



HAL
open science

Multi-taxa environmental DNA inventories reveal distinct taxonomic and functional diversity in urban tropical forest fragments

Julian Donald, Jérôme Murienne, Jérôme Chave, Amaia Iribar, Eliane Louisanna, Sophie Manzi, Melanie Roy, Shengli Tao, Jérôme Orivel, Heidy Schimann, et al.

► To cite this version:

Julian Donald, Jérôme Murienne, Jérôme Chave, Amaia Iribar, Eliane Louisanna, et al.. Multi-taxa environmental DNA inventories reveal distinct taxonomic and functional diversity in urban tropical forest fragments. *Global Ecology and Conservation*, 2021, 29, pp.e01724. 10.1016/j.gecco.2021.e01724 . hal-03282383

HAL Id: hal-03282383

<https://hal.science/hal-03282383>

Submitted on 9 Jul 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

1 **Multi-taxa environmental DNA inventories reveal distinct taxonomic and functional**
2 **diversity in urban tropical forest fragments**

3
4 Julian Donald^{a,b*}, Jérôme Murienne^a, Jérôme Chave^a, Amaia Iribar^a, Eliane Louisanna^c, Sophie
5 Manzi^a, Melanie Roy^a, Shengli Tao^a, Jérôme Orivel^c, Heidy Schimann^c, Lucie Zinger^d

6
7 ^a Evolution et Diversité Biologique (EDB UMR 5174), Université Toulouse 3 Paul Sabatier,
8 CNRS, IRD - Toulouse, France

9
10 ^b Centre for Ecology and Conservation, University of Exeter, Penryn TR10 9FE, UK

11
12 ^c INRAE, CNRS, AgroParisTech, CIRAD, Université de Guyane, Université des Antilles, UMR
13 Ecologie des Forêts de Guyane (EcoFoG), Campus agronomique, Kourou, France

14
15 ^d Institut de Biologie de l'ENS (IBENS), Département de Biologie, École Normale Supérieure,
16 CNRS, INSERM, Université PSL, 75005 Paris, France

17
18 *Corresponding Author : julesdonald@gmail.com

19
20 **ORCID:**

21 Julian Donald: 0000-0001-6900-3777

22 Jerome Murienne: 0000-0003-1474-7829

23 Jerome Chave: 0000-0002-7766-1347

24 Amaia Iribar: 0000-0002-4709-648X

25 Eliane Louisanna: 0000-0002-2082-5726

26 Sophie Manzi: 0000-0001-5907-6999

27 Jerome Orivel: 0000-0002-5636-3228

28 Melanie Roy: 0000-0002-4565-2331

29 Heidy Schimann: 0000-0002-9139-920X

30 Shengli Tao: 0000-0002-2145-4736

31 Lucie Zinger: 0000-0002-3400-5825

32
33 **Abstract**

34 Urban expansion and associated habitat transformation drives shifts in biodiversity, with declines
35 in taxonomic and functional diversity. Forests fragments within urban landscapes offer a number
36 of ecosystem services, and help to maintain biodiversity and ecosystem functions. Here, we
37 focus on a tropical forest environment, and on the soil biota. Using eDNA metabarcoding, we
38 compare forest fragments within the city of Cayenne, French Guiana, with a neighbouring
39 continuous undisturbed forest. We wished to determine if urban forest fragments conserve high
40 levels of alpha and beta diversity as well as similar functional composition for plants, soil
41 animals, fungi and bacteria. We found that alpha diversity is similar across habitats for plants
42 and fungi, lower in urban forests for metazoans and higher for bacteria. We also found that urban

43 forests communities differ from undisturbed forests in their taxonomic composition, with urban
44 forests exhibiting greater turnover between fragments potentially caused by ecological drift and
45 limited dispersal. However, their functional composition exhibited limited differences, with an
46 enrichment of palms, arbuscular mycorrhizal fungi and bacteria and a depletion of climber plants
47 and termites. Thus, although urban forest fragments do shelter soil biodiversity that differs from
48 native forests, the losses of soil functions may be relatively limited. This study demonstrates the
49 strong potential of a multi-taxa eDNA approach for rapid inventories across taxonomic
50 kingdoms, in particular for cryptic soil diversity. It also demonstrates the key role of urban forest
51 fragments in conserving biodiversity and ecosystem function, and points to a need for more
52 systematic monitoring of these areas in urban management plans.

