Title: Biogenic factors explain soil carbon in paired urban and natural ecosystems worldwide

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Abstract:

Urban greenspaces support multiple nature-based services, many of which depend on the amount of soil carbon (C). Yet, the environmental drivers and sensitivity to the warming of soil C are still poorly understood globally. Here, we use soil samples from 56 paired urban greenspaces and natural ecosystems worldwide and combine soil C concentration and size fractionation measures with metagenomics and warming incubations. We show that surface soils in urban and natural ecosystems sustain similar C concentrations that follow comparable negative relationships with temperature. Plant productivity's contribution to explaining soil C was higher in natural ecosystems, while in urban ecosystems, the soil microbial biomass had the greatest explanatory power. Moreover, the soil microbiome supported a faster C mineralization rate with experimental warming in urban greenspaces compared to natural ecosystems. Consequently, urban management strategies should consider the soil microbiome to maintain soil C and related ecosystem services.

Main text

Introduction

Urban greenspaces, such as urban forests, parks, gardens and lawns, are a common feature of cities and represent important ecosystems that could help offset the carbon (C) footprint of urban areas by storing C in their soils[1]. Despite their importance at both local and global scales[2,3], examples of natural solutions to changing climates are dominated by natural and agricultural ecosystems[4] and fail to account for the potential soil C in urban greenspaces. Management practices in urban greenspaces such as planting of horticultural plants, mowing and irrigation, may alter the balance between soil C outputs from microbial decomposition and soil C inputs from plant photosynthesis and litter entrance[5]. This balance regulates the size of the soil C pool[6,7], and management-induced changes (e.g., mowing and pruning) may compromise the ability of urban greenspaces to store soil C by altering the sensitivity (i.e., the degree to which a given ecosystem property responds to a particular environmental disturbance) of soil C and its drivers to changing global climates. Yet, whether the controls and sensitivities of soil C in urban greenspaces are similar to those of natural ecosystems across global gradients in climate and soil properties remains largely unexplored.

Uncertainties about the concentrations and sensitivity of soil C in urban greenspaces result from three main reasons. First, global- and regional-scale biotic and abiotic controls on soil C stocks and concentrations are poorly characterized for urban greenspaces. Although soil C has been studied for decades in urban environments, most studies have been conducted at the local level[8], while global patterns remain unknown (but see refs.[1,3]). A previous study[1] represented the first attempt to compare soil C in natural vs. urban using a meta-analytical approach. However, as in all meta-analyses, the analysis was shaped by the nature of the studies available to be synthesized from the literature. Thus, available meta-analytical data differ study-to-study in sampling design, methods and data analysis[1]. This paper highlighted trends and research gaps that needed to be filled in future urban vs. natural comparisons. Second, the influence of classic controls on soil C such as soil microbial decomposition and plant photosynthesis may be different in urban areas compared to natural ecosystems because of different management practices. The relative contribution of biotic factors such as plant productivity[9,10] and the soil microbiome need to be assessed across contrasting abiotic conditions in a standardized way to compare the main controls on soil C between natural environments and greenspaces. Moreover, we lack studies comparing the responses of soil microbes to warming in urban environments, and how microbial genes associated with soil C formation (e.g., photosynthesis genes) and mineralization (e.g., enzymes involved in the degradation of lignin and carbohydrates) traits differ between urban and natural environments. Finally, soil C comprises multiple C pools. Studies focusing on estimates of bulk soil C may fail to capture the effects of land management and climate change on important C fractions such as particulate (POC) and mineral-associated (MAOC) organic C[11]. These fractions differ in their turnover rate and persistence because organo-mineral associations in the MAOC fraction help protect C against warming and physical disturbances[12-14].

Our study aims to provide the first standardized survey of paired urban greenspaces and natural ecosystems across a worldwide spatial distribution, integrating novel microbial aspects (i.e., metagenomics and warming incubations) and emerging trends in soil C persistence (i.e., POC and MAOC fractions) aiming at helping to tease apart commonalities and differences between factors controlling soil C in urban and natural ecosystems. Addressing all of these knowledge gaps is necessary to assess the potential of soil C in urban greenspaces as a natural climate solution to mitigate and adapt to climate change. Specifically, we combined a field survey of paired urban and natural ecosystems with measures of soil organic C concentration (SOC), size fractionation (POC and MAOC), metagenomics and soil warming incubations. We selected 56 paired urban

greenspaces and adjacent natural ecosystems from locations in 17 countries and six continents across environmental gradients (Fig. 1; see also Supplementary Figs. 1 and 2, and Supplementary Table 1). Our study provides a global field survey including paired urban-natural ecosystems across a worldwide spatial distribution, yet we acknowledge potential limitations of our reduced number of paired sites at a global scale. We analyzed composite samples from surface soils (five soil cores to ~5-cm depth were pooled to account for spatial heterogeneity; Methods) collected beneath the dominant vascular vegetation (trees, shrubs or grasses) in 30 m × 30 m plots located in urban greenspaces and paired natural ecosystems (Methods; Supplementary Fig. 2). We focused on surface soils because (a) the uppermost layer is typically the most biologically active in terms of soil C turnover, plant roots, microbial biomass, and atmospheric C exchange; (b) city parks and gardens can have shallow soils due to extensive surface preparation and disturbance; and (c) surface soils are exposed to the direct influence of atmospheric temperature and could be more vulnerable to global warming. We hypothesized that the link between plant productivity and soil C may be altered in urban greenspaces, making soil C more dependent on microbial turnover in these systems. In brief, plant productivity and soil C are known to be connected in natural ecosystems[15], especially in the range of climatic conditions where many cities are found. In fact, plant productivity is often used as a predictor of C distribution in global soil models[16]. Plants fix C from the atmosphere, and soil microbes and animals decompose plant litter and incorporate this C into the soil. This link, however, may be strongly altered in urban environments by green space management (e.g., mowing and pruning) that can systematically remove litter and deadwood and thus reduce the input of plant-associated organic matter into soils.

Results and Discussion

The SOC concentration in the surface soil of the surveyed 56 well-established urban greenspaces (48.9 ± 7.7 g C kg^{-1} of soil) was similar to that found in adjacent natural ecosystems (57.1 ± 8.5 g C kg^{-1} of soil) (Fig. 2A; $P > 0.05$; Methods for nested Permanova; Supplementary Figs. 3-5 for global distribution of SOC and additional analyses). Thus, even though the global area of urban greenspaces is much lower than that of natural ecosystems, the role of urban greenspaces in C storage could help to support the efforts of cities to implement natural climate solutions to mitigate their C footprint. Soil C is also critical for ecosystem resilience, such as the maintenance and enhancement of biodiversity, plant growth and soil functions such as nutrient supply, water regulation and purification, suggesting that the comparable C concentrations in urban systems might also support climate adaptation efforts. Notably, the concentrations of POC and MAOC also did not differ between urban greenspaces and adjacent natural ecosystems (Fig. 2A; $P > 0.05$; Supplementary Table 2; Supplementary Figs. 3-4). Similar concentrations of SOC, POC and MAOC in soils from both systems were detected when we used linear mixed-effects modelling to account for differences in climatic, soil, microbial and plant productivity drivers (Supplementary Fig. 4). This similarity also holds between urban and natural forests, as suggested by the non-significant interaction between urban vs. natural predictors in the linear mixed models (Supplementary Fig. 5). Taken together, our global findings suggest that urban greenspaces have concentrations of SOC, POC and MAOC – at least in the surface 5 cm – comparable with the paired natural ecosystems from where they originated, challenging the notion that urban greenspace soils are C depleted[18].

We used structural equation modelling to examine whether the similarity in C concentrations between urban and natural areas can be attributed to similar environmental factors. We found that temperature was a strong and consistent predictor of soil C in both ecosystem types, with its effects operating directly and indirectly (Fig. 3; Supplementary Figs. 6-7 for a priori model

and rationale). In particular, we found that mean annual temperature is negatively related to the concentrations of SOC, POC and MAOC in both urban greenspaces and paired natural ecosystems worldwide (Fig. 2B), a result also supported by the linear mixed-effects modelling (Supplementary Figs. 4-5). The influence of temperature was independent of how we represented this variable in the models, presumably because mean annual temperature was strongly positively correlated with other metrics such as soil temperature, maximum temperature, and recent mean air and land surface temperatures (Supplementary Tables 3-4). These inferences were supported in the Variation Partitioning Modelling (see Methods). Specifically, mean annual temperature explained a unique portion of the variation in the global distribution of SOC, POC and MAOC in urban greenspaces and adjacent natural ecosystems (Supplementary Fig. 8). A similar negative spatial association between temperature and soil C has been previously described in global natural ecosystems[7,19,20]. Our findings extend this finding to the behavior of soil C in urban greenspaces across the globe. Further, we also identified similar temperature thresholds (i.e., 17-18ºC) associated with the global distribution of soil C content in urban and natural ecosystems (Supplementary Fig. 9; Supplementary Table 5 for AIC values of segmented compared with linear models). The commonality in the temperature dependence of soil C in both systems is noteworthy given stark differences between urban and natural contexts, and suggests that the negative effects of warming on the capacity of natural soils to store C extend to urban ecosystems worldwide.

Despite the similarities in the responses of soil C to temperature variation, we found that the influence of biotic processes in urban greenspaces differed from that in natural ecosystems. Our structural equation (Fig. 3; Supplementary Figs. 6-7) and Variation Partitioning (Supplementary Fig. 8) modelling revealed that SOC, MAOC and POC concentrations in natural ecosystems were significantly correlated with plant productivity (measured using high-resolution satellite NDVI information; see Methods). Our results are restricted to the range of climatic conditions supporting cities and their nearby natural environments, and do not necessarily represent a universal pattern across global biomes, which is still under debate and needing further research. Unlike for natural ecosystems, microbial biomass[21] was the strongest controller or, at least, the most strongly correlated predictor in urban greenspaces (Fig. 3; Supplementary Fig. 7). We also considered an alternative SEM including a two-path association between soil microbial biomass and C which yielded similar results (Supplementary Fig. 10; Supplementary Table 5). Put simply, we show that soil microbial biomass explained more variation in soil C in urban environments compared with natural ecosystems. Microbial biomass was also positively correlated with glucose and lignin-induced respiration (Methods), and with the biomass of bacteria and fungi (Supplementary Table 6). These findings suggest that soil C in urban greenspaces may be more dependent on microbial activity in these ecosystems. On the contrary, soil C seems to be more dependent on plant productivity in natural ecosystems (e.g., litter inputs). The increased importance of soil microbial biomass in predicting C in urban environments may be associated with the direct management of plants in these ecosystems. The management of urban ecosystems often involves manipulating plant communities by pruning, mowing, fertilization and re-vegetation, potentially weakening the connection between plant productivity and soil C concentrations in these systems. Interestingly, although the contribution of microbial biomass and plant productivity to soil C is shifted in urban ecosystems, both environments support similar levels of C (Fig. 2), suggesting that microbial communities may compensate for the reduced contribution of plants to support soil C in urban environments.

