- 1 **Title:** Urban food forestry transforms fine-scale soil function for rapid and uniform carbon
- 2 sequestration.
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23 Abstract

24 Urbanization displaces agriculture and natural ecosystems, constraining food security and carbon 25 (C) sinks. A proposed solution, Urban Food Forestry (UFF), promises local food from trees that 26 can sequester C faster than other land cover types as long as soil function can sustain increased 27 above and belowground productivity. We compared fine-scale variation in soil physical, 28 chemical and biological properties within and between UFF and traditional lawn for evidence of 29 changes in belowground ecosystem services. Both land covers sequestered C, but UFF did so 30 834% faster, especially in upper soil strata where soil bulk density fell by 50% and microbial 31 activity increased by 1167%. Species richness of both soil fungi and bacteria increased along 32 with nutrient concentrations. Contrary to expectations, that different tree traits would drive 33 increasing fine scale variability in C density, soils beneath the UFF became more uniform, which 34 is consistent with the rapid emergence of system-level regulation. Soil C mass balance may 35 distinguish forests from collections of trees and determine how long UFF helps cities store their carbon and eat it too. 36

37 Keywords: agroforestry, carbon sequestration, permaculture, soil function, urban agriculture

38 Introduction

Both population growth and anthropogenic warming are concentrated in cities. Emerging 39 solutions to these related issues reimagine where and how to grow food. Relocating production 40 41 to underutilized urban areas like vacant lots and lawns can increase yields (McDougall et al. 42 2019) while reducing greenhouse gas emissions (Cleveland et al. 2017). Further incorporating 43 useful trees diversifies agricultural outputs while storing more C in wood and soils (De Stefano 44 and Jacobson 2018). Combining the high provisioning services of urban agriculture with the climate regulating services of agroforestry may be possible in Urban Food Forests (UFF). Clark 45 and Nicholas (2013) defined UFF as "the intentional and strategic use of woody perennial food 46 producing species in urban edible landscapes to improve the sustainability and resilience of 47 48 urban communities." Because this broad definition includes isolated trees, other authors 49 emphasize a more systems-based approach by stipulating that UFF should further mimic the 50 structural and functional complexity of natural forests where diverse perennials grow together 51 (Salbitano et al. 2019). Early assessments of UFF are promising. A mature 1 ha food forest can feed 5-6 people per year (Nytofte and Henriksen 2019) while sequestering 40,000 kg of C in 52 53 living plant tissues (Schafer et al. 2019). Whether or not UFF can approximate the C dynamics of 54 other forest ecosystems while maintaining yields remains unknown.

A major factor that could limit services from UFF is soil function. Soils supply roots with key resources and provide a medium for interactions with microbial symbionts and pathogens. Moreover, soils can store C in more chemically stable forms than living vegetation (Trumbore 2000). However, the physical, chemical and biological properties of both urban and agricultural soils tend to reduce a range of soil functions (Cardoso et al. 2013). Urban soil compaction limits root growth and water availability (Nawaz et al. 2013). Whether or not trees planted in UFF can either tolerate or reverse compaction remains unknown. Furthermore, urban and agricultural soils
can have low microbial diversity and activity, which could limit nutrient availability and C
cycling (Guilland et al. 2018; Díaz-Vallejo et al. 2021). Finally, UFF explicitly incorporates
woody species with contrasting functional traits, which could change fine-scale soil properties
relative to more homogenous land cover types, like lawns.

66 To understand how belowground changes support ecosystem services from UFF, we analyzed fine-scale variation in soil structure and function in an urban land use experiment. The 67 New College Food Forest and Carbon Farm is a small (0.057 ha) demonstration UFF in a rapidly 68 69 urbanizing area of Florida, U.S.A. Shortly after installation in 2017, we measured soil C at 56 70 locations across the UFF and adjacent lawn in different vertical strata. We compared C 71 measurements using a novel before-after impact-control (BACI) design that incorporated 72 uncertainty from calibration curves while controlling for spatial autocorrelation in a heirarchical 73 Bayesian framework. Our research objectives were to (1) document differences in physical, 74 chemical and biological aspects of soil function between UFF and adjacent lawn and (2) to analyze fine-scale variation in soil C sequestration within and between land cover types. We 75 76 expected that conversion to UFF would improve soil function by decompacting soils and 77 increasing nutrient concentrations, microbial activity and richness relative to lawns, which had 78 experienced consistent, low-intensity management for at least 70 years. Furthermore, we 79 expected to observe higher rates of soil C sequestration beneath UFF, with increasing spatial 80 variation driven by contrasting tree traits.

81 Methods

102

82 Site Description

The land use experiment took place on the New College of Florida Campus at 83 [27.380446°N, -82.562416°W] in southwestern Florida approximately 100 m east of Sarasota 84 85 Bay and 600 m west of the Sarasota-Bradenton International Airport. The climate is humid 86 temperate-subtropical with mean annual temperature of 22.8°C and annual precipitation of 1346 87 mm varying between a hot summer wet season and a cool winter dry season. Local soils are Eau 88 Galle - Myakka Fine Sands, with sandy and loamy marine deposits as the main parent materials 89 (Soil Survey Staff, 2023). Prior to urbanization, the predominant land cover types in the area were pine flatwoods, a savannah-like ecosystem maintained by periodic fire with a discontinuous 90 91 canopy of *Pinus elliottii*, a dense shrub layer dominated by *Serenoa repens*, and a rich mixture of 92 grasses and forbs (Sparkman and Bryant, 2016). In 1921, the study area was developed by a real 93 estate mogul who constructed an estate with two major buildings and roadways. By 1948, when 94 the oldest aerial imagery that is available today was captured (USDA 1948), several large pines remained but the groundcover on the property was more uniform than surrounding undeveloped 95 96 parcels, consistent with conversion to lawn (Figure S1). Lawn management in the United States 97 typically promotes uniform cover by desirable species of grasses by some combination of 98 periodic mechanical mowing, irrigation, and nutrient enrichment while controlling undesirable 99 species with herbicide, fungicide or pesticides (Thompson and Kao-Kniffin 2019). 100 The establishment of the UFF began in February 2016 with sheet mulching over the 101 existing lawn. Community volunteers then combined locally sourced topsoil, compost and