53

54 **Keywords:**

55 Tropical, urban forest, land use, soil diversity, community assembly, DNA metabarcoding

56

57 **1. Introduction**

58 Over the course of the next decade, the rate of natural habitat conversion as a result of
59 urbanisation may double, owing to the continued increase in human populations and the global
60 expansion of urban sprawl (DeFries et al. 2010; Seto et al. 2012; McDonald et al. 2020).
61 Dependant on the scale of habitat disturbance, fragments of natural habitat found within the
62 expanding urban matrix can shelter species, either native, thus serving as local refuges, or
63 adapted to novel conditions created by human modification. Whilst ecological studies of
64 urbanization effects have revealed declines in the density and/or diversity of birds, plants and
65 fungi (Aronson et al. 2014; Abrego et al. 2020), a broad range of taxa inhabit urban or suburban
66 areas, and their response to urbanization remains unknown, thus biasing environmental impact
67 assessments but also the potential role of urban habitat fragments for conservation. The biota
68 inhabiting soils provide numerous ecosystem processes (Bardgett & Van Der Putten, 2014), and
69 have previously been shown to persist in temperate urban forests (Ramirez et al. 2014). Whether
70 urban forests can conserve the soil biota in tropical regions is not known, in spite of well
71 documented evidence that biodiversity is under threat in these regions, including that of soils
72 (McDonald et al. 2008; Barnes et al. 2017; Barlow et al. 2018; Franco et al. 2019).

73 The reduced connectivity and isolation of forest fragments from undisturbed, continuous
74 forests is expected to modify the ability of species to disperse, colonise and persist (Simberloff &
75 Abele 1982; Saunders et al. 1991; Tschardt et al. 2012). It is often thought that the high forest
76 fragmentation in urban spaces likely reduces the survival of populations and thus, should change
77 the composition of species found in fragments, as is sometimes observed (Ewers & Didham
78 2006). However, the biotic response to fragmentation varies among species (Fahrig et al., 2019).
79 Urban environments experience modified abiotic conditions relative to undisturbed forests,
80 exposed to increased air pollution, air temperature, and soil structural modification, with higher
81 compaction, soil pollution such as heavy metal content and acidity, and reduced organic matter
82 content (Jim 1998; McKinney, 2002; Arnfield, 2003). Such modifications impact on soil
83 dwelling species, from the short-lived microscopic fungi and bacteria through to long-lived trees
84 (Newbound et al. 2010; Sinsabaugh 2010; Rodrigues et al. 2013; Hendriksen et al. 2016; de
85 Carvalho et al. 2016; Vieira et al. 2018; Abrego et al. 2020). The heterogeneity of abiotic
86 conditions within forest fragments is also known to increase as a result of edge effects, resulting
87 in smaller fragments becoming uninhabitable for inner-forest adapted species (Laurance et al.
88 2002; Canale et al. 2012; Aerts et al. 2016). All these different aspects may lead to reduced
89 diversity and biotic homogenisation through the proliferation of species that are either urban-
90 specific/tolerant or native pioneers in urban forests (McKinney et al. 2002; Lôbo et al. 2011).