To further investigate the mechanisms behind the importance of soil microbial biomass as a predictor of surface soil C concentrations in urban greenspaces, we conducted metagenomic analyses[22] on composite soil samples collected from a subset of the study sites (27 pairs of natural

and urban ecosystems covering the entire biogeographic range; Supplementary Fig. 11). We targeted microbial genes associated with soil C formation (e.g., photosynthesis genes) and mineralization (e.g., enzymes involved in the degradation of lignin and carbohydrates) traits. Soils in urban greenspaces supported a larger proportion of genes associated with both photosynthesis and C mineralization than in natural areas (Fig. 4D). Indeed, consistent with the knowledge that soils in urban greenspaces support a greater proportion of Chlorophyta than natural ecosystems[23], we found that urban soils had a greater proportion of genes associated with the Photosystem II type photosynthetic reaction center (Fig. 4D). To further explore the C mineralization gene findings while considering the importance of temperature in soil C concentration (Fig. 3), we assessed the temperature sensitivity of soil heterotrophic respiration in laboratory incubations at 0, 10, 20 and 30ºC (Fig. 4A-B). Soil C losses via soil respiration were significantly more sensitive to temperature (i.e., evaluated with the Q10 coefficient[24], which represents an increase in soil respiration with a temperature increase of 10°C; Fig. 4B) in urban greenspaces than in natural ecosystems (Fig. 4A-B). The greater C formation and mineralization activities in urban greenspace soils suggest that the positive relationship between microbial biomass and C concentrations in urban greenspaces is likely driven by enhanced microbially-mediated formation and mineralization of soil C relative to plant productivity controls (Fig. 3; Supplementary Figs. 7-8). Given the important role of soil microbes in controlling C fluxes under climate warming[7,22,25], our findings suggest that urban soil C might be particularly sensitive to climate warming.

Our urban greenspaces structural equation modelling also considered the influence of management on soil microbial biomass and soil C. Management was not considered in the SEM of natural environments as, to the best of our knowledge, the studied ecosystems were not subjected to active management. Our analyses provided evidence that management practices can provide opportunities to indirectly manage soil microbial biomass. For example, urban greenspaces subjected to mowing practices showed higher soil microbial biomass, the most important biotic predictor of soil C. There are potential mechanisms by which mowing could, at least partially, support such an effect. For example, frequent mowing is known to negatively impact alive aboveground biomass, increasing allocation to roots that are an important precursor for soil microbial biomass and for soil C. Moreover, rapid regrowth of aboveground biomass after mowing (typically observed in grass lawns) may stimulate rhizodeposition, which supplies labile C compounds that fuel microbial growth. Our study also highlights that the multiple aspects associated with the influence of management on soil carbon need to be considered in an integrative manner. For example, management practices other than mowing impacting vegetation, such as removal of grass clippings, leaf litter and deadwood inputs, might help to explain the altered link between aboveground plant productivity and soil C in urban greenspaces, as these managements reduce aboveground inputs of organic matter into urban soils. All these aspects need to be considered simultaneously when planning the sustainable management of urban greenspaces. Overall, our results suggest the need for research that investigates the mechanisms underlying the influence of management on soil microbes in urban ecosystem. Yet regardless of the specific mechanism(s), our analysis reinforces the notion that the main environmental factors associated with soil C concentrations in urban greenspaces can differ from those in natural systems, suggesting that urban greenspaces may need to tackle more microbial-oriented approaches for the conservation of soil C.

Conclusions

In summary, we show that urban greenspaces are important reservoirs of surface soil C, supporting similar concentrations to those in adjacent natural ecosystems across a worldwide spatial

distribution. We further reveal that mean annual temperature is the most consistent environmental predictor of soil C concentrations in both urban and natural greenspaces. As demonstrated in natural ecosystems[7], warming temperatures can also trigger microbial-induced soil C losses in urban greenspaces. However, we also showed that plant productivity and soil microbes contribute differently to explaining the distribution of surface soil C in natural and urban ecosystems, with soil microbes appearing central to soil C and its sensitivity to warming in urban greenspaces worldwide. It is important to note that urban soils were characterized by microbial traits associated with faster C cycling, such as high C mineralization capacities. Warming may therefore increase microbial-induced soil C losses in urban greenspaces to a larger extent than in natural ecosystems, limiting the potential of greenspaces to offset the C footprint of urban areas as climate changes. To combat such warming-induced soil C losses, our findings suggest a focus on microbial-based, rather than plant-based management for sustaining soil C, given that microbial biomass was a much stronger predictor of soil C in urban soils. Given that urban greenspaces are more intensively managed than most natural systems, there seems to be a greater potential to develop management strategies that steer the soil microbiome to sustain soil C in urban systems and the multiple ecosystem services that it provides.

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Competing interests:

Authors declare that they have no competing interests.

Figure legends

Figure 1 | Location of the 112 ecosystems surveyed in this study. These ecosystems include 56 paired urban greenspaces and adjacent natural ecosystems. An alternative figure with additional details can be found in Supplementary Fig. 1.

Figure 2 | Soil organic carbon (SOC) concentrations in urban greenspaces and adjacent natural ecosystems. First row shows mean values for concentration (robust linear regressions; rlm) of different C fractions (POC, particulate organic C; MAOC, mineral-associated organic C) in urban greenspaces and adjacent natural ecosystems (n = 56 urban and 56 natural ecosystems). Second row shows the relationship between mean annual temperature (MAT) and soil C concentrations of urban greenspaces and adjacent natural ecosystems. The correlations between MAT and other temperature variables can be found in Supplementary Table 3 (n = 56 urban and 56 natural ecosystems).

Figure 3 | Drivers of soil organic carbon (SOC) concentration in urban greenspaces and adjacent natural ecosystems. The first row shows structural equation modelling, including standardized direct effects of climate (MAT and MAP), plants (forest/non-forest and NPP), texture (sand %) and microbial biomass (sum of bacterial and fungal biomass) on SOC. Numbers adjacent to arrows indicate standardized effect size of the relationship. Only significant relationships are shown ($P < 0.05$). n = 56 urban and 56 natural ecosystems. See a priori model in Supplementary Fig. 6. The second row shows the standardized total effects (STE, sum of direct and indirect effects) of climate, vegetation, texture and microbial biomass on SOC (n = 56 urban and 56 natural ecosystems). MAT, mean annual temperature; MAP, mean annual precipitation; NPP (measured as NDVI; see Methods), plant productivity.

Figure 4 | Microbial-driven losses in soil organic carbon under experimental warming. Panel A shows the relationship between experimental increases in temperature and soil respiration in natural ecosystems (blue) and urban greenspaces (red) (robust linear regressions; rlm, n = 56 urban and 56 natural ecosystems). Panel B shows carbon sensitivity to warming (Q_{10} coefficient, mean ± SE, n = 56 urban and 56 natural ecosystems). Panel C represents microbial biomass (mean ± SE, n = 56 urban and 56 natural ecosystems). Panel D shows the percentage of functional genes associated with carbon cycling in natural ecosystems and urban greenspaces (n = 27 urban and 27 natural ecosystems).

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Methods

Study sites

We conducted a global field standardized survey in urban greenspaces and adjacent natural ecosystems from 56 municipalities across six continents and 17 countries (Supplementary Table 1; Fig. 1; Supplementary Figs. 1 and 2)[23]. Urban greenspaces included well-established urban parks and large residential gardens. Adjacent natural ecosystems had relatively undisturbed natural ecosystems such as semi-natural forests, grasslands and shrublands close to cities, or relict forests maintaining their original vegetation and embedded within urban spaces. Natural ecosystems were ~25 km apart from urban greenspaces. Adjacent natural ecosystems were selected to represent the most common ecosystem type in each location without urbanization. Our survey also included a wide range of climatic conditions supporting cities (Fig. 1). For instance, mean annual temperature and precipitation ranged from 1.2-26.4ºC and 210-1577 mm, respectively. Our study includes a wide range of soils from non-anthropized soils to Technosols. Paired natural and urban ecosystems showed similar levels of mean annual precipitation, sand content and soil C:N ratios. However, temperature is slightly higher in urban spaces supporting the well-known heat-island effect, and urban environments are located in slightly lower elevations (Supplementary Fig. 12).

In each location, we surveyed a 30 m × 30 m representative plot of each ecosystem type (e.g., a grass lawn or an urban forest for 'urban greenspace'). Composite surface soil samples (top ~5 cm depth) were collected from these ecosystems between 2017 and 2019 (Supplementary Fig. 2). To account for spatial heterogeneity in our plots, a composite soil sample (from five soil cores) was collected under the dominant vegetation at each plot (Supplementary Fig. 2). After field collection, each composite soil sample was divided into two sub-samples - one sub-sample was immediately frozen at -20 ºC for molecular analyses while the other sub-sample was air-dried for chemical analyses. Soil samples were sieved (2 mm) and roots were manually removed when present.

Carbon concentrations and fractionation

The total concentration of soil organic C was measured by dry combustion and gas chromatography using a ThermoFlash 2000 NC Soil Analyzer (Thermo Fisher Scientific, MA)[26]. Carbonates were removed prior to analysis by acid fumigation. Soil samples were subjected to a size fractionation method[27] to separate the particulate (not protected by minerals from microbial decomposition) and mineral-associated (protected by minerals) C fractions. In particular, 30 mL of sodium hexametaphosphate (5%) was added to 10 g of soil and shaken for 18 h to disperse aggregates. After dispersion, the mixture was thoroughly rinsed through a 53 μm sieve to separate the particulate (> 53 μm) and mineral-associated (< 53 μm) C fractions using an automated wet sieving system. The isolated fractions were oven-dried at 60 ºC, weighed, and ground with a ball mill. The C fractions were analyzed for organic C concentrations following the same procedure as for total soil organic C (Thermo Fisher Scientific, MA). Soil C concentrations, both in urban and natural greenspaces were, on average, dominated by the MAOC fraction (Supplementary Fig. 13).

Environmental factors included in statistical models

Mean annual temperature and mean annual precipitation data were obtained from WorldClim 2.0 database[28], a high resolution (30 seconds, ~1 km^2) database based on a large number of climate observations and topographical data for the 1970-2000 period. We also determined alternative temperature measurements, including soil mean annual temperature (SBIO1; 1-km resolution)[29], maximum temperature (BIO5; WorldClim v2; 1-km resolution) and recent (2016-2020) mean air and land surface temperatures (30-m resolution; Landsat) (Supplementary Table 3). Plant productivity (NPP) was estimated using the mean annual Normalized Difference Vegetation Index (NDVI) from Landsat (averaged values between 2016 and 2020 at a resolution of 30 m)[30]. We are working at a 30m resolution to match the resolution of our field survey (30m x 30m plots). NDVI is commonly used to investigate vegetation patterns and dynamics in urban greenspaces across a worldwide spatial distribution[31, 32]. Sand content was also determined in the lab, as done in ref.[33]. Forest structure (1 = forest vs. 0 = non-forested ecosystems) and management practices (irrigation, fertilization and mowing) were determined in the field.

Soil microbial biomass

The biomass of bacteria and fungi were measured using microbial phospholipid fatty acids (PLFAs)[21] according to ref[34]. The extracted PLFA samples were quantified using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). The peaks were identified using a Sherlock Microbial Identification System (MIDI, Inc., Newark, NJ, USA). Total biomass of fungi and bacteria were determined as the sum of bacterial and fungal PLFAs, respectively[35]. Total biomass was positively correlated with substrate-induced respiration using glucose (Spearman ρ = 0.39; $P < 0.001$; n = 112 ecosystems) and lignin (Spearman ρ = 0.48; $P < 0.001$; n = 112

ecosystems) from MicroResp analyses (measured absorbance at 570 nm after 5 h of incubation; 25 ºC and 60% water holding capacity).

Soil respiration and Q_{10}

Soil respiration rates were measured after 10-h incubations in triplicate at four increasing temperatures (0, 10, 20 and 30 ºC) in 96- deep- well microplates, using the MicroResp technique[36]. We calculated the β and R_0 coefficients for the exponential relationship between heterotrophic soil respiration rate (R_s, in μg CO_2-C g^{-1} h^{-1}) and temperature (T, in ºC): $R_s = R_0 \times \exp(\beta \times T)$ (24); and used β to compute Q_{10} using the equation $Q_{10} = \exp(10 \times \beta)$. The Q_{10} coefficient represents the increase in soil respiration as temperature increases by 10 °C. Higher soil respiration rates and Q_{10} values were interpreted as higher soil C sensitivity to microbial decomposition and increases in temperature.

