28 | Agra, Uttar Pradesh, India* | Yes | Yes | Yes | 1585704 | Grassland | Dryland | Arid |
| 29 | Beijing, China* | Yes | No | No | 21450000 | Forest | Dryland | Continental |
| 30 | Tai'an, Shandong, China | No | No | No | 1735425 | Forest | Dryland | Continental |
| 31 | Tianjin, China | No | No | No | 12938224 | Forest | Dryland | Continental |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 32 | Ürümqi, Xinjiang, China* | No | No | No | 3519600 | Grassland | Dryland | Arid |
| 33 | Alice Springs, Northern Territory, Australia* | Yes | No | Yes | 26534 | Forest | Dryland | Arid |
| 34 | Brisbane, Queensland, Australia | Yes | No | Yes | 2514184 | Grassland | Mesic | Temperate |
| 35 | Mildura, Victoria, Australia | Yes | Yes | Yes | 51903 | Grassland | Dryland | Arid |
| 36 | Cecil Hills, Sydney, New South Wales, Australia | No | No | Yes | 7018 | Grassland | Dryland | Temperate |
| 37 | Heathcote, Sydney, New South Wales, Australia* | Yes | No | Yes | 6013 | Grassland | Mesic | Temperate |
| 38 | Barcelona, Catalunya, Spain* | Yes | No | No | 1620343 | Forest | Dryland | Temperate |
| 39 | Pullman, Washington, USA | No | No | No | 34019 | Shrubland | Dryland | Continental |
| 40 | Corvallis, Oregon, USA* | No | Yes | Yes | 58641 | Grassland | Mesic | Temperate |
| 41 | Coyoacán, Mexico City, Mexico | No | No | No | 608479 | Shrubland | Dryland | Temperate |
| 42 | Tlalpan, Mexico City, Mexico | No | No | Yes | 677104 | Forest | Dryland | Temperate |
| 43 | Miguel Hidalgo, Mexico City, Mexico* | No | No | Yes | 364439 | Forest | Dryland | Temperate |
| 44 | Madrid, Comunidad de Madrid, Spain* | Yes | Yes | No | 3266126 | Forest | Dryland | Arid |
| 45 | Esa-Odo, Osun state, Nigeria* | Yes | Yes | Yes | 1069 | Forest | Mesic | Tropical |
| 46 | Obafemi Awolowo University, Osun state, Nigeria | No | No | No | 35000 | Forest | Mesic | Tropical |
| 47 | Ife city, Osun state, Nigeria | Yes | No | No | 509035 | Shrubland | Mesic | Tropical |
| 48 | Lakeland, Florida, USA* | Yes | Yes | Yes | 110516 | Forest | Mesic | Temperate |
| 49 | Sebring, Florida, USA | Yes | Yes | Yes | 10937 | Forest | Mesic | Temperate |
| 50 | Punta Gorda, Florida, USA | Yes | Yes | Yes | 20057 | Forest | Mesic | Temperate |
| 51 | Utrera, Andalusia, Spain* | No | Yes | Yes | 50728 | Forest | Dryland | Temperate |
| 52 | Coimbra, Portugal | No | No | No | 143396 | Forest | Mesic | Temperate |
| 53 | Porto, Portugal* | No | No | Yes | 237559 | Forest | Mesic | Temperate |
| 54 | Jerusalem, Israel* | Yes | Yes | Yes | 919438 | Forest | Dryland | Temperate |
| 55 | Be'er Sheva, Israel | Yes | No | Yes | 209002 | Forest | Dryland | Arid |
| 56 | Ofakim, Israel | Yes | No | No | 29021 | Forest | Dryland | Arid |

**Table S2. Dominant vegetation genus in natural ecosystems and urban greenspaces.**

| Site | Dominant plant (genus) | Land use | Ectomycorrhizal plant |
|---|---|---|---|
| 1 | *Quercus* | Natural | Yes |
| 1 | *Pinus* | Urban greenspace | Yes |
| 2 | *Acer* | Natural | No |
| 2 | *Acer* | Urban greenspace | No |
| 3 | *Betula* | Natural | Yes |
| 3 | *Pinus* | Urban greenspace | Yes |
| 4 | *Quercus* | Natural | Yes |
| 4 | *Larix* | Urban greenspace | Yes |
| 5 | *Tilia* | Natural | Yes |
| 5 | *Larix* | Urban greenspace | Yes |
| 6 | *Schinus* | Natural | No |
| 6 | *Acacia* | Urban greenspace | Yes |
| 7 | *Eragrostis* | Natural | No |
| 7 | *Zoysia* | Urban greenspace | No |
| 8 | *Eragrostis* | Natural | No |
| 8 | *Merostachys* | Urban greenspace | No |
| 9 | *Echinolaena* | Natural | No |
| 9 | *Brachiaria* | Urban greenspace | No |
| 10 | *Pinus* | Natural | Yes |
| 10 | *Poa* | Urban greenspace | No |
| 11 | *Juniperus* | Natural | No |
| 11 | *Poa* | Urban greenspace | No |
| 12 | *Poa* | Natural | No |
| 12 | *Poa* | Urban greenspace | No |
| 13 | *Quercus* | Natural | Yes |
| 13 | *Poa* | Urban greenspace | No |
| 14 | *Quercus* | Natural | Yes |
| 14 | *Cynodon* | Urban greenspace | No |
| 15 | *Sorbus* | Natural | No |
| 15 | *Poa* | Urban greenspace | No |
| 16 | *Picea* | Natural | Yes |
| 16 | *Poa* | Urban greenspace | No |
| 17 | *Quercus* | Natural | Yes |