91 Such effects remain to date unknown for the soil biota in Amazonia. Only a few studies
92 have assessed the drivers of soil communities in Amazonian native forests (e.g. Ritter et al. 2019;
93 Vleminckx et al. 2019, Zinger et al. 2019). Some reports also suggest that agricultural conversion
94 of forests reduces macrofauna biomass and diversity (Decaens et al. 2018), a tendency that seems
95 to hold true for both soil meso- or micro- fauna in the few studies published thus far (Franco et
96 al. 2019, Paula et al. 2014). One study reported increased bacterial diversity in agricultural sites
97 at local scales, although these were more homogenous at larger scales due to the loss of forest
98 taxa with restricted ranges (Rodrigues et al. 2013). However, urban forest fragments do not
99 experience the drastic land-use changes of agricultural conversion, and may respond differently.
100 The position of a forest fragment within an urban landscape could also promote local diversity,
101 by providing a broader range of resources and habitats as was found for pollinators
102 (Sritongchuay et al. 2019).

103 Regardless of whether local diversity is above or below a baseline in urban forest
104 fragments, changes in community composition may impact whole functional groups, and hence
105 potentially lead to altered ecosystem functioning. Increased fragmentation and habitat
106 disturbance promote fast-growing plants such as lianas (Laurance et al. 2001; Martin, Sherman
107 & Fahey 2004; Magrach et al. 2016; Campbell et al. 2018), which reduce the capacity of the
108 forest to store carbon (van der Heijden et al. 2015; de Lima et al. 2020). Such changes lead to
109 reduced organic matter availability in urban forest soils (Shen et al. 2019), which should reduce
110 the abundance of detritivore organisms such as blattodea and collembola (Fountain & Hopkin
111 2004; Jones et al. 2003; Santorufo et al. 2012) and increase organisms able to tolerate nutrient-
112 poor soils such as arbuscular mycorrhizal (AM) fungi (McGee et al. 2019), oligotrophic bacteria
113 and nitrogen fixing bacteria (Rodrigues et al. 2013; Mendes et al. 2015; McGee et al. 2019).
114 Functional changes may thus operate across the whole soil biota through biotic interactions,
115 requiring simultaneous assessment which can be particularly challenging.

116 Environmental DNA (eDNA) metabarcoding on soil samples offers a method to assess
117 the effects of habitat modification in tropical regions and beyond across soil trophic and
118 functional groups (Taberlet et al. 2012; 2018; Geisen et al. 2019; Zinger et al. 2020a). The
119 community assembly processes of meso-fauna and microbial communities in tropical forests can
120 be explored by means of eDNA (Ritter et al. 2019, Zinger et al. 2019), where the gaps in our
121 taxonomic knowledge otherwise remain large. eDNA hence holds great potential for quantifying
122 ecosystem health and perform long-term monitoring even in places where baseline biodiversity is
123 poorly quantified (Cordier et al. 2021).

124 We used DNA metabarcoding on soil samples to ask whether tropical urban forest
125 fragments harbour levels of taxonomic and functional diversity across the whole soil biota that
126 are comparable with those of nearby continuous forest. More specifically, we compared
127 communities of plants, soil meso-fauna, fungi and bacteria in forest fragments within the mid-
128 sized tropical city of Cayenne, French Guiana, referred to as urban forests, with nearby lowland
129 tropical forest sites that have experienced low levels of disturbance in recent history, referred to
130 as control forests. We hypothesised that:

131 H1) plants and meso-fauna show a lower alpha diversity at the forest fragment scale in
132 urban compared to control sites, due to habitat degradation and dispersal limitation, causing local
133 extirpations. As dispersal is expected to be less limited for microbes, urban forests and control

134 forests should exhibit similar levels of diversity, or even higher diversity in the former due to
135 local environmental heterogeneity of the surrounding forest patches and higher sensitivity of
136 microbes to changes in environmental conditions.

137 H2) At the scale of the city (i.e. including all forests patches), we expected lower gamma
138 diversity for all groups due to biotic homogenization.

139 H3) Turnover in community taxonomic composition between control and urban forests is
140 high for all clades, with increased turnover within urban forests compared to within control ones,
141 as a result of urban sites experiencing increased fragmentation and more varied soil chemistry.