Microbial functional traits

A composite soil sample per plot was sequenced for the entire metagenome[37,38] in 27 paired urban/natural ecosystems (54 samples). These composite soil samples came from the same plots and sampling dates as those analyzed above, and were collected in open spaces between plant patches. According to the manufacturer's protocol, more than 500 ng DNA per soil sample was isolated for shotgun metagenomic sequencing using the DNeasy PowerSoil DNA Isolation Kit (QIAGEN Inc., USA). Sequencing was performed using an Illumina HiSeq (Illumina Inc., USA) at Majorbio in Shanghai, China. Raw reads (PE150, 150 bp paired-end reads) were trimmed to remove low-quality reads as follows. First, the SeqPrep software (https://github.com/jstjohn/SeqPrep) was used to remove the adapter sequences. Second, the library sickle (https://github.com/najoshi/sickle) was used to trim the reads from the 5' end to 3' end using a sliding window (size 50 bp, 1 bp step). If the mean quality of bases inside a window dropped below 20, the remainder of the read below the quality threshold was trimmed. Quality-trimmed reads that were shorter than 50 bp or containing N (ambiguous bases) were discarded.

The original sequences of the 54 samples were annotated using Subsystem Technology (MG-RAST; https://www.mg-rast.org)[39] to perform quality control, automated annotation, and produce taxonomic and functional assignments. MG-RAST generates taxonomic assignments based on the SEED subsystem database by DIAMOND software (version 0.9.32) by best-hit classification with a maximum E-value of $1e^{-5}$, a minimum identity of 60%, and a minimum alignment length of 25 amino acids for proteins and functional categories. The resulting table was parsed at SEED Subsystem Level3 by software SUPER-FOCUS. This information was used to investigate the proportion of genes associated with respiration and the degradation of C sources.

Statistical analyses

Relationship between MAT and soil C

The relationship between mean annual temperature and soil C was first tested using a robust linear model using the MASS package in R (https://www.r-project.org/) in urban and natural environments. This approach was used to avoid any potential influence of outliers in our results.

Nested permanova

All comparisons between C cycling and functional gene variables between natural ecosystems and urban greenspaces explicitly took into consideration our sampling design (paired urban and natural

ecosystems). In particular, we conducted Nested PERMANOVA analyses[23] using a block design (to account for our paired natural/urban ecosystem design) testing for differences in the values associated with C concentrations and proportion of functional genes in urban greenspaces vs. natural ecosystems. We used the function "adonis" in the R package "Vegan"[40] and the term "strata" (block) to conduct these analyses.

Linear mixed modelling

Differences in the contents of the total, mineral-associated and particulate soil organic C between urban and natural ecosystems were also tested by linear mixed-effects modelling to control for climate, net primary production, soil texture and microbial biomass. For these analyses, we used the R packages lme4 and lmerTest[41-43]. The paired design was accounted for by incorporating an intercept structure in the random term of the model. The numeric predictors were standardized by subtracting the mean and dividing it by two standard deviations, and the binary predictors were rescaled to -0.5 and 0.5. The coefficients and 95% confidence intervals were calculated using the restricted maximum likelihood method and bootstrapping (1000 simulations). Variance inflation factors (VIF) showed valued lower than 5 for all the predictors, indicating low multicollinearity[45].

Structural equation modelling

The main objective of this analysis was to provide a system-level understanding of the total, direct and indirect effects of mean annual temperature on total soil organic, particulate and mineral C concentrations considering multiple environmental factors such as mean annual precipitation, sand content, forest ecosystems, and management (i.e., in the case of urban greenspaces: mowing, irrigation and fertilization). The Forest/non-forest (lawns and gardens) ecosystems were included in our SEM as categorical variables with two levels: 1 (forest) and 0 (non-forest). These analyses were done independently for urban and natural ecosystems (n = 56 urban and 56 natural ecosystems). Because some of the variables introduced were not normally distributed, we used bootstrap tests in these SEMs. We evaluated the fit of these models using the model χ2-test, the root mean squared error of approximation and the Bollen–Stine bootstrap test[44]. All models showed a good fit. Natural ecosystems: χ^2 / df = 0.70, P = 0.40; RMSEA = 0.00, P = 0.44, and Bootstrap P = 0.42. Urban greenspaces: χ^2 / df = 0.33, P = 0.57; RMSEA = 0.00, P = 0.59, and Bootstrap P = 0.60. We did not find multicollinearity in our models. In particular, the results of a multiple regression model shows that variance inflation factors (VIF) of the correlates used in our SEM models are always lower than 5[45]. This indicates low collinearity (considered to be high when VIF >5 and problematic if >10)[45] (Supplementary Table 7).

Variation partitioning

The main goal of this analysis was to quantify the relative contribution of mean annual temperature, plant productivity and microbial biomass to explain total soil organic, particulate and mineral C concentrations in soils from urban and natural greenspaces after controlling for other important environmental factors such as mean annual precipitation, sand content, forest ecosystems, and management (i.e., in the case of urban greenspaces: mowing, irrigation and fertilization). These analyses were done independently for urban and natural ecosystems (n = 56 urban and 56 natural ecosystems). We also included spatial influence (location: latitude and longitude) in these analyses. Variation partitioning analyses were conducted with the R package Vegan[40].

Temperature threshold analyses

To search for the existence of thresholds in the relationship between soil temperature and soil C concentrations we fitted linear and threshold regressions to the relationship between mean annual temperature and soil C fractions (POC and MAOC). We used the Akaike information criteria (AIC) to decide which model best fitted the data. This criterion penalizes model fit (loglikelihood) by the number of parameters used in the model, and is minimum for the type of model that best fits the data. In general, differences in the AIC larger than 2 indicate clearly different model fits[46]. To estimate the threshold, we used segmented models[46]. These models allow both the slope and the intercept to change at a given point of the predictor (here annual mean temperature) which is called breakpoint and is identified as a threshold in temperature producing a discontinuous sudden change in the response of soil C concentrations to temperature[47,48]. We selected segmented models based on prior knowledge of the response of soil organic carbon[48]. Once determined that the fitting of segmented models was better than that of linear regressions, we bootstrapped 100 times the segmented regression to find the confidence interval of the breakpoint parameter (thus retrieving an estimation of the threshold error). We performed this procedure for POM and MAOC and independently for natural and urban ecosystems. We used the chngpt (v2021.5–12)[49] packages in R to fit segmented regressions.

Data availability:

The raw data associated with this study is available in https://figshare.com/s/1eadef6619e74a8f2904 (DOI: 10.6084/m9.figshare.21025615)[50].

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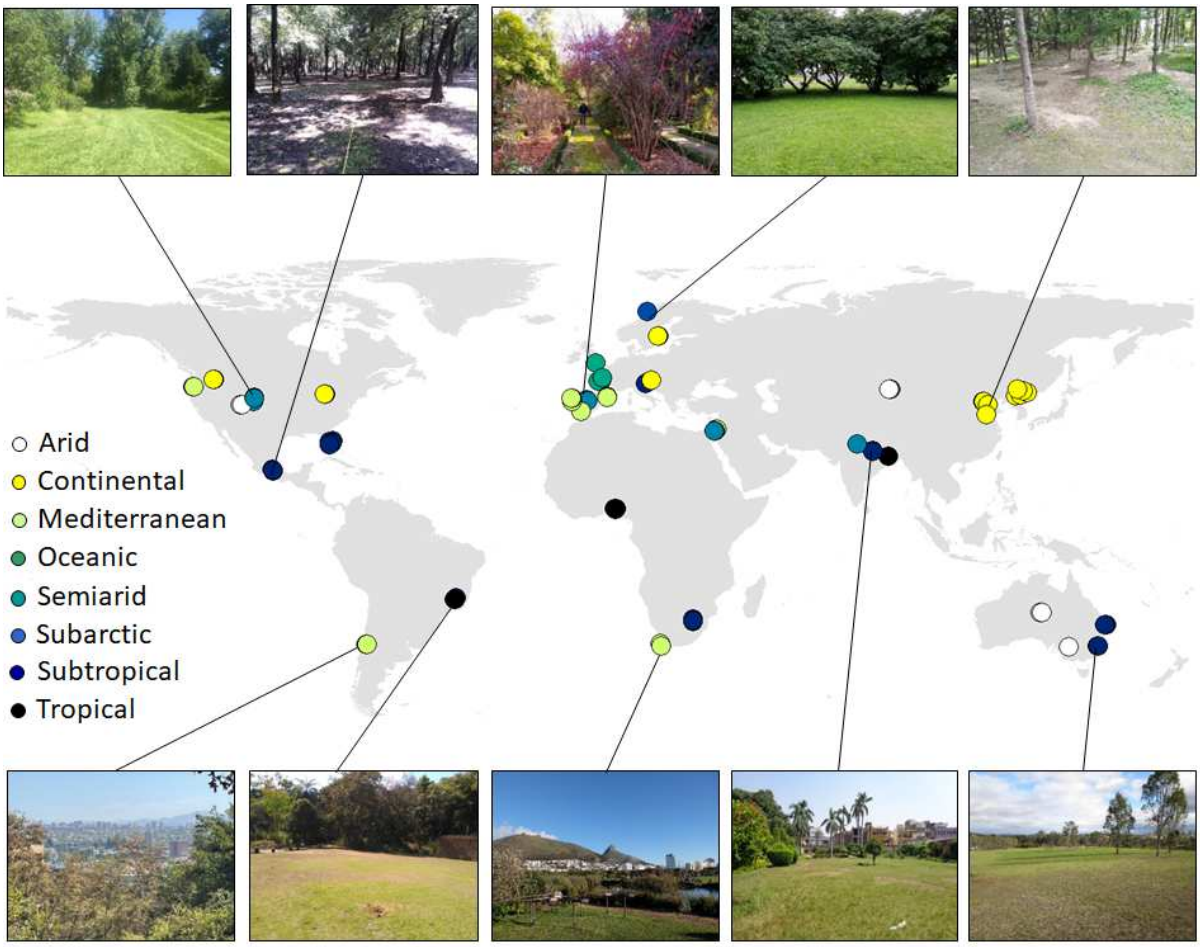
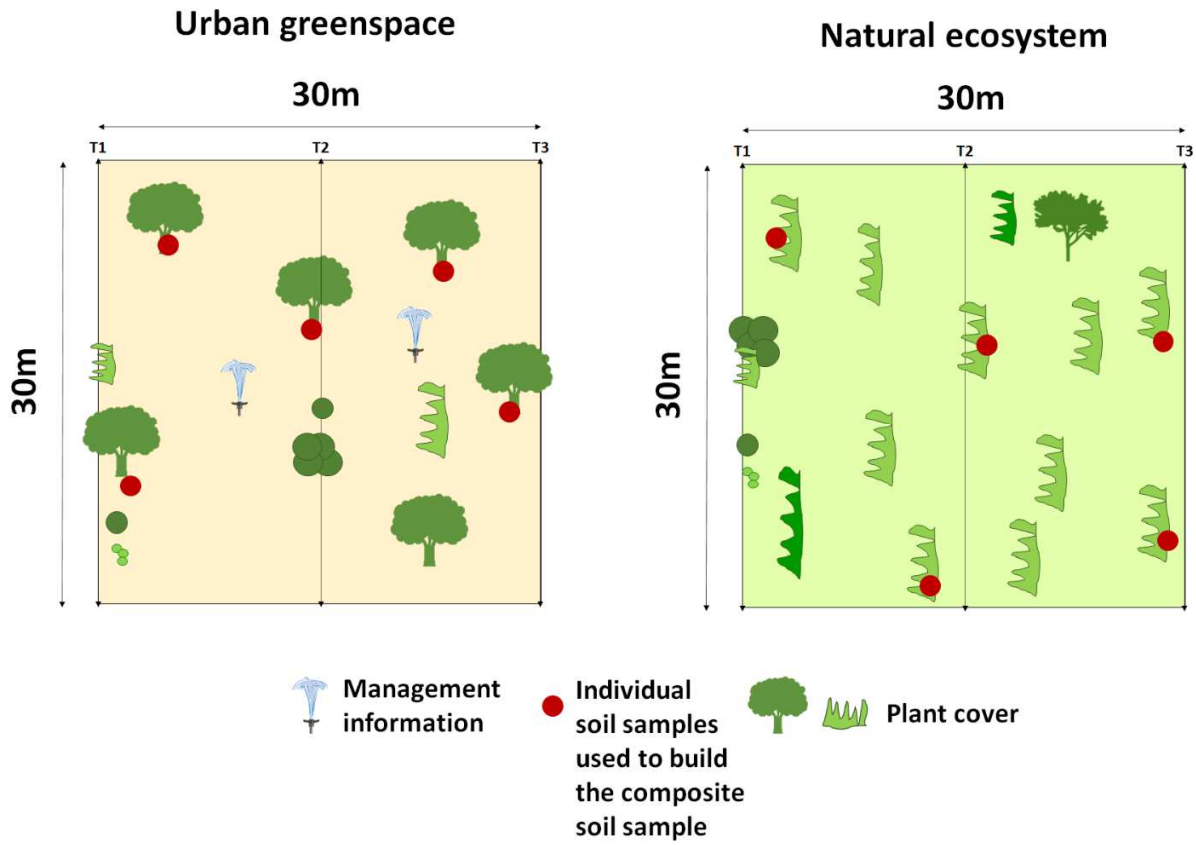
Supplementary Information