103 students planted 50 seedlings representing 33 different useful woody species (Table S1) into

biochar into berms that were approximately 10 cm high and 1 m wide. Starting in May 2016,

104 rows along the berms along with assorted short-lived woody and pseudo-woody plants, vines, 105 and herbaceous plants. Ongoing maintenance consisted of watering, trail-making, mulching and 106 spreading compost generated by students on campus from food scraps. During this period, the 107 adjacnt lawn area was mowed biweekly at a 6 - 12 cm height, retruing clippings to the turf and 108 without irrigation or chemical fertilizer application. An informal survey of plants present in lawn 109 habitats identified 36 species, with grasses (Poaceae) being the most diverse plant family, 110 represented by at least 8 different taxa. The most frequently encountered species in the footprint 111 of the study area were the common turfgrass, *Paspalum notatum* Flüggé (Poaceae), and the 112 introduced subshrub, Sida acuta Burm.f. (Malvaceae) (unpublished data). 113 Sampling design 114 To understand how implementation affected soil function, we employed a novel Before-115 After-Control-Impact (BACI) sampling design. Originally developed to estimate the

116 environmental effects of unique interventions, BACI compares the magnitude of change in 117 repeated measurements before and after implementation to similar measurements at a control 118 location while statistically controlling for autocorrelation from non-random sampling (Conner et 119 al. 2015). We applied different aspects of the BACI design to our different research objectives. 120 For our primary research objective, which was to quantify fine-scale variation in C sequestration, 121 we established impact and control sampling arrays in the UFF and adjacent lawn, respectively. 122 We use fixed landmarks to collect soil samples at 4 m intervals along each berm for 28 sampling 123 locations in a hexagonal array, which we reproduced in the lawn. We sampled soils in both 124 arrays shortly after construction in 2017 and after two years of managing the UFF in 2019. We 125 controlled for spatial autocorrelation using a hierarchical Bayesian model (see Supplementary 126 Methods).

127 In addition to representing spatial variation in the horizontal dimensions, we also 128 analyzed changes across vertical strata. At each sampling location and time interval, we removed 129 herbaceous vegetation and detritus and then used a gauge auger to extract a soil core. In the UFF, 130 we split the upper 30 cm of each core into three vertical strata representing the berm stratum of 131 imported soils (0-10 cm), the upper stratum of original lawn soils (10-20 cm) and the lower 132 stratum of original lawn soils (20-30 cm) (n=84). In the lawn, we only sampled the top 20 cm of 133 soils and split samples into two strata (0-10 cm and 10-20 cm, n=56). Berm soils should show 134 the greatest impact effect, followed by the original upper stratum and finally the original lower 135 stratum compared to the corresponding control strata in the lawn.

For the secondary research objective, comparing other physical, chemical and biological differences we only sampled after implementation in 2019 but again compared measurements between the impact and control areas using a spatial model to account for autocorrelation. In each area, we added four sampling sites that corresponded to an expanded margin of the UFF and an equivalent area of the lawn for 64 sampling locations. At all locations, we measured soil bulk density (SBD) in every stratum (n=160). We also measured soil respiration at every location and stratum except for the lowest (n=96).

For soil nutrients and microbial diversity, we used different sampling approaches. In a random subset of 20 sites from each area, we collected additional soil samples that we physically aggregated and analyzed for soil nutrient concentrations. Finally, we leveraged preliminary results from the soil C analysis to identify six sampling locations—four in the UFF and two in the lawn—from which we remeasured soil nutrients along with microbial diversity. For a summary of the sampling strategy for each dataset and analysis, refer to Table S2.

149 Soil C measurements, calibration and projection

150	During the 2017 campaign, we air-dried all 140 samples to constant weight and analyzed
151	them for organic carbon (OC) content (%) using chromic acid wet oxidation (Walkley and Black
152	1934). During the 2019 campaign, we transferred all 160 samples to sealed polyethylene bags for
153	storage up to two days at 4°C. After measuring soil respiration from a subset of samples, we
154	dried samples to 105°C for a minimum of 8 hours and calculated Soil Bulk Density (SBD) as the
155	ratio of sample dry mass to fresh volume. We then measured soil organic matter via loss on
156	ignition (LOI). Specifically, we combusted up to 15 mL of dried sample for 4 hours at 550°C in
157	a Lindberg Blue M muffle furnace (Fisher Scientific, Waltham MA). We calculated LOI as the
158	ratio of the combusted sample mass to its initial dry mass.
159	Because we measured different but related variables in each sampling campaign (OC in
160	2017; SBD and LOI in 2019), we generated calibration relationships using a stratified dataset. In
161	2019, we collected an additional 15 samples representing the highest, lowest, and approximate
162	mean LOI from each stratum and area, where we measured SBD and LOI using the methodology
163	described above and Walkley-Black OC using the same analytical lab as in 2017 (Figure S2). To
164	identify the most adequate calibration relationships, we used the function "Im" in R-package
165	"stats" to fit pairwise regressions between original and log-transformed values of OC and LOI as
166	well as between SBD and OC while including every combination of area, stratum and their
167	interactions as covariates. We selecting the functional form of the relationship with the lowest
168	AIC (Table S3) for further analysis.
169	Uncertainty from calibration curves and spatial autocorrelation are common in soil C

169 Uncertainty from calibration curves and spatial autocorrelation are common in soil C
 170 analyses and BACI designs (Conner et al. 2015). To address each of these complications in a
 171 coherent statistical framework, we used a Hierarchical Bayesian (HB) approach. HB models are
 172 modular and integrate uncertainty from multiple sources into posterior distributions of key

quantities (Ogle and Barber 2012). We describe the model in detail in Supplementary Methods S1, which also includes all code and data necessary to reproduce the analysis. We note that this approach tends to broaden credible intervals without biasing the means. Therefore, estimates of the statistical significance of differences between land cover types and years are conservative relative to conventional analyses that would use calibration constants, censor observations or ignore spatial autocorrelation.