142 H4) differences in the functional composition of soil biota should be observed between
143 urban and control forests, with greater abundance of lianas, fewer detritivores due to declines in
144 available organic matter, higher levels of AM fungi and bacteria adapted to reduced soil
145 nutrients.

146

147 **2. Methods**

148 ***2.1 Sample sites***

149 This study was conducted in French Guiana, an overseas territory of France of 84500 km²
150 mostly covered by Amazonian tropical forest. Urbanisation of natural habitats in French Guiana
151 is low, with a population size of 250,000, but is quickly increasing (INSEE 2016). The urban
152 area of Cayenne (4°56'0 N, 52°20'0 W), the regional capital city of French Guiana, accounts for
153 about half of the total population. The city is positioned on a peninsula with the Atlantic to the
154 north, and the Cayenne and Mahury rivers to the east and west respectively. Within the city lie
155 four substantial forest fragments (Rorota, Montravel, Montabo, Mont Bourda) positioned on
156 outcrops with underlying metamorphic granite/diorite, which benefit from a protection status of
157 'zones of natural interest for the ecology of flora & fauna' (ZNIEFF). All these sites are
158 documented as having been cleared of forest cover at some stage over the past 400 years since
159 the first European settlement. Since that time, forest patches have recovered and include some
160 mature trees, indicative of advanced forest recovery. The urban forest fragments vary in their
161 overall surface area, as follows: Montabo (CAY1): 19.4ha; Mont Bourda (CAY2): 29.9ha, Mont
162 Ravel (CAY3): 3.5ha, and Rorota (CAY4) 762.2ha.

163 In order to compare these with undisturbed forest, four control sites were selected near
164 the main urban centre of Cayenne ("FOR1": Balata; "FOR2": Cambior; "FOR3": Coralie &

165 “FOR4”: Guyakaw; Supplementary 1). These sites were selected due to their proximity to
166 Cayenne, in order to reduce climatic variation effects. Geology varies across all study sites. Soil
167 Chemistry for the sites was determined as outlined in Supplementary 2. Analysis determined
168 sample pH, granulometry, total Carbon and Nitrogen and the derived C:N ratio, available
169 Phosphorus, and five major elements (Aluminium, Iron, Magnesium, Potassium, Manganese,
170 Calcium).

171

172 ***2.2 Field sampling and bioinformatic processing of samples***

173 A detailed description of sample collection, molecular and bioinformatics procedures are
174 provided in Supplementary 3. Briefly, sampling of soils was conducted within 1 ha plots at each
175 sample site, whereby 16 sample points were arranged on a 20 x 20 m grid. The arrangement of
176 sites allowed for the subdivision of the grid into 4 subplots each containing four sample points (n
177 =32), in order to account for variation in both soil chemistry and biotic communities at the plot
178 level.

179 Extracellular DNA was extracted from 15 g of soil per sample, following a method
180 shown to retrieve a similar signal to more expensive total DNA extraction protocols at a fraction
181 of the cost (detailed in Taberlet et al. 2012; Zinger et al. 2016; 2019). PCR amplifications were
182 then conducted for four DNA molecular markers, with primers targeting either viridiplantae
183 (subsequently referred to as plants), eukaryotes, fungi or bacteria. Negative extraction and PCR
184 controls were simultaneously amplified, with two replicates per PCR, and a use of a double
185 indexing strategy of tags to enable retrieval of sequences and reassignment to their samples,
186 including non-used combinations as sequencing controls.

187 Sequencing was conducted on different Illumina platforms (San Diego, CA, USA)
188 depending on the marker considered, using paired-end technology. Bioinformatic analyses of
189 generated sequences were performed on the GenoToul bioinformatics platform (Toulouse,
190 France), with the OBITOOLS package (Boyer et al. 2016). After basic sequencing data
191 manipulation and curation, we built OTUs (Operational Taxonomic Units) using the ‘sumacust’
192 clustering algorithm (Mercier et al. 2013) at a set threshold of 97% for eukaryotes, fungi and
193 bacteria, and lowered to 95% for plants to account for sequence length.