Table of contents

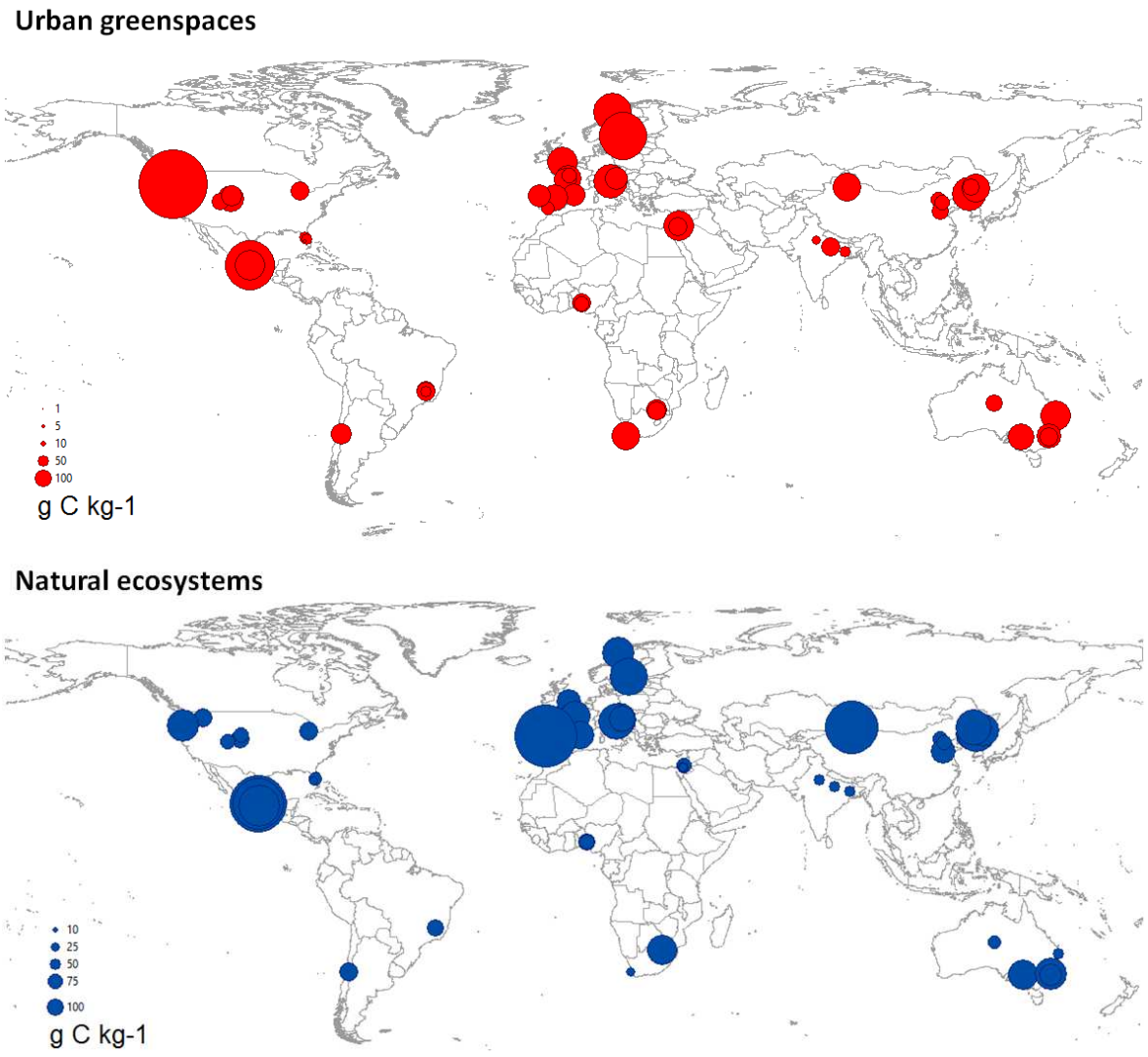
Preprint submitted to Nature Climate Change



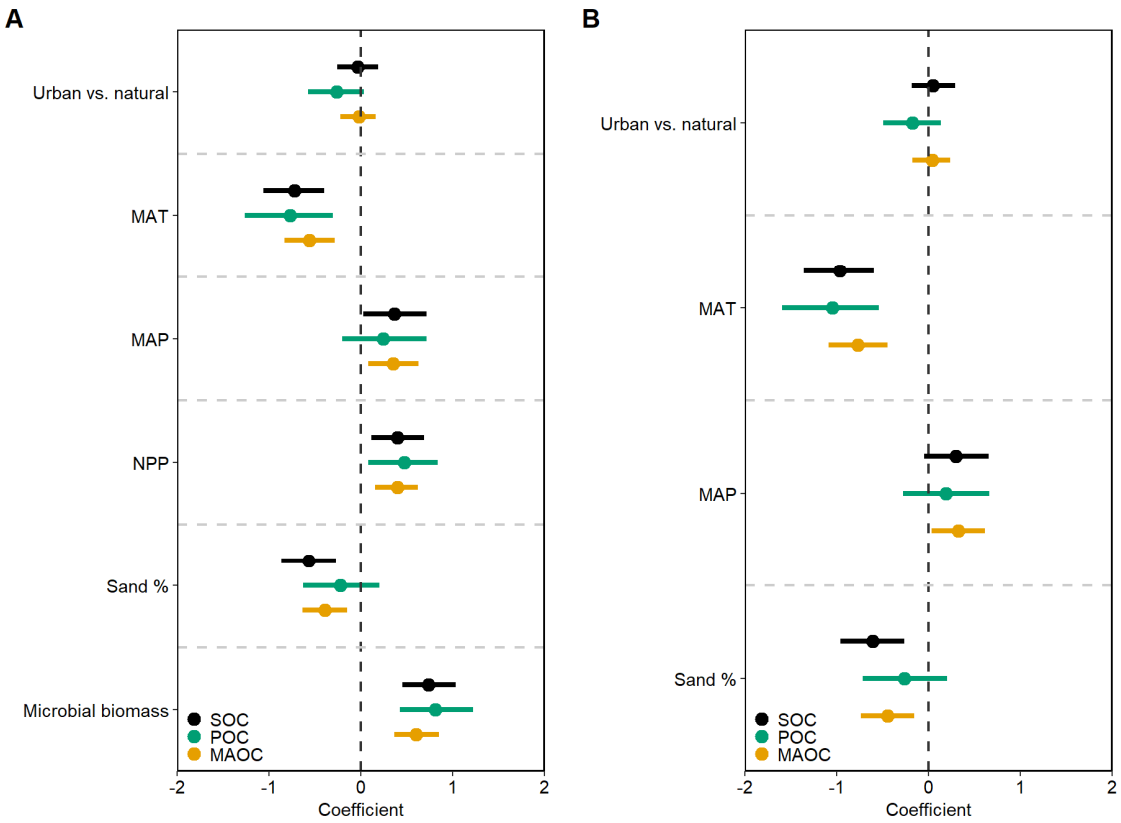
Supplementary Figure 1 | Location of the 112 ecosystems surveyed in this study. These ecosystems include 56 paired urban greenspaces and adjacent natural ecosystems. Pictures show examples of urban greenspaces.



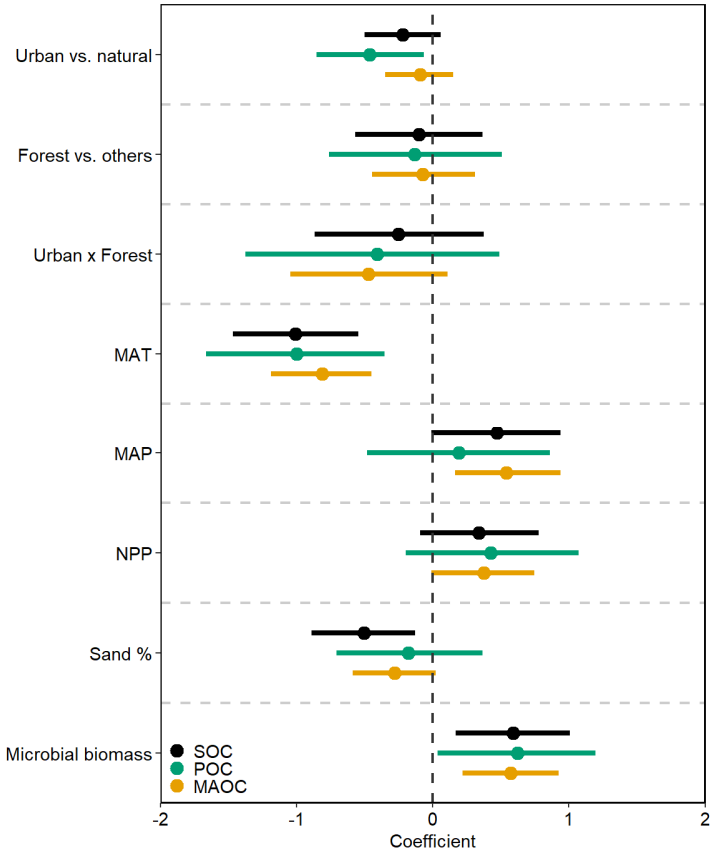
Supplementary Figure 2 | Summary of the survey design for each of the 56 natural and urban paired ecosystems used in this study. This figure is a visual example of our survey design.



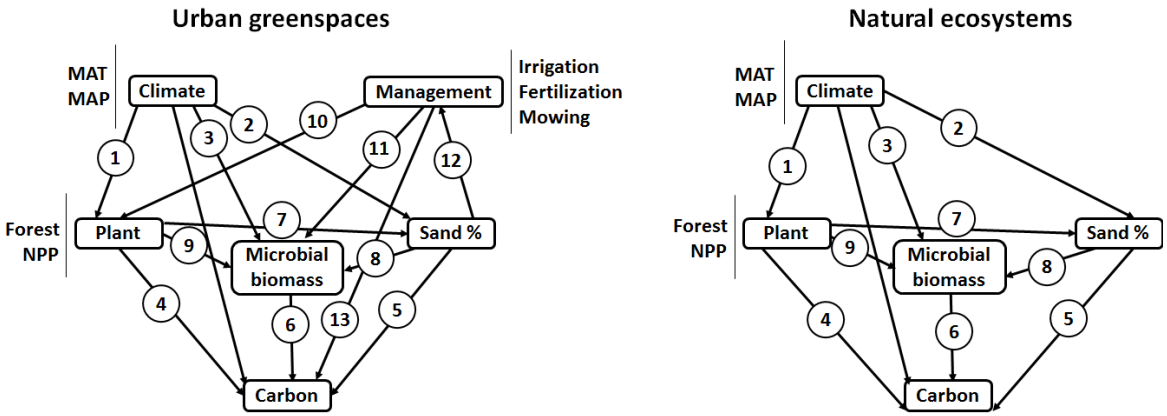
Supplementary Figure 3 | Global distribution of soil C concentrations in natural and urban greenspaces (n = 56 per ecosystem type).