To estimate the attainable SOC stocks in the UFF, we used fine-scale variation in C
sequestration rates to parameterize a simple mass balance equation for SOC (Morais et al. 2019).
The model assumes that the change in SOC depends on the difference between the SOC input
(*K*) and the mineralization rate (*α*):

183
$$\frac{d\text{SOC}}{dt} = K - \alpha * \text{SOC}$$
(Eq. 1)

We calculated these quantities in the UFF by applying the moment-based estimators for the intercept and slope parameters to the spatially detrended estimates of SOC in 2017 and 2019 across all (o=1...28) sampling locations (See Supplementary Methods, Eq. S1-S8):

187
$$\alpha = \frac{Cov(SOC17_o, SOC19_o - SOC17_o)}{Var(SOC17_o)}$$
(Eq. 2)

188
$$K = \overline{\text{SOC19}_o - \text{SOC17}_o} - \alpha * \overline{\text{SOC17}_o}$$
(Eq. 3)

189 In addition to using these values as estimates for SOC input and mineralization in the 190 UFF, we projected SOC accumulation through time to its equilibrium value (K/α) using the 191 solution to the differential equation:

192
$$\operatorname{SOC}(t) = \frac{\kappa}{\alpha} (1 - e^{-\alpha t}) + e^{-\alpha t} * \overline{\operatorname{SOC17}_o}$$
(Eq. 4)

where *t* is time in years. To relate the quantities from the Mass Balance Equation (Eq. 1) to
system level regulation of the UFF, we estimated the minimum quantity of mulch necessary to
meet the estimated C supply rate (*K*). Specifically, we used the standard C content of wood of

196	50% (w/w) (USDA 2011) and bulk density of dry wood chips of 200 kg m ⁻³ (Forest Products
197	Laboratory 2010) to convert supply rate estimates into mass and depth of mulch.
198	To propagate uncertainty from all aspects of the model, including calibration curve
199	estimation (Supplementary Methods Eq. S1, S3, S7, S9), and spatial detrending (e.g.
200	Supplementary Methods Eq. S5, S6), we calculated the SOC input and mineralization parameters
201	(Eq. 1) during Markov Chain Monte Carlo (MCMC) sampling and drew 1200 samples from the
202	MCMC chains for the projection (Eq. 4). For details of model fitting, see Supplementary
203	Methods.
204	Soil respiration
205	We measured soil respiration in 2019 as the mass specific respiration rate (MSRR) under
206	standard laboratory conditions. Within 48 hours of collection, we allowed samples to equilibrate
207	to room temperature inside 500 ml glass tissue culture flasks, which served as static respiration
208	chambers. We measured initial headspace CO2 concentrations by injecting 10 mL of soda-lime
209	scrubbed air and pumping the syringe three times to mix. We measured the resulting 10 mL
210	headspace sample using a PP Systems EGM-4 Infrared Gas Analyzer (Amesbury, MA) and then
211	repeated the procedure after an incubation of approximately 20 minutes. We calculated the
212	headspace C masses by applying the ideal gas law:
213	mass $C = 12.05 * PPM \frac{pressureATM * (jarVolume - coreLength * \pi * augerRadius2)}{(273.15 + labTemp) * R}$ (Eq. 5)
214	Where $R = 82.05 \text{ cm}^3 \text{ atm g}^{-1}\text{mol}^{-1}\text{K}^{-1}$. We then calculated MSRR as the increase in headspace C
215	min ⁻¹ g ⁻¹ of dry soil.

To assess the biological relevance of differences in SBD and MSRR between upper strata of the lawn and UFF in 2019, we quantified the frequencies with which samples exceeded US Department of Agriculture thresholds for each soil health metric (Soil Science Division Staff, 219 2017). Soil compaction limits plant growth in sandy soils with bulk densities greater than 1.6 g 220 cm⁻³ and in sandy loams with bulk densities greater than 1.4 cm⁻³. Those with respiration rates 221 below 0.032 μ g C min⁻¹ g⁻¹ are considered to have very low soil activity relative to a typical 222 value of 0.056 μ g C min⁻¹ g⁻¹ in agricultural soils.

223 Soil nutrients and microbes

To represent differences in soil nutrients, we air dried samples and mailed them to the
University of Georgia Soil, Plant, and Water Laboratory for quantification of soil pH,
phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), iron
(Fe), copper (Cu), molybdenum (Mo) and cation exchange capacity (CEC) by Mehlich I sum
with percent base saturation. We summarized variation in scaled nutrient concentrations using
Principal Component Analysis (PCA) using the function "prcomp" in R v 4.0.2 packages "stats."
(R Core Team 2020).

231 To describe soil microbial diversity, we collected six total samples using the stratified 232 approach described above. At each location, we collected five cores to 20 cm depth with a 233 sterilized gauge auger. After removing the top 5 cm, the soil was homogenized and frozen at -80 234 °C. DNA was extracted using a Qiagen DNeasy PowerSoil kit (Qiagen USA, Germantown, MD) 235 following the standard protocol except for increasing the vortexing time to 10 min to improve 236 inhibitor removal. DNA was extracted into 100µL nanopure water. We sent 25µL of total DNA 237 to Molecular Research LP Laboratory (MR DNA, Shallowater, TX) for diversity assays based on 238 DNA barcoding. For bacteria identification, 16S rRNA V4 region was amplified (515/806 239 primers, Parada et al. 2016) with a HotStar Taq Plus Master Mix Kit following manufacturer 240 instructions (Qiagen, USA). For fungal identification, the ITS region was amplified (ITS1 and 241 ITS4 primers, White et al., 1990). Amplicons were barcoded using bTEFAP (Dowd et al., 2008),