194 Taxonomy was assigned to each OTU using either a local database of sequences from
195 French Guiana, or global databases derived from EMBL

196 (<https://www.ebi.ac.uk/ena/browser/home>; release 141) using OBITOOL's ecotag programme
197 (Boyer et al. 2016). For bacteria and eukaryotes, the SILVA taxonomic database was used
198 (version 1.3; Quast et al., 2012).

199 Datasets were subsequently filtered to remove contaminants as well as artefacts such as
200 PCR chimeras and remaining sequencing errors, following Zinger et al. (2019) and using the
201 metabarR R package (Zinger et al 2021), in R version 3.6.1 (R Development Core Team, 2013).
202 Upon filtering completion, the sequencing read counts of remaining PCRs were summed across
203 technical replicates, before this total was normalized in line with all samples. This step was done
204 to reduce potential bias caused by PCR stochasticity and differential sequencing efforts.
205 Standardization consisted in randomly resampling (with replacement) a number of reads that
206 corresponded to the first quartile of the total read number for reads per samples. This returns
207 samples with a read count equal across all samples, whilst maintaining sample specific OTU
208 relative abundances. Finally, we sought to reduce stochastic variation of taxa across soil samples,
209 we aggregated the four replicate samples within each subplot by summing reads (after
210 normalisation). Soil chemistry data were also provided for bulk samples of four replicate samples
211 within each subplot.

212

213 ***2.3 Assigning Functional Groups***

214 Each dataset was processed to assign OTUs a functional group based on their taxonomy, when
215 possible (see Supplementary 4 for a comparison between forest types). Plant OTUs were
216 assigned a life form following Cardoso et al. (2017). Since life form information is only
217 available at the species level, we considered the life form of a genus or a family to be the one
218 that is the most common amongst its associated species. For metazoans, we used broad
219 taxonomic clades as proxies of key functional groups in the soil. We focused on annelids for
220 their role in soil bioturbation, on Blattodea, Nematoda, Acari, Collembola, Millipede as
221 detritivores, on Araneae as predators (see Supplementary 5). Fungal OTUs were assigned a
222 function by using FunGuild (Nguyen et al. 2016). OTUs assigned to multiple guilds were
223 removed. For OTUs without a FunGuild annotation but assigned to a species, we searched for
224 their putative host and ecology in the literature. Some FunGuild functional groups were
225 aggregated into the following group: Saprotroph, Parasite, AM fungi, Plant Pathogen,
226 Ectomycorrhizal fungi. Bacterial OTUs were assigned a functional group mainly from

227 FAPROTAX (Louca et al. 2016), but also by using other resources (i.e. Burns et al. 2013; Madin
228 et al. 2020; Stein & Klotz 2016; Strous et al.1999; De Boer & Kowalchuk 2001; Wardeh et al.
229 2015). We also assigned bacterial OTUs a growth strategy following Ho et al. (2017) in order to
230 see whether control forests, which we expect to be richer in organic matter, are enriched in
231 copiotrophic clades. We finally also assigned bacterial OTUs N-cycling pathways using
232 FAPROTAX and Nelson et al. (2016). Further details on the functional classification and
233 number of assigned OTUs for each clades/kingdom are given in Supplementary 5.

234

235 *2.4 Statistical analyses*

236 All statistical analyses were conducted with R version 3.6.1.

237 *2.4.1 Removal of outliers*

238 A visual inspection of soil physiochemistry and of eDNA derived taxonomic data
239 revealed urban forest Mont Ravel (CAY3) to be a significant outlier, displaying soil properties
240 and communities of organisms distinct from the other urban sites. To avoid confounding the
241 effects extreme soil chemistry variation at one site with more broader effects of urban
242 fragmentation, this site was removed from subsequent analysis (Supplementary 2.2, 2.3, 2.4).