Supplementary Figure 4 | Linear mixed model testing the influence of ecosystem, climate, texture, plant and microbial data on soil carbon concentrations. Panel A shows effects of urban greenspaces versus natural ecosystems on the concentration of total, particulate and mineral-associated soil organic C (SOC, POC, MAOC) controlling for mean annual temperature (MAT), mean annual precipitation (MAP), net primary productivity (NPP), and content (Sand %) and soil microbial biomass (n = 112; 56 urban and 56 natural ecosystems). Dots and bars represent standardized coefficients and 95% confidence intervals for fixed effects obtained by mixed-effects modeling and bootstrapping. Panel B shows effects of urban greenspaces versus natural ecosystems on total soil organic C and C fractions controlling for MAT, MAP and Sand %, but not for NPP and microbial biomass (n = 112; 56 urban and 56 natural ecosystems). Dots and bars represent standardized coefficients and 95% confidence intervals for fixed effects obtained by mixed-effects modeling and bootstrapping.



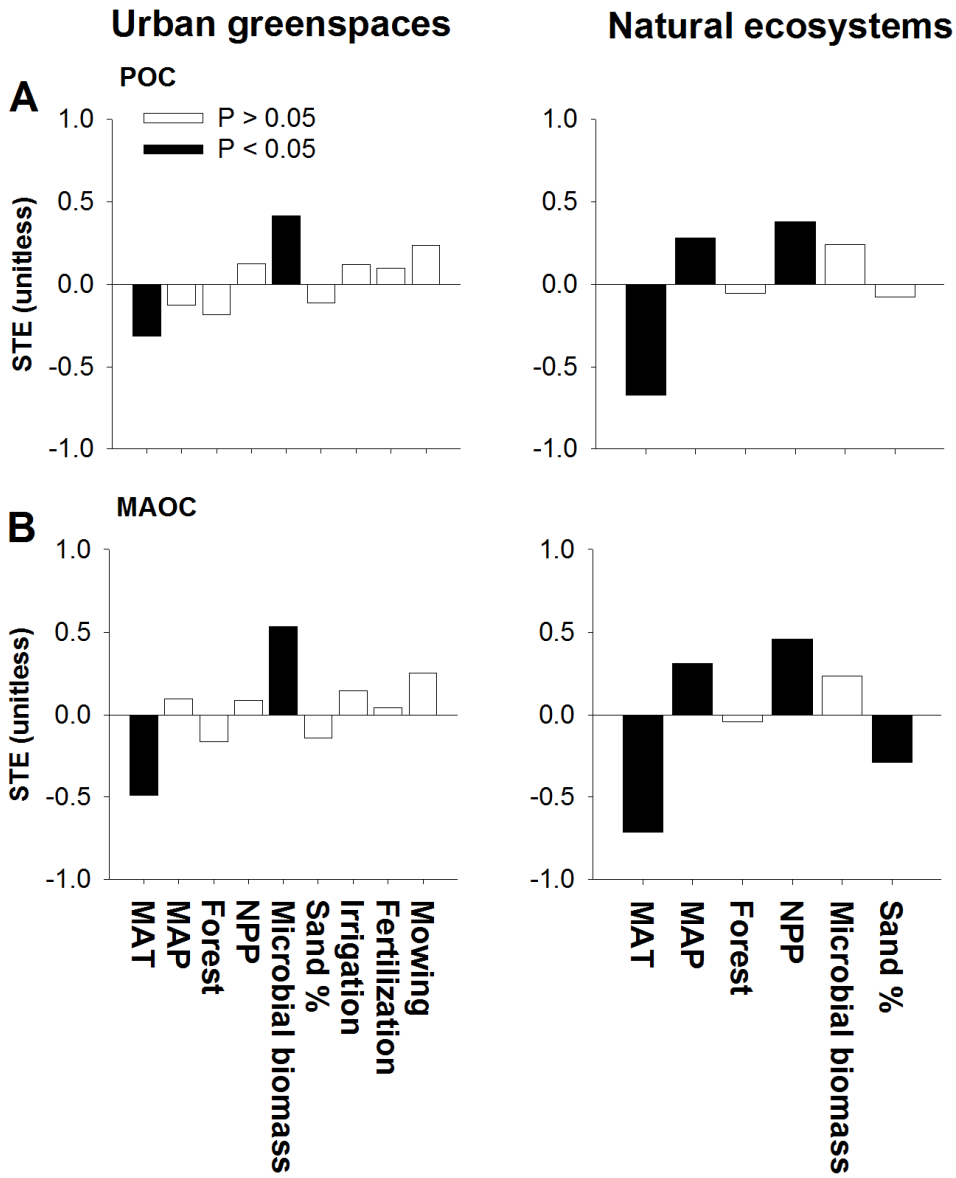
Supplementary Figure 5 | Linear mixed model testing the influence of ecosystem, climate, texture, plant and microbial data on soil carbon concentrations after accounting for vegetation interactions. Effects of urban greenspaces versus natural ecosystems on the concentration of total, particulate and mineral-associated soil organic C (SOC, POC, MAOC) controlling for climate, plant and soil variables (n = 112; 56 urban and 56 natural ecosystems). Dots and bars represent standardized coefficients and 95% confidence intervals for fixed effects of urban greenspaces, mean annual temperature (MAT), mean annual precipitation (MAP), net primary productivity (NPP), sand content and soil microbial biomass obtained by mixed-effects modeling and bootstrapping.



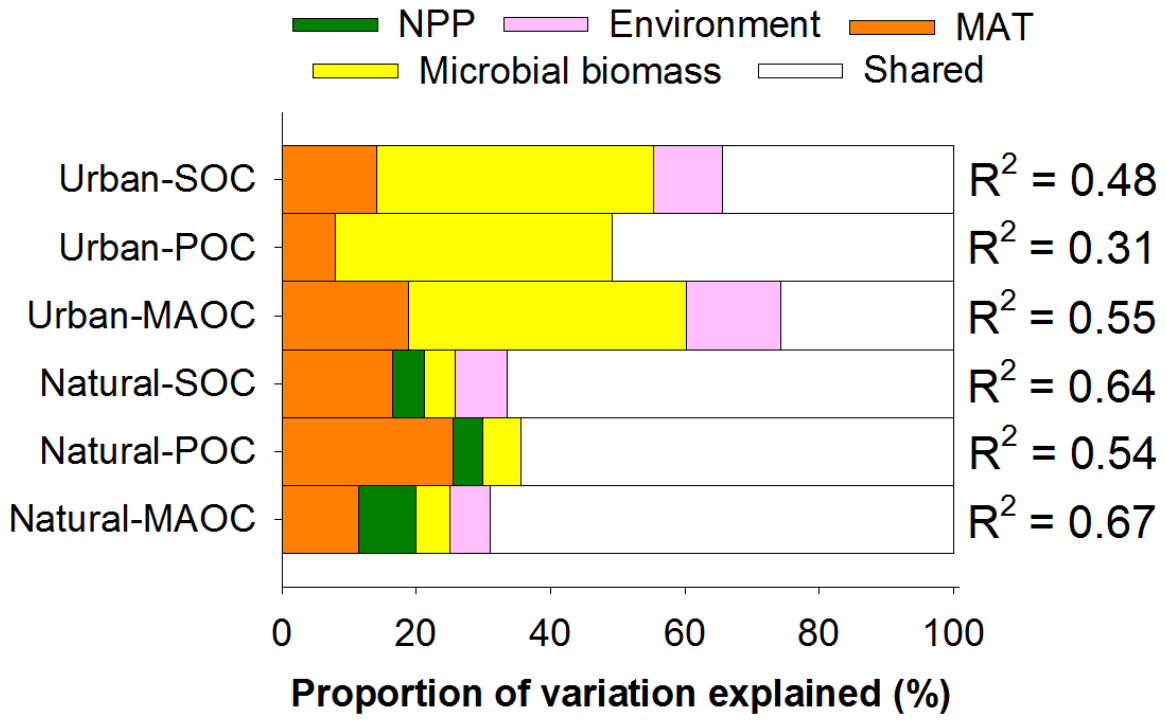
#	Factor	Rationale
1	Climate → Plant	Mean annual precipitation and temperature is well known to control the development of vegetation structure (e.g., forests) and plant productivity in terrestrial ecosystems
2	Climate → Sand %	Climate is known to control soil texture through weathering
3	Climate → Microbial biomass	Mean annual precipitation and temperature drives the biomass of soil organisms. Drier and hotter terrestrial ecosystems often support lower microbial biomass
4	Plant → Carbon	Plant structure and productivity control the storage of soil carbon by fixing carbon from the atmosphere and incorporating this carbon to the soil through important processes such as litter decomposition and rhizodeposition. This link could be strongly altered (lessen or broken) in managed urban greenspaces: no litter, no deadwood generally is available as input of organic matter for soils because they are systematically removed.
5	Sand % → Carbon	Soil texture is fundamental in the sequestration of carbon. Soils with sandy texture are known to retain less carbon than those with fine texture
6	Microbial biomass → Carbon	Microbes drive the concentration of soil carbon through important processes such as organic matter decomposition and in being an important part of the living and dead biomass of soils
7	Plant → Sand %	Plant structure influences soil texture by regulating key processes such as soil erosion that negatively affects the percentage of small soil particles

8	Sand % → Microbial biomass	Soil texture is known to influence microbial biomass. Sandy soils, for example, have a reduced capacity to build soil microbial biomass
9	Plant → Microbial biomass	Plant structure and productivity drive the biomass of microbial communities by constituting an important source of energy (e.g., litter) and habitat for soil microbes. Forest often supports larger microbial biomass than non-forested ecosystems
10	Management → Plant	Management types such as irrigation, mowing and fertilization can influence plant productivity and vegetation structure by changing resource availability and through anthropogenic disturbance. For example, irrigation and fertilization are expected to promote plant productivity
11	Management → Microbial biomass	Management types such as irrigation, mowing and fertilization can influence microbial biomass by disturbing soils and changing resource accessibility (e.g., water and nutrient availability)
12	Sand % → Management	Soil texture can largely influence the type of management. For example, sandy soils, often poor in nutrients and water holding capacity, would require more irrigation and fertilization than soils with fine texture
13	Management → Carbon	Management can influence the amount of carbon in the soil through processes such as fertilization and irrigation, but also through anthropogenic disturbance