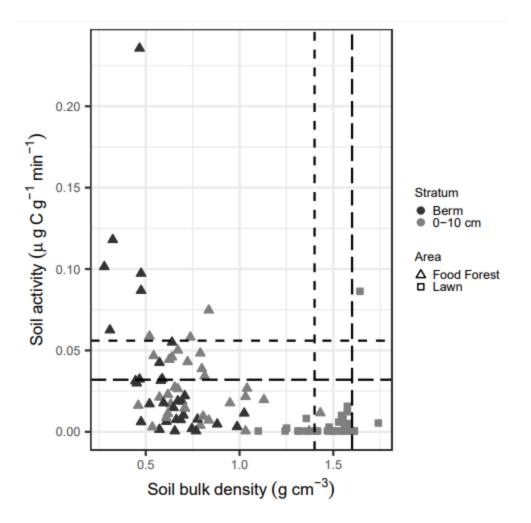
adapted for the sequencing technology used here. Sequencing was performed with ThermoFisher
Scientific Ion S5, following manufacturers' instructions. Sequences were processed by removing
barcodes, primers, short sequences <150 bp, sequences with ambiguous base calls and sequences
with homopolymer runs exceeding 6bp. Then sequences were denoised, OTUs were generated
by clustering at 97% similarity (3% divergence), and singleton sequences and chimeras were
removed. The resulting OTUs were taxonomically classified using BLASTn against a curated
database. The number of sequences per taxonomic level were compiled as counts.

249 We calculated observed richness and the Chao 1 alpha diversity metric from fungal and 250 bacterial samples using the function "estimate richness" in the R package phyloseq (McMurdie 251 and Holmes 2013) and considered areas different if their estimated standard errors did not 252 overlap. To represent overall differences in community composition, we used Principal 253 Coordinate Analysis (PCoA) of Bray-Curtis distances implemented using "ordinate" in phyloseq. 254 To identify distinctive fungal taxa in each area, we used indicator species analysis as 255 implemented by the "multipatt" function in R package "indicspecies" (De Cáceres and Legendre 256 2020), correcting for differences in sample numbers in each area. We selected any taxa with 257 marginally significant point biserial correlation coefficients (p < 0.1) for further functional 258 characterization by cross referencing fungal OTUs to the FunGuild Database (Nguyen et al. 259 2016). We analyzed evidence for functional differentiation by comparing the trophic guild 260 classification of distinctive fungal OTUs in the UFF versus all others using Fisher's Exact Test 261 in R package "stats".

262 **Results**

263 Converting lawn to UFF precipitated a dramatic change in soil structure and function
264 after only two years (Fig. 1). The majority of samples from the top 10 cm of lawn soil (24/32)

265	exceeded the soil compaction standard for sandy loams (>1.4 g cm ⁻³) and five samples exceeded
266	the soil compaction standard for sands (>1.6 cm ⁻³). In contrast, just $1/32$ samples from the same
267	stratum beneath the UFF exceeded 1.4 cm ⁻³ , despite the added weight of plants and soils on
268	berms. Taking the spatial structure of the sampling locations into account, the average SBD of
269	the upper stratum of the lawn was 1.478 g cm ⁻³ ([1.435, 1.521] 95%CI), which was nearly double
270	that of the corresponding stratum beneath the UFF, 0.751 g cm ⁻³ ([0.689, 0.820] 95%CI). The
271	differences in the original lower strata were less extreme but still significantly lower under the
272	UFF (Lawn 10-20 cm mean SBD=1.336 g cm ⁻³ [1.297, 1.375] 95% CI, UFF original 10-20 cm
273	mean SBD= 0.969 g cm^{-3} [0.885, 1.058] 95%CI). The lowest average SBD occurred in the
274	imported soils in the berm stratum of the UFF (mean SBD=0.583 g cm ⁻³ [0.534, 0.637] 95%CI).



285

276 Fig. 1 Urban food forest soils were more active and less compacted than lawn soils. Dashed lines 277 correspond to agronomically relevant soil function thresholds (see Materials and Methods) with 278 long dashes corresponding to the least functional range, and short dashes corresponding to 279 intermediate function. Triangles correspond to samples from the Urban Food Forest and squares 280 to samples from the lawn. Dark fill indicates samples from the imported berm stratum in the 281 Urban Food Forest. Light fill corresponds to the original soil strata in either area 282 Rapid soil decompaction in the UFF was associated with an agriculturally relevant 283 increase in microbial activity (Fig. 1). Taking the spatial structure of the 32 sampling locations 284 into account, the average mass specific respiration rate in the upper stratum of lawn soil was

0.0015 µg C g⁻¹ min⁻¹ ([0.0012, 0.0019] 95%CI), approximately 20 times slower than the

286	common threshold for the least healthy category of soil microbial activity of 0.032 μg C $g^{\text{-1}}$ min^{\text{-}}
287	¹ . In the corresponding stratum under the UFF, average respiration rates were an order of
288	magnitude faster, 0.0175 μ g C g ⁻¹ min ⁻¹ ([0.0112, 0.0260] 95%CI) and similar to the respiration
289	rate of berm soil (0.0156 µg C g ⁻¹ min ⁻¹ [0.0100, 0.0230] 95%CI). Among 64 UFF samples, 21
290	exceeded the threshold for the least healthy category of soil activity compared to just one sample
291	of 32 from the lawn. Among all 96 samples, 11/12 with healthy levels of soil activity (>0.056 μg
292	C g ⁻¹ min ⁻¹) occurred in the UFF.
293	Compared to control sites in the lawn, UFF soils also had higher concentrations of soil
294	nutrients (Fig. 2A). Cation exchange capacity (CEC), which measures overall nutrient
295	availability, was only 7.75 meq per 100g in a physically aggregated sample from the lawn. In
296	contrast, CEC in the UFF was 4.63 times higher. Every measurement of K, Ca, Mg, Mn and Fe
297	concentrations from the five UFF samples exceeded those from the three lawn samples.
298	

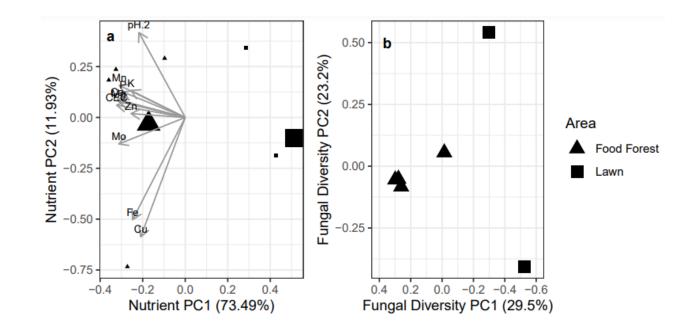




Fig. 2 Urban food forest soils have higher nutrient concentrations (a) and distinctive fungal
communities (b) compared to lawn soils. Large symbols in panel (a) correspond to physically
aggregated samples from each area. Small symbols in panel a correspond to 6 additional
stratified samples. Triangles correspond to samples from the Urban Food Forest and squares to
samples from the lawn.