| | | | |
|---|---|---|---|
| 17 | *Celtis* | Urban greenspace | No |
| 18 | *Fraxinus* | Natural | No |
| 18 | *Alnus* | Urban greenspace | Yes |
| 19 | *Quercus* | Natural | Yes |
| 19 | *Acer* | Urban greenspace | No |
| 20 | *Carpinus* | Natural | Yes |
| 20 | *Tilia* | Urban greenspace | Yes |
| 21 | *Quercus* | Natural | Yes |
| 21 | *Pinus* | Urban greenspace | Yes |
| 22 | *Fagus* | Natural | Yes |
| 22 | *Acer* | Urban greenspace | No |
| 23 | *Senegalia* | Natural | No |
| 23 | *Cynodon* | Urban greenspace | No |
| 24 | *Poa* | Natural | No |
| 24 | *Rorippa* | Urban greenspace | No |
| 25 | *Cynodon* | Natural | No |
| 25 | *Populus* | Urban greenspace | Yes |
| 26 | *Shorea* | Natural | Yes |
| 26 | *Cynodon* | Urban greenspace | No |
| 27 | *Cassia* | Natural | No |
| 27 | *Cynodon* | Urban greenspace | No |
| 28 | *Cynodon* | Natural | No |
| 28 | *Cynodon* | Urban greenspace | No |
| 29 | *Adenanthera* | Natural | No |
| 29 | *Ginkgo* | Urban greenspace | No |
| 30 | *Quercus* | Natural | Yes |
| 30 | *Pinus* | Urban greenspace | Yes |
| 31 | *Ulmus* | Natural | No |
| 31 | *Ulmus* | Urban greenspace | No |
| 32 | *Picea* | Natural | Yes |
| 32 | *Ulmus* | Urban greenspace | No |
| 33 | *Triodia* | Natural | No |
| 33 | *Pennisetum* | Urban greenspace | No |
| 34 | *Eucalyptus* | Natural | Yes |
| 34 | *Cynodon* | Urban greenspace | No |
| 35 | *Chenopodium* | Natural | No |

| | | | |
|---|---|---|---|
| 35 | *Pennisetum* | Urban greenspace | No |
| 36 | *Eucalyptus* | Natural | Yes |
| 36 | *Pennisetum* | Urban greenspace | No |
| 37 | *Angophora* | Natural | Yes |
| 37 | *Cynodon* | Urban greenspace | No |
| 38 | *Quercus* | Natural | Yes |
| 38 | *Pinus* | Urban greenspace | Yes |
| 39 | *Pinus* | Natural | Yes |
| 39 | *Acer* | Urban greenspace | No |
| 40 | *Ulmus* | Natural | No |
| 40 | *Acer* | Urban greenspace | No |
| 41 | *Abies* | Natural | Yes |
| 41 | *Buddleja* | Urban greenspace | No |
| 42 | *Cupressus* | Natural | No |
| 42 | *Cupressus* | Urban greenspace | No |
| 43 | *Pinus* | Natural | Yes |
| 43 | *Ligustrum* | Urban greenspace | No |
| 44 | *Quercus* | Natural | Yes |
| 44 | *Buxus* | Urban greenspace | No |
| 45 | *Theobroma* | Natural | No |
| 45 | *Marantochloa* | Urban greenspace | No |
| 46 | *Elaeis* | Natural | No |
| 46 | *Elaeis* | Urban greenspace | No |
| 47 | *Elaeis* | Natural | No |
| 47 | *Musa* | Urban greenspace | No |
| 48 | *Pinus* | Natural | Yes |
| 48 | *Ficus* | Urban greenspace | No |
| 49 | *Quercus* | Natural | Yes |
| 49 | *Quercus* | Urban greenspace | Yes |
| 50 | *Pinus* | Natural | Yes |
| 50 | *Pinus* | Urban greenspace | Yes |
| 51 | *Pinus* | Natural | Yes |
| 51 | *Citrus* | Urban greenspace | No |
| 52 | *Pinus* | Natural | Yes |
| 52 | *Tilia* | Urban greenspace | Yes |
| 53 | *Quercus* | Natural | Yes |

| | | | |
|---|---|---|---|
| 53 | *Acer* | Urban greenspace | No |
| 54 | *Cistus* | Natural | Yes |
| 54 | *Olea* | Urban greenspace | No |
| 55 | *Noaea* | Natural | No |
| 55 | *Eucalyptus* | Urban greenspace | Yes |
| 56 | *Noaea* | Natural | No |
| 56 | *Atriplex* | Urban greenspace | No |

**Table S3.** List of identified microbial phylotypes associated with the soil microbiome of urban greenspaces across the globe. This list contains information on the taxonomic identity of each phylotype, representative sequences, and the most closely related reference genome.

*Table S3 is available online as a Separate .XLS file under the Supplementary Materials for this article.*