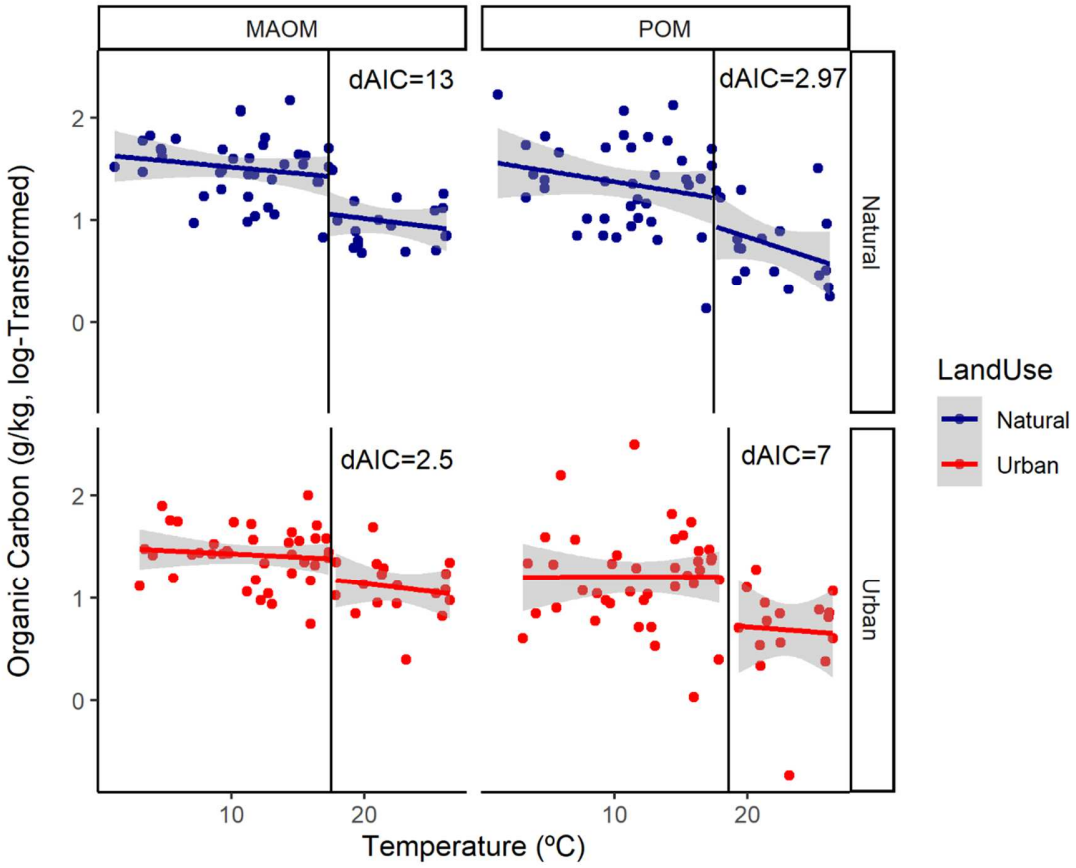
Supplementary Figure 6 | *A priori* structural equation modelling including the direct and indirect effects of environmental factors on soil carbon concentrations.



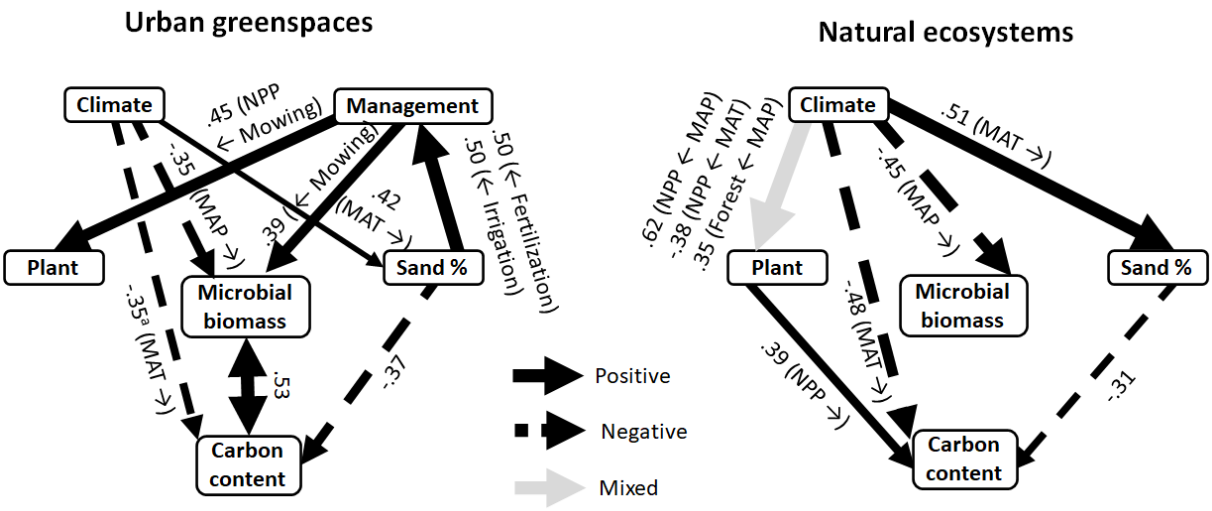
Supplementary Figure 7 | Standardized total effects (sum of direct and indirect effects) of environmental factors on the concentration of mineral (MAOM) and particulate soil carbon (POM) (n = 56 urban and 56 natural ecosystems).



Supplementary Figure 8 | The unique contribution of mean annual temperature (MAT) to explaining soil carbon concentrations in urban greenspaces and natural ecosystems. The environment includes location (latitude and longitude), mean annual precipitation, sand content, forest ecosystems, and management (i.e., in the case of urban greenspaces: mowing, irrigation, and fertilization) (n = 56 urban and 56 natural ecosystems). NPP, Plant productivity; SOC, soil organic carbon; POM, particulate organic matter; MAOM, mineral-associated organic matter. Shared variation is attributed to more than one group of predictors and cannot be distinguished to what group this variation belongs to.

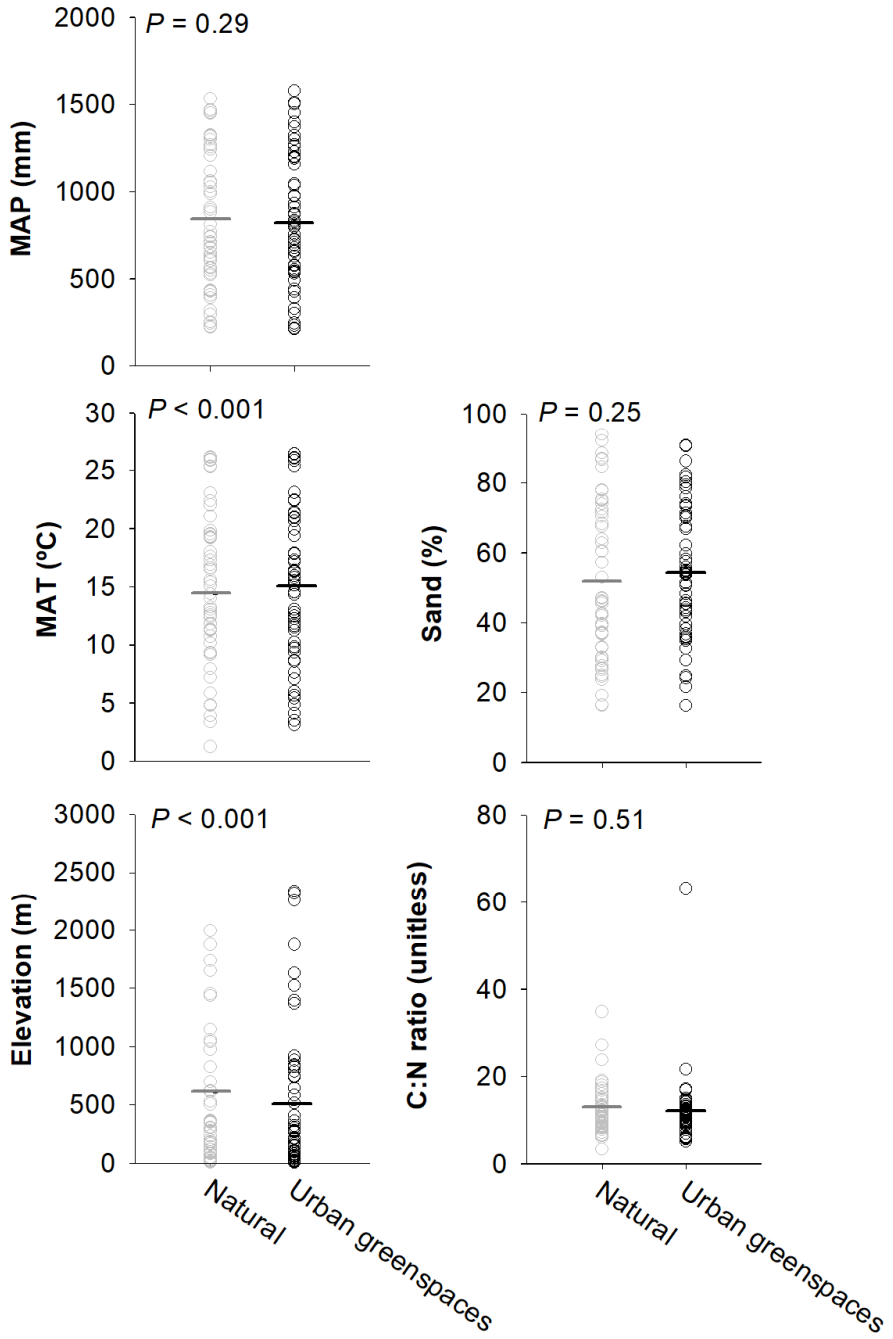


Supplementary Figure 9 | Mean annual temperature thresholds associated with soil C concentrations in natural and urban greenspaces. dAIC represents the difference in AIC between segmented (showed in this figure) and linear models (see Supplementary Table 5) (n = 56 urban and 56 natural ecosystems). The shade in these panels corresponds to the 95% confidence interval.

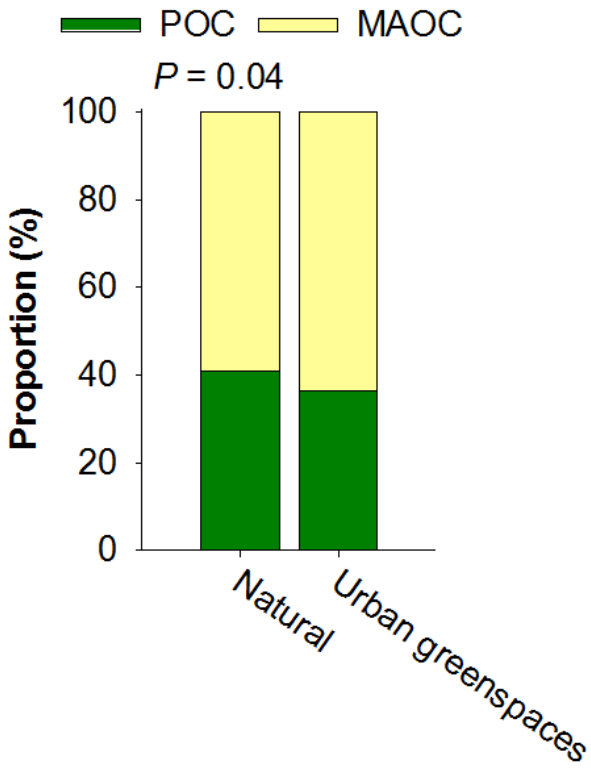


Supplementary Figure 10 | Alternative structural equation model (SEM) to that showed in Fig. 3 considering a two-path association between soil microbial biomass and C in our a priori model (Supplementary Fig. 6) (n = 56 urban and 56 natural ecosystems). [a]*P* = 0.05. Numbers adjacent to arrows indicate standardized effect size of the relationship. The rest of the caption as in Fig. 3.

Supplementary Figure 11 | Location for the 54 ecosystems (27 paired urban and adjacent natural ecosystems) including soil metagenomic data.



Supplementary Figure 12 | Environmental variables in urban and natural ecosystems (mean ± SE; n = 112; 56 natural greenspaces and 56 natural ecosystems). Significance is determined from a nested Permanova considering our paired design.



Supplementary Figure 13 | Proportion of particulate (POC) and mineral (MAOC) soil organic C in natural and urban greenspaces (n = 112; 56 urban and 56 natural ecosystems). P = Permanova P.

Supplementary Table 1 | Information of the 56 municipalities included in this study.