307 Microbial communities from more functional UFF soil were diverse and distinctive 308 compared to lawn soil (Fig. 2B). Among 417 unique fungal OTUs, the Chao 1 index of the four 309 UFF samples was 423.1 (\pm 4.4 s.e.) compared to 395.3 (\pm 18.5 s.e.) in two samples from the 310 lawn. Overall, 99 OTUs were unique to the UFF compared to just 24 from the lawn. The UFF 311 included 26 marginally significant indicator taxa that matched the FunGuild traits database. The 312 trophic modes for the 26 distinctive UFF soil fungi were significantly different from 224 other 313 fungi (Fisher's Exact Test, p=0.012). Fungal communities exhibited fine scale variability, with 314 different taxa dominating specific samples (Supplemental Table S4 A). For example, 315 Trechispora was the most abundant taxon in one UFF soil sample (16.95%) and was present at 316 low abundance in the rest of the samples (0.04-0.09%). Similarly, *Pisolithus* was the most 317 abundant taxon present in a lawn sample (29.82%) but was present at low abundance in the rest 318 of the samples (0.08-0.13%). 319 Bacterial communities from the UFF were also more diverse and distinctive. Among 320 2323 bacterial OTUs, the Chao 1 index for the UFF samples was 2317.5 (\pm 10.7 s.e.) with 130 321 322

unique taxa compared to an alpha diversity of 2291.3 (±19.4 s.e.) and 39 unique taxa in the lawn. The most abundant bacterial taxa in each sample were variable with some overlap between UFF 323 samples and the lawn samples (Supplemental Table S4,B). Steroidobacter were the most 324 abundant bacteria in UFF soils (1.40-4.91%) compared to the lawn soils (0.17-1.24%). This 325 genus has been previously reported present in farmed soils (Huang et al. 2019). Holophaga, 326 which has been reported from natural forest soils (Hackl et al. 2004), was also abundant in UFF 327 soils (1.18-2.89%) but had low abundance in lawn soil (0.06-0.42%). The most abundant genus 328 in the lawn soil was Actinoallomurus, which was reported present in mangrove soils (Tang et al. 329 2013), however it was found in small amounts (2.4%).

330	As soil functions improved, and distinctively rich microbial communities developed, the
331	UFF sequestered much more organic C belowground than traditionally managed lawn. Shortly
332	after establishment, all of the difference in SOC stocks between areas was attributable to C added
333	with the berm stratum (Fig. 3). After two years of management for useful tree growth, SOC more
334	than doubled among 168 samples from the top 30 cm of the UFF. Taking the spatial structure
335	into account, the average soil C concentration in the UFF increased from 5.88 ([4.48, 7.65]
336	95%CI) to 14.14 ([8.94, 22.28] 95%CI) kg C m ⁻² . In contrast, soil C concentrations in the lawn
337	increased only 25% from 3.92 ([3.00, 5.02] 95%CI) to 4.91 ([3.29, 7.25] 95%CI) kg C m ⁻² (Fig.
338	3), which was not significantly different. The original soil strata beneath the UFF sequestered
339	slightly more C than corresponding areas of the lawn (UFF original soil C sequestration = 0.77
340	([0.22,1.72] 95%CI) kg m ⁻² y ⁻¹ ; Lawn soil C sequestration = 0.49 ($[0.14,1.11] 95%$ CI kg m ⁻² y ⁻¹),
341	while the vast majority of C was sequestered in the berm stratum (3.35 ([1.38,6.56] 95%CI) kg
342	$m^{-2} y^{-1}$).

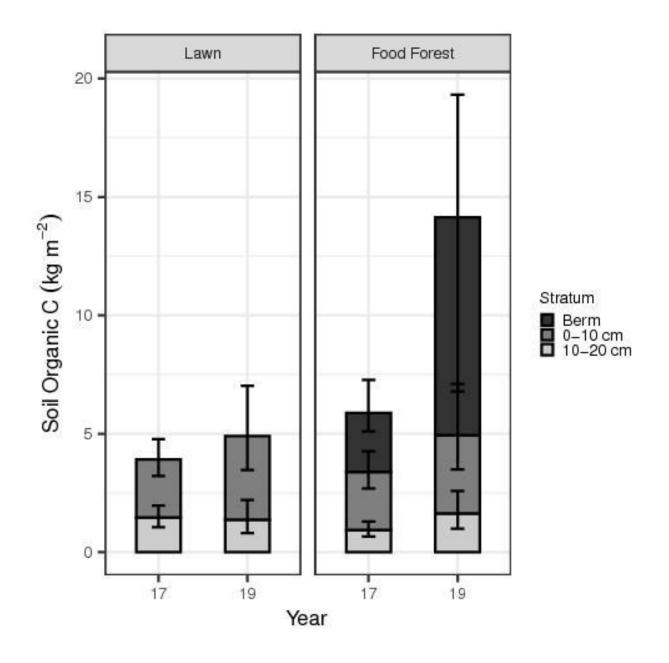


Fig. 3 Soil carbon density approximately doubled in the Urban Food Forest compared to a
negligible increase in the lawn. Error bars represent posterior 95% credible intervals for each
stratum and area. Dark fill corresponds to carbon concentration within the berm stratum of the
Urban Food Forest. Medium fill corresponds to carbon concentration in the original upper
stratum of soil in either area (0-10 cm depth). Light fill corresponds to the original lower soil
stratum in either area (10-20 cm depth).