243 *2.4.2 Alpha diversity / abundance patterns*

244 We measured the alpha diversity of each clade/functional group in each subplot with the
245 Shannon index. Calculations were performed using the vegan package (Oksanen et al., 2013).
246 This index was chosen since it has been shown to best quantify diversity in eDNA
247 metabarcoding data compared with other indices (Calderón- Sanou et al. 2020). For each forest
248 type and each of the four clades/kingdoms, we ensured that the diversity coverage was adequate
249 by checking saturation of rarefaction curves using the metabaR package (Supplementary 6).
250 Gamma diversity was estimated with the iNEXT package, where input data was abundance, and
251 the output rarefaction curve plotted the exponential Shannon index (Hill number $q = 1$; Hsieh et
252 al. 2016).

253 To determine whether alpha diversity or relative abundance differed between urban and
254 control forests, we built Linear Mixed Models (LMMs) with forest type (urban vs. control) as a
255 fixed effect, and sample site as a random effect to account for potential site specificities in the
256 analysis that would lead to a higher similarity of subplots from a same site. Prior to model fitting,

257 residuals were tested for normality. Significance of variation was determined by conducting a
258 Wald chi-squared test. This was conducted with the lme4 R package (Bates et al. 2019)

259 For visualization purposes, the influence of forest type on Shannon diversity or functional
260 group relative abundance was also analysed using a log₂-fold change approach, i.e. the log₂
261 transformed ratio of the diversity of each of the 12 urban forest subplots over the average
262 diversity of the control forest subplots.

263 *2.4.3 Beta diversity patterns*

264 In order to test the effect of forest type on the turnover of species, and its importance
265 relative to sample sites, we first computed Bray–Curtis dissimilarities for each of the four clades
266 (plant, metazoa, fungi, and bacteria) using the vegan package. Dissimilarities were computed
267 from Hellinger-transformed community data to downweigh the effects of rarely occurring OTUs
268 (Legendre & Legendre, 2012). Principal Coordinates Analysis (PCoA) was used to visualize the
269 effect of forest type on OTU composition. The effect of forest type, site, their interaction, and of
270 soil chemistry (summarized by the first two principal components of a PCA, Supplementary 2)
271 were tested with a permutational multivariate analysis of variance (PERMANOVA, Anderson,
272 2017), using the adonis function in the vegan package.

273 To further determine whether patterns in beta diversity were rather due to the
274 replacement of some species by others between samples (i.e. turnover; Qian et al., 2005) or a
275 reduction in richness between samples (i.e. nestedness; Baselga et al. 2007), we used the
276 beta.pair function of the betapart R package (Baselga & Orme 2012; Baselga 2013) and focused
277 on the control versus urban samples comparisons (CvU). We also assessed control versus control
278 samples (CvC), and urban versus urban samples (UvU) to test if urban forests exhibit biotic
279 homogenization. Differences were tested for using an ANOVA. Because this analysis is based on
280 presence/absence data, and hence potentially sensitive to remaining false positive OTUs, we
281 made sure that rare OTUs had no effect in modifying the conclusions drawn from this analysis,
282 by repeating it on datasets where OTUs representing less than 0.0001 - 0.01% of the total read
283 count were incrementally removed (Supplementary 7).