Site	City	Latitude	Longitude
1	Tonghua City, Jilin, China	41.74	125.94
2	Baishan City, Jilin, China	42.18	127.5
3	Yanji City, Jilin, China	42.91	129.49
4	Dunhua City, Jilin, China	43.38	128.22
5	Jilin City, Jilin, China	43.84	126.52
6	Santiago, Santiago Metropolitan Region, Chile	-33.37	-70.61
7	Belo Horizonte, Minas Gerais State, Brazil	-19.87	-43.97
8	Contagem, Minas Gerais State, Brazil	-19.94	-44.04
9	Betim, Minas Gerais State, Brazil	-19.94	-44.18
10	Longmont, CO, USA	40.16	-105.12
11	Grand Junction, CO, USA	39.11	-108.61
12	Cheyenne, WY, USA	41.16	-104.83
13	South Lyon, MI, USA	42.44	-83.68
14	Oxford, England, UK	51.75	-1.29
15	Bodø, Norway	67.28	14.39
16	Uppsala, Sweden	59.85	17.63
17	Poitiers, France	46.58	0.34
18	Niort, France	46.33	-0.47
19	Tours, France	47.4	0.68
20	Ljubljana, Slovenia	46.05	14.48
21	Koper, Slovenia	45.54	13.73
22	Maribor, Slovenia	46.57	15.65
23	Pretoria, South Africa	-25.76	28.22
24	Germiston, South Africa	-26.16	28.13
25	Cape Town, South Africa	-33.9	18.4
26	Durgapur, West Bengal, India	23.56	87.3
27	Mirzapur, Uttar Pradesh, India	25.14	82.56
28	Agra, Uttar Pradesh, India	27.2	78.01
29	Beijing, China	40.01	116.39
30	Tai'an, Shandong, China	36.22	117.02
31	Tianjin, China	39.08	117.69
32	Ürümqi, Xinjiang, China	43.83	87.66
33	Alice Springs, Northern Territory, Australia	-23.71	133.87
34	Brisbane, Queensland, Australia	-27.5	153.02
35	Mildura, Victoria, Australia	-34.19	142.17
36	Cecil Hills, Sydney, New South Wales, Australia	-33.88	150.85
37	Heathcote, Sydney, New South Wales, Australia	-34.08	151.01
38	Barcelona, Catalunya, Spain	41.42	2.15

39	Pullman, Washington, USA	46.74	-117.18
40	Corvallis, Oregon, USA	44.53	-123.26
41	Coyoacán, Mexico City, Mexico	19.31	-99.18
42	Tlalpan, Mexico City, Mexico	19.29	-99.19
43	Miguel Hidalgo, Mexico City, Mexico	19.42	-99.19
44	Madrid, Comunidad de Madrid, Spain	40.41	-3.69
45	Esa-Odo, Osun state, Nigeria	7.76	4.81
46	Obafemi Awolowo University, Osun state, Nigeria	7.52	4.53
47	Ife city, Osun state, Nigeria	7.49	4.59
48	Lakeland, Florida, USA	28.04	-81.97
49	Sebring, Florida, USA	27.48	-81.42
50	Punta Gorda, Florida, USA	26.93	-82.06
51	Utrera, Andalusia, Spain	37.19	-5.77
52	Coimbra, Portugal	40.21	-8.42
53	Porto, Portugal	41.17	-8.68
54	Jerusalem, Israel	31.77	35.22
55	Be'er Sheva, Israel	31.23	34.79
56	Ofakim, Israel	31.31	34.63

Supplementary Table 2 | Correlation (Pearson; two-tailed) between the concentration of total soil organic C and C fractions (POC, particulate organic C; MAOC, mineral-associated organic C) in natural and urban greenspaces.

		Natural	**Urban greenspaces**
POC	r	.914	.930
	P	<0.001	<0.001
	n	56	56
MAOC	r	.918	.724
	P	<0.001	<0.001
	n	56	56

Supplementary Table 3 | Correlation (Pearson; two-tailed) between mean annual temperature (BIO1; MAT; average of the last 50 years; 1-km resolution; WorldClim v2) and soil mean annual temperature (1-km resolution; Lembrechts et al. 2022), maximum temperature (BIO5; WorldClim v2), and recent (2016-2020) mean surface temperatures (30-m resolution; Landsat) in natural and urban greenspaces.

	Natural	**Urban**
Soil mean annual temperature (SBIO1)	0.973	0.981
	< 0.001	< 0.001
	56	56
Maximum temperature (BIO5)	0.711	0.699
	< 0.001	< 0.001
	56	56
Land surface temperature_(2016-2020)	0.674	0.692
	< 0.001	< 0.001
	52	50
Mean air temperature (2016-2020)	0.825	0.843
	< 0.001	< 0.001
	56	56

Supplementary Table 4 | Correlation (Spearman; two-tailed) between maximum temperature and concentrations of soil organic C (SOC), microbial, bacterial and fungal biomass, particulate organic C (POC) and mineral-associated organic C (MAOC) in natural and urban greenspaces.

Soil carbon	Parameter	Natural	Urban
SOC	ρ	-.709	-.541
	P-value	<0.001	<0.001
	n	56	56
Microbial biomass	ρ	-.352	-.361
	P-value	.008	.006
	n	56	56
Bacterial biomass	ρ	-.382	-.456
	P-value	.004	.000
	n	56	56
Fungal biomass	ρ	-.343	-.346
	P-value	.010	.009
	n	56	56
POM	ρ	-.598	-.441
	P-value	<0.001	.001
	n	56	56
MAOM	ρ	-.687	-.589
	P-value	<0.001	<0.001
	n	56	56

Supplementary Table 5 | Akaike index associated with the models included in Supplementary Figure 9 (n = 56 urban and 56 natural ecosystems).

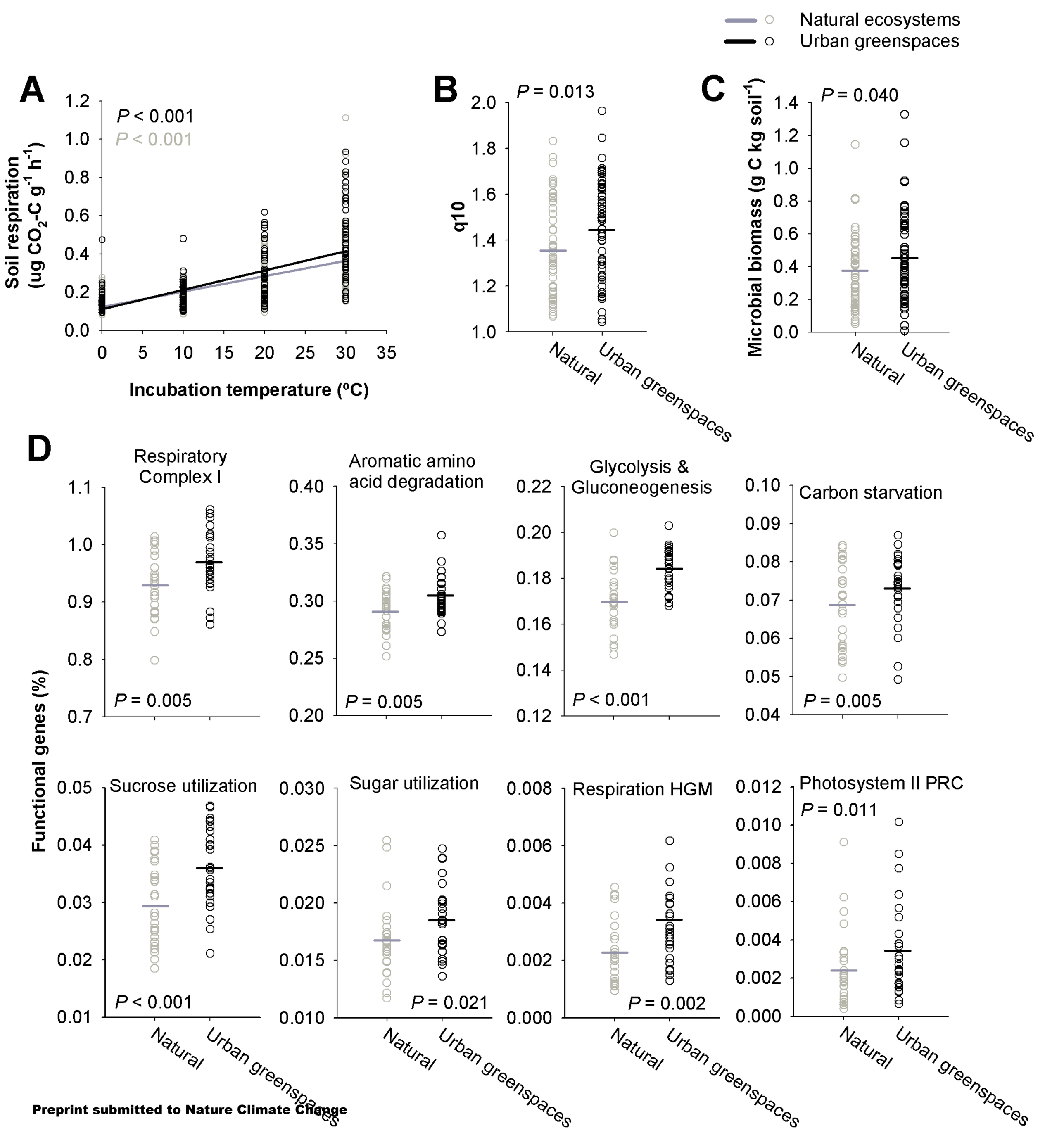
Ecosystem	Variable	AIC Lineal model	AIC Segmented model	Delta AIC	Selected model
Natural	MAOC	30.98	19.14	11.85	*Segmented*
Natural	POC	63.28	56.90	6.39	*Segmented*
Urban	MAOC	20.91	18.49	2.42	*Segmented*
Urban	POC	80.22	72.94	7.28	*Segmented*