351 While differences between areas demonstrated how quickly UFF adds SOC, fine scale 352 variation within the UFF indicated approaching limits to its SOC sequestration capacity. 353 Contrary to our expectation that different tree functional traits would exaggerate fine-scale 354 variation in C sequestration, C densities became more homogenous because locations that began 355 with relatively high SOC sequestered less C than locations with relatively low initial SOC (Fig. 356 4A). The negative linear relationship between initial SOC stocks and SOC sequestration is 357 consistent with a simple soil C mass balance model (Eq. 1). After parameterizing the model from 358 spatially detrended C stock and sequestration values, the SOC supply rate was estimated as 6.57 kg C m⁻² yr⁻¹ ([3.73, 10.9] 95%CI) and the SOC mineralization rate was estimated as 0.41 yr⁻¹ 359 360 ([0.04, 0.70] 95% CI). Meeting the estimated SOC supply rate from added mulch alone would have required 13.2 kg m⁻² yr⁻¹ ([7.46, 21.9] 95%CI) corresponding to a layer 6.6 cm deep 361 362 converted directly into soil C each year. Given the estimated SOC supply and mineralization rates, the mass balance equation projected an equilibrium SOC stock of 14.48 kg C m⁻² ([8.94, 363 364 19.02] 95% CI). After just two years, the UFF had attained nearly 90% of its estimated SOC 365 stock capacity and was projected to achieve 95% of its capacity after only 4 years. By contrast, 366 the estimated SOC supply rate in the lawn was significantly lower than in the UFF, 2.56 ([1.63,3.87] 95%CI) kg C m⁻² yr⁻¹ with a slightly higher mineralization rate (0.526 [0.41,0.67]367 95%CI yr⁻¹) and was within 1% of its estimated equilibrium, 4.88 ([3.31,7.20] 95% CI) kg C m⁻² 368 369 (Figure S3), which aligns with our statistically insignificant estimated increase in SOC. 370

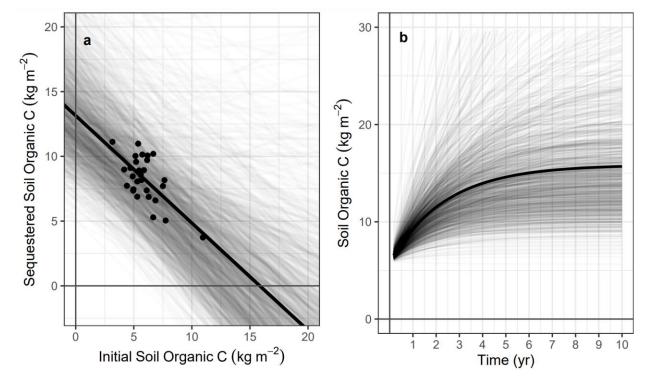




Fig. 4 Fine scale changes in soil carbon concentration over two years implies that the Urban
Food Forest exhibits system-level mass balance (a) and a rapidly approaching limit to
sequestration capacity (b). Transparent line overlay represents 1200 draws from the posterior
distribution for the estimated SOC supply rate (*K*) and mineralization rate (*α*) parameters (Eq. 1).

376 Discussion

377 Established ecological theory (Loreau 2010; Hulvey et al. 2013) and emerging empirical 378 syntheses (Feliciano et al. 2018; Salbitano et al. 2019) agree with policy analysis (McElwee et al. 379 2020) that replacing unproductive urban land uses with UFF can mitigate related problems of 380 urban food security and climate change. Only two years after converting lawn to UFF, our land 381 use experiment demonstrated agriculturally relevant improvements in multiple dimensions of soil 382 function with especially rapid soil C sequestration. However, fine-scale changes in C stocks 383 generated more uniform conditions, despite the high diversity of tree species involved, implying 384 that system level constraints have rapidly emerged in the UFF. Comparing our results to existing 385 analyses of urban agriculture and agroforestry contextualizes the promise and limitations of UFF 386 as a sustainable solution to major environmental challenges.

387 UFF improves soil function

388 Soil compaction, whereby stress from mechanical loads reduces soil pore space and 389 associated biological functions, is the most widespread and important physical means for soil 390 degradation in urbanized and agricultural soils (Nawaz et al. 2013; Smith et al. 2016; Thompson 391 and Kao-Kniffin 2019). Natural recovery can take decades of physical cycling and 392 bioperturbation (Nawaz et al. 2013). However, we found rapid decompaction following land use 393 change, as measured by significant differences in SBD between the same soil strata under the 394 UFF and at control sites in the lawn. The magnitude of change was similar to that reported in 395 other recent studies of land use change. Two years after converting vacant lots in Ohio, U.S.A. to vegetable gardens, surface SBD decreased from 1.79 g cm⁻³ to 0.98 g cm⁻³ (Beniston et al. 2016), 396 397 a change approximately 11% greater than what we observed. Rapid soil decompaction in the 398 UFF without direct mechanical perturbation probably reflects a combination of processes,

including exclusion of heavy lawn mowers, increased woody root growth and infiltration oforganic matter produced *in situ* and added with management.

401 Converting lawn to UFF also improved biological and chemical metrics for soil function. 402 Soil activity, as measured by short-term respiration, was significantly higher in both the imported 403 berm soil and original topsoil than it was in the topsoil of the lawn. Similar increases in soil 404 respiration have been documented close to established trees in both experiments and 405 observations of agroforestry systems (Lee and Jose 2003; Hoosbeek et al. 2018). We note that 406 the short duration of our *ex situ* measurements exclude soil C efflux from more recalcitrant 407 compounds and root respiration. Longer incubations and *in situ* measurements would be 408 necessary to estimate ecosystem-level soil C efflux in a way that is comparable to the 409 mineralization rates that we estimated using the mass balance model (Eq. 1).

410 Higher soil activity tends to increase nutrient availability (Van Der Heijden et al. 2008), 411 which is consistent with the higher nutrient concentrations and CEC that we measured in the 412 UFF compared to the lawn. In the control lawn area, leaf clippings were retained, which tends to 413 increase nutrient availability and SOC storage relative to management that removes clippings 414 (Thompson and Kao-Kniffin 2019), but lawn management did not add fertilizer. Some of the 415 improvement in soil activity and nutrient availability in the UFF could directly reflect input from 416 compost, which was instrumental in improving C stocks in experimental reforestation plots in 417 New York City (Ward et al. 2021). However, a forest garden in France without exogenous inputs 418 exhibited higher CEC and nutrient availability than conventional agricultural soils, likely 419 because tree roots improved mineral weathering and plant litter retained nutrients within the 420 system (de Tombeur et al. 2018). Determining whether improved soil chemistry sustainably

421 increases yields will require more detailed analysis of soil nutrient speciation as well as an422 assessment of nutrient losses with harvest compared to input from exogenous sources.

423 Improvements in both soil activity and nutrient availability coincided with the 424 development of a more diverse and functionally distinctive soil microbial community in the UFF 425 compared to the lawn. The differences that we observed may combine the known beneficial 426 effects of both agroforestry and urban agriculture. Converting forests to agriculture generally 427 decreases the diversity of soil bacteria, which recovers following reforestation (Díaz-Vallejo et 428 al. 2021). These changes can have important functional consequences. Compared to farmland, 429 agroforestry systems in China had higher soil C and respiration, which correlated with increased 430 diversity of both bacteria and fungi (Ren et al. 2018). The increases in bacteria were also 431 associated with changes in nutrient composition (Ren et al. 2018). Urbanization also impacts soil 432 microbial community structure and function, such that urban soils tend to have reduced bacterial 433 biomass and diversity (Guilland et al. 2018). However, microbial diversity within urban 434 greenspaces varies greatly depending on cultivation and the presence of woody plants 435 (Thompson and Kao-Kniffin 2019). In Adelaide, Australia, nutrient availability and bacterial 436 richness were highest in urban garden plots and much lower in lawns of sports fields (Baruch et 437 al. 2021). Sports fields also had lower fungal richness, particularly among saprotprohic taxa, 438 compared to urban greenspaces with woody vegetation (Baruch et al. 2020). Taken together with our analysis, these results strongly suggest that cultivating diverse woody plant species in urban 439 440 settings changes microbial communities in ways that promote physical and chemical dimensions of soil function relative to traditionally managed lawn. 441

442 Rapid SOC sequestration exhibits system-level constraints

443 Gains in soil function coincided with rapid SOC sequestration, reinforcing the potential 444 for urban land use to mitigate climate change. While lawns can store more SOC than other land 445 covers, depending on their management and biogeography (Thompson and Kao-Kniffin 2019), we measured relatively low SOC concentrations (4.91 kg C m⁻²) that were nearly identical to 446 those measured in urban lawns in another region of Florida (4.9 kg C m⁻², Nagy et al. (2014)). 447 Moreover, lawn SOC did not significantly increase after two years, implying that 70 years of 448 449 consistent management generated an SOC equilibrium, as has been documented after just 30 450 years in other urban grasslands (Shi et al. 2012). Compared to the relatively low equilibrium 451 SOC concentrations in the lawn, establishing the UFF precipitated a dramatic increase in SOC. 452 The magnitude was high but within the range observed for certain agroforestry systems. An early modelling analysis estimated potential soil C sequestration of up to 23 kg m⁻² by converting 453 454 traditional agriculture to agroforestry (Albrecht and Kandji 2003), which is consistent with empirical measurements from Canada which found over 20.1 kg C m⁻² in soils from forested 455 sites compared to just 15 kg C m⁻² in herblands (Baah-Acheamfour et al. 2015). The comparable 456 457 land cover types in our study showed a similar difference in surface SOC stocks. Our 458 measurements were also similar to values reported from coffee polyculture in Costa Rica (16.8 kg C m⁻², Alpizar et al., 1986), which is very similar to the maximum attainable SOC that we 459 projected of approximately 16 kg C m⁻². Our measurements differ from others primarily due to 460 the rapid initial rate of sequestration in berm soils (3.35 kg C m^{-2} yr⁻¹), which was nearly five 461 462 times faster than observed in most agroforestry systems (Feliciano et al. 2018). However, the rate of C sequestration that we observed in the original upper stratum of soil (0.77 kg m⁻² v⁻¹) is much 463 464 closer to values reported from other agroforestry systems.

465 Several factors could contribute to the high values for SOC stocks and sequestration that 466 we observed in our study. First, our measurements occurred along berms where soil amendments 467 and tree plantings were concentrated. Because SOC concentrations tend to increase closer to 468 trees (Cardinael et al. 2020; Clivot et al. 2020), our values represent a maximum SOC stock for a 469 subset of the UFF soils. Additional sampling from intervening alleys would more accurately 470 estimate the SOC stocks of the entire project area and would probably yield lower average SOC 471 concentrations that are closer to values that have been reported for other agroforestry 472 experiments. Geographic and biological factors could also contribute to the high maximum 473 values for SOC concentrations that we observed. Agroforestry projects in warmer locations tend 474 to exhibit higher soil C concentrations (Feliciano et al. 2018). Also, ecological theory and 475 experiments indicate that increasing woody plant diversity and associated functional trait 476 variation tends to increase SOC sequestration (Hulvey et al. 2013). Our experiment included 33 species of trees and many more perennial pseudowoody herbs, vines and grasses with contrasting 477 478 biogeographic origins. The extreme functional diversity of species involved could have increased 479 the maximum SOC concentration. Additional experiments that vary species composition across 480 climatic gradients are necessary to estimate how and why the SOC capacity of UFF may vary 481 from other land use types.