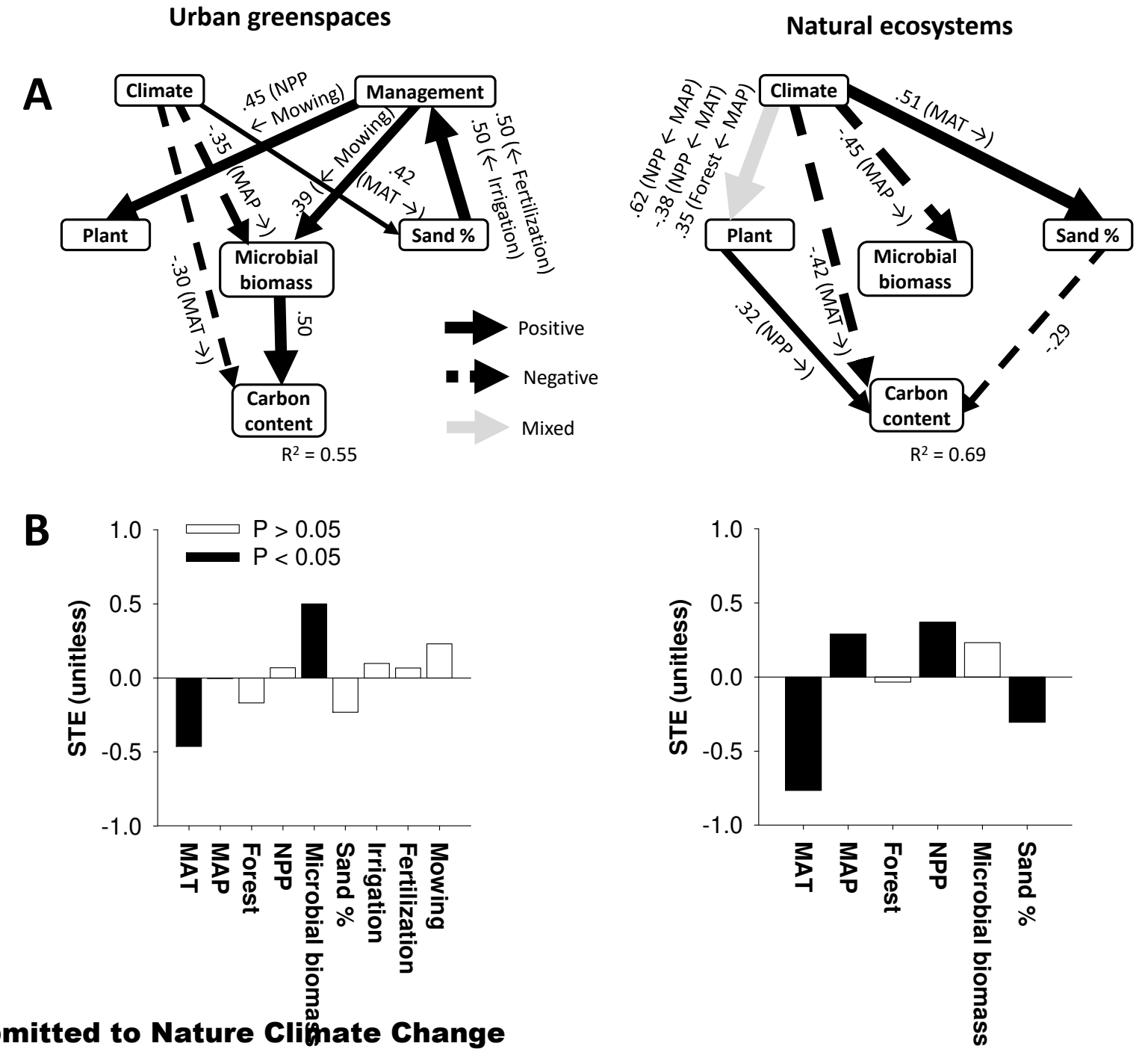
Supplementary Table 6 | Correlation (Pearson; two-tailed) between total microbial biomass with bacterial and fungal biomass in natural and urban greenspaces.

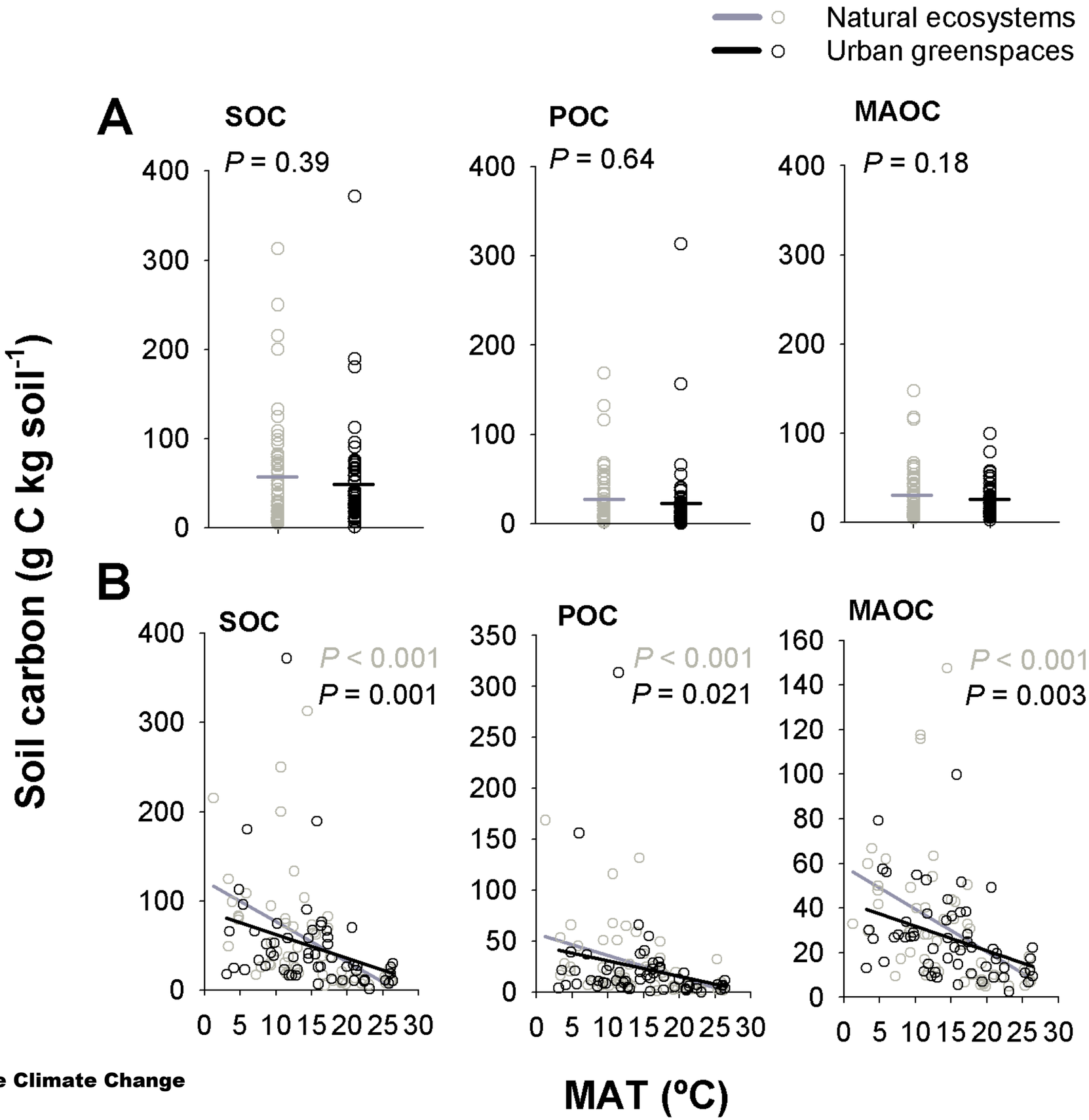
		Natural	**Urban**
Bacterial biomass	r	.929	.925
	P-value	< 0.001	< 0.001
	N	56	56
Fungal biomass	r	.999	.999
	P-value	< 0.001	< 0.001
	N	56	56

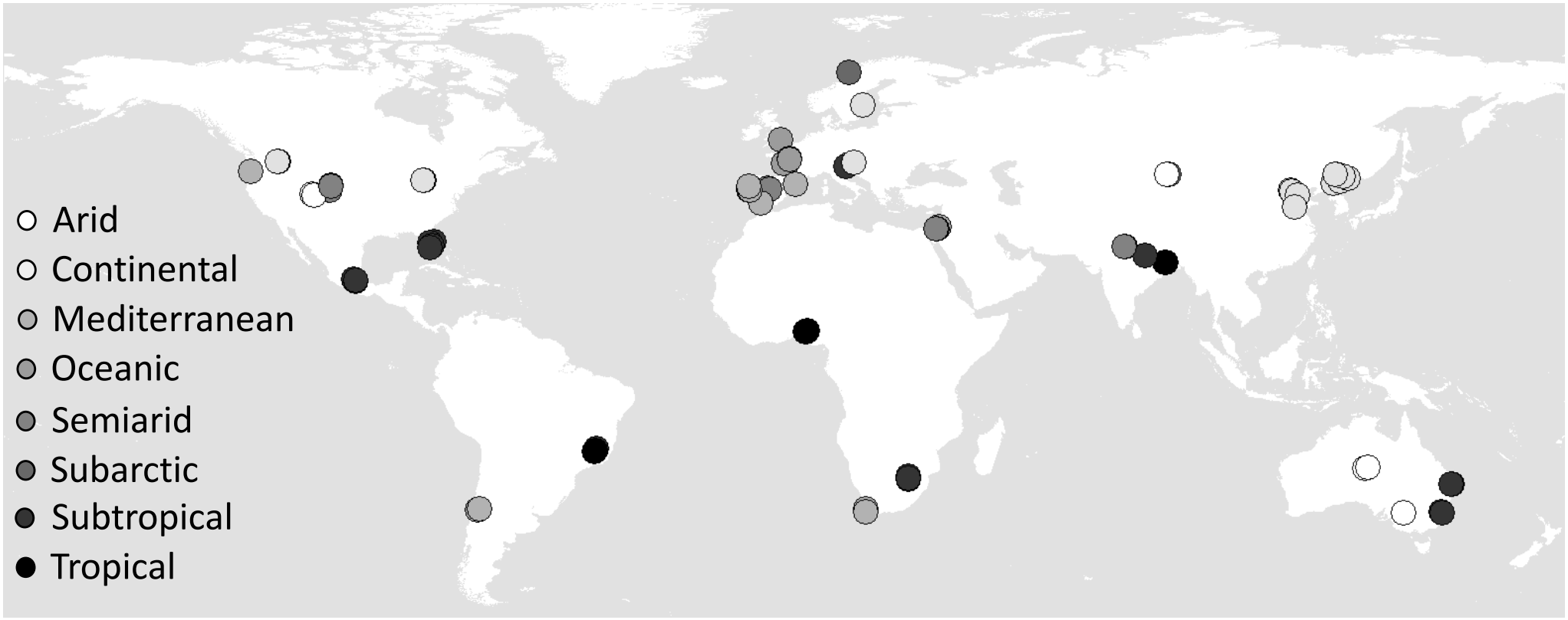
Supplementary Table 7 | Variance inflation factors (VIF) calculated for the saturated SEMs (see Supplementary Figure 6), for urban greenspaces (left row) and natural ecosystems (right row). Values with VIF < 5 indicate low multicollinearity[45].

	Urban	**Natural**
NPP	1.25	2.79
Sand	1.79	1.37
Microbial biomass	1.16	1.12
Precipitation	1.37	1.83
Temperature	1.7	1.79
Forest	1.13	2.09
Irrigation	1.99	NA
Fertilization	1.51	NA
Mowing	1.8	NA


Natural ecosystems
Urban greenspaces
A
Soil respiration (ug CO_2-C g^{-1} h^{-1})
P < 0.001
P < 0.001
Incubation temperature (ºC)
B
q10
P = 0.013
C
Microbial biomass (g C kg $soil^{-1}$)
P = 0.040
Natural
Urban greenspaces
D
Functional genes (%)
Respiratory Complex I
P = 0.005
Aromatic amino acid degradation
P = 0.005
Glycolysis & Gluconeogenesis
P < 0.001
Carbon starvation
P = 0.005
Sucrose utilization
P < 0.001
Sugar utilization
P = 0.021
Respiration HGM
P = 0.002
Photosystem II PRC
P = 0.011


Urban greenspaces
Natural ecosystems
A
Climate
Management
Plant
Microbial biomass
Sand %
Carbon content
.45 (NPP ← Mowing)
-.35 (MAP →)
.39 (← Mowing)
.42 (MAT →)
.50 (← Fertilization)
.50 (← Irrigation)
-.30 (MAT →)
.50
Positive
Negative
Mixed
R2 = 0.55
.62 (NPP ← MAP)
-.38 (NPP ← MAT)
.35 (Forest ← MAP)
.51 (MAT →)
-.45 (MAP →)
-.42 (MAT →)
.32 (NPP →)
-.29
R2 = 0.69
B
P > 0.05
P < 0.05
STE (unitless)
1.0
0.5
0.0
-0.5
-1.0
MAT
MAP
Forest
NPP
Microbial biomass
Sand %
Irrigation
Fertilization
Mowing


Natural ecosystems
Urban greenspaces
A
SOC
POC
MAOC
P = 0.39
P = 0.64
P = 0.18
Soil carbon (g C kg soil-1)
B
SOC
POC
MAOC
P < 0.001
P = 0.001
P < 0.001
P = 0.021
P < 0.001
P = 0.003
MAT (ºC)


Arid
Continental
Mediterranean
Oceanic
Semiarid
Subarctic
Subtropical
Tropical