While maximum SOC stocks and sequestration rates that we estimated were high, finescale variability implied a rapidly approaching limit to SOC sequestration. Contrary to our prediction that contrasting tree functional traits would exaggerate initial differences in SOC concentrations, with some species storing more C belowground than others, fine-scale variability in SOC concentrations actually decreased through time. The negative correlation between initial SOC concentration and subsequent SOC sequestration was consistent with a mass balance model (Eq. 1). The model, which has been used to estimate SOC capacity for global land cover types
(Morais et al. 2019), is considerably simpler than widely used mechanistic models for soil C
cycling (e.g. CENTURY, Parton et al. 1988; RothC, Coleman et al. 1997), and assumes uniform
conditions with constant SOC supply and proportionally constant mineralization. Our finding
that these assumptions approximately hold in a two-year old land use experiment has two major
implications.

494 The first implication is that the system has fixed C sequestration capacity under current 495 management and biological conditions. Indeed, 90% of attainable SOC had been reached just 496 two years after establishment. For this reason, UFF soils may quickly meet discrete C 497 sequestration goals, for instance as carbon credits, but not for mitigating ongoing emissions. It is 498 possible that succession above and belowground could alter the parameters of the mass balance 499 model to change the equilibrium SOC concentration or break the assumptions of the model 500 altogether such that ongoing SOC sequestration is more likely. A recent metanalysis indicated 501 that agroforestry soil C sequestration rates may be multiphasic, with an initial decelerating trend 502 yielding to a transitory increase before a long term steady state (Feliciano et al. 2018). For a 503 holistic picture of how UFF can factor into mitigation schemes, further measurements and 504 modelling will be necessary to estimate the capacity and rate of C sequestration aboveground, 505 which are reported to be considerable (Schafer et al. 2019), as well as the extent to which local 506 food production offsets GHG emissions as has been demonstrated for other forms of urban agriculture (Cleveland et al. 2017). 507

The second major implication is that converting lawn to UFF precipitated the emergence
of system-level regulation in just two years. Exogenous and endogenous factors could contribute.
Added compost and mulch supply SOC and could homogenize microbial communities in a way

511 that promotes constant proportional mineralization. The mass balance model constrains the 512 quantity of exogenous inputs necessary to explain the increase in UFF SOC concentrations. 513 Assuming that mulch was the main exogenous source of SOC during establishment, several tons 514 added per year would be necessary to account for all of the additional C, which is inconsistent 515 with the management history of the site. However, differences in management intensity and 516 duration certainly contributed to the differences in stocks and sequestration between the UFF and 517 the lawn. The lawn received neither irrigation nor fertilizer, which tend to increase SOC concentrations and sequestration (Thompson and Kao-Kniffin 2019). Elsewhere in Florida, 518 519 where lawns likely received more inputs and urban forests, less, differences in soil SOC 520 concentrations between these two land covers were not significantly different (Nagy et al. 2014). 521 Given that short term changes in management can modify long-term equilibrium conditions in 522 both lawns and forests (Peach et al. 2019), strategic use of irrigation and fertilizers may increase 523 soil function and carbon sequestration across a range of urban land covers 524 While management may contribute to system-level regulation in both lawn and UFF, the 525 speed with which it emerged in the UFF despite the extreme functional diversity of plants raises 526 the possibility that endogenous mechanisms played some role. The community of woody plants 527 and associated organisms, including people, may reinforce negative feedback regulation over 528 belowground C cycling at the system-level. For example, shared mycorrhizal networks can 529 redistribute photosynthetic resources to reduce variation in resource stress among trees in forests 530 (Klein et al. 2016). Analogous exchanges involving SOC supply and mineralization may operate

within the UFF. In other words, from the perspective of soil C cycling, the collection of plants

under current management began to behave like a forest. As such, soil C mass balance could

serve as an objective criterion for delineating forest ecosystems independent of arbitrary

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- 534 definitions that depend on tree stem density and size distributions. Identifying which
- 535 mechanisms generate this pattern, as well as their characteristic temporal and spatial scales,
- 536 could be a productive avenue for basic and applied research on C cycling and sequestration in
- 537 forest ecosystems.

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- 711 Supplementary Information Caption:
- 712 This article includes Supplementary Information
- 713 Table S1 Inventory of woody species plantings
- 714 **Table S2** Sampling strategy
- 715 Table S3 Information theoretic criteria (Akaike Information Criterion, AIC) for alternative
- regression relationships in calibration dataset. Models compare both Organic Carbon (OC) and
- 717 Soil Bulk Density (SBD), against explanatory factors, Area and Stratum, and Loss-on-Ignition
- 718 (LOI). Most adequate models correspond to lowest AIC and are highlighted in bold.
- 719 Table S4 A: Heat map of fungal OTU relative abundance for the most dominant taxa identified
- in four soil samples from UFF and two from Lawn.
- 721 **Table S4 B:** Heat map of bacterial OTU relative abundance for the most dominant taxa
- identified in four soil samples from UFF and two from Lawn.
- **Figure S1:** Aerial imagery of land cover at the study site in 1948 (a) and 2019 (b) with the study
- area footprint approximately represented by opposed trapezoids, with the UFF and lawn north
- and south, respectively, of the contemporary roadway. The scale bars are approximately 150 m
- 726 long and north is vertical as depicted. Historical aerial imagery available at
- 727 <u>http://ufdc.ufl.edu/UF00071781/00001</u> accessed 17 January 2023. Contemporary aerial imagery
- provided by Landsat available at <u>https://landsat.visibleearth.nasa.gov/</u> accessed 17 January 2023.
- **Figure S2:** Calibration relationships between ln(Loss-on-Ignition) and both ln(Organic Carbon)
- (a) and ln(Soil bulk density) (b) among 15 samples representing low, high and intermediate LOI
- samples from each area and stratum.
- **Figure S3**: Fine scale changes in soil carbon concentration over two years in the lawn is
- consistent with mass balance (A) and is close to equilibrium (B). Transparent line overlay

- represents 1200 draws from the posterior distribution for the estimated SOC supply rate (*K*) and
- 735 mineralization rate (α) parameters (Eq. 1).
- 736 **Supplementary Methods:** Detailed description of the heirarchical Bayesian statistical model.
- 737 Supplementary Code: Annotated code for R statistical computing environment necessary to
- reproduce all results and figures.