284 In each forest type, the effect of dispersal limitation in influencing community
285 composition was tested using partial Mantel tests based on Pearson's product-moment correlation
286 with 999 permutations, where soil chemistry effects were partialled out. The reverse was done
287 for testing the effect of environmental filtering. Euclidean distance of normalized and scaled soil

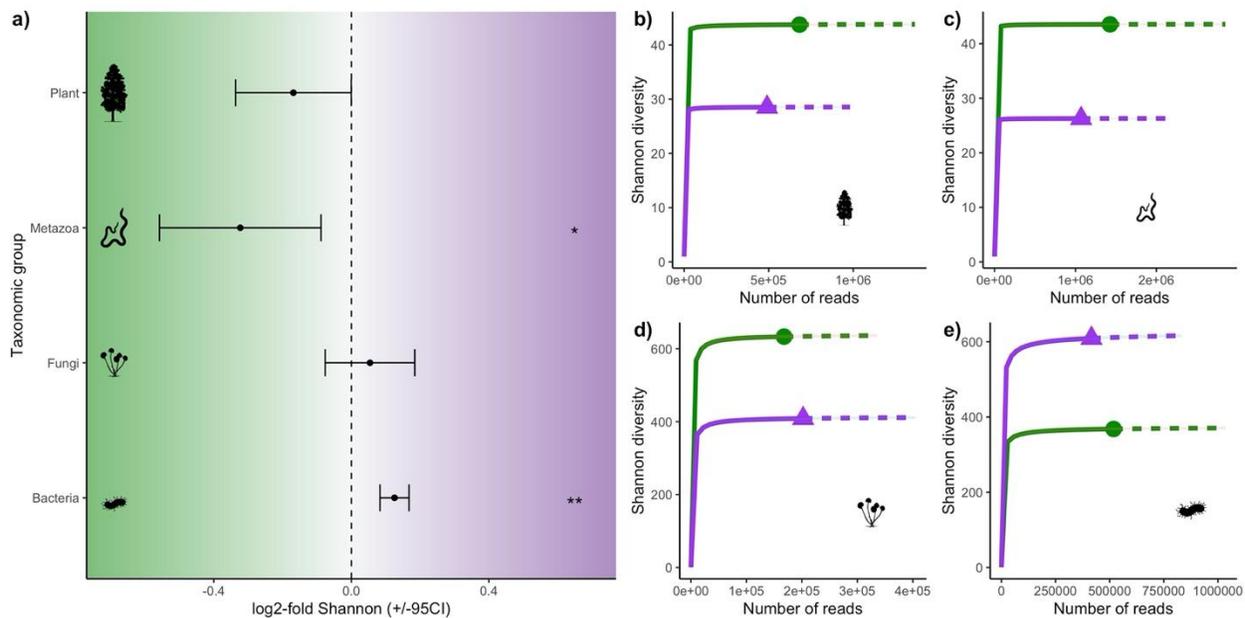
288 chemistry data (see supplementary 2) was calculated to generate an environmental distance
 289 matrix. Geographic distance between sample plots in km was calculated by converting sample
 290 site GPS coordinates into a sample by sample distance matrix using the `rdist.earth` function in the
 291 `fields` package (Nychka et al. 2005).

292

293 **3. Results**

294 **3.1 Alpha and Gamma Diversity**

295 Sequencing of eDNA from metabarcoding soils revealed that alpha diversity did not significantly
 296 differ between control and urban forests for plants and fungi. However, metazoan communities
 297 were *ca.* 40% less diverse in urban compared to control forests, whilst bacteria were 10% more
 298 diverse in urban compared to control forests (Figure 1a, Supplementary 8). At the scale of the
 299 study, plant, metazoan and fungal gamma diversity was higher in control forests, whilst for
 300 bacteria was higher in urban forests (Figure 1b,c,d,e).



301

302 Figure 1: Alpha and gamma exponential Shannon diversity of sample sites a) log-2 fold change
 303 in alpha diversity between forest types. Dots represent the mean values and error bars, their 95%
 304 confidence interval. Values tending towards -1 correspond to a diversity twice lower in urban
 305 forests as compared to control forests. Values tending towards +1 represent diversity twice
 306 higher in urban forests. Confidence interval bars indicate whether differences are significant,
 307 assessed by LMM, where asterisks represent significance levels (* indicates a p-value of <0.05,
 308 and ** p<0.01, detailed in Supplementary 7). Gamma diversity estimates rarefied (solid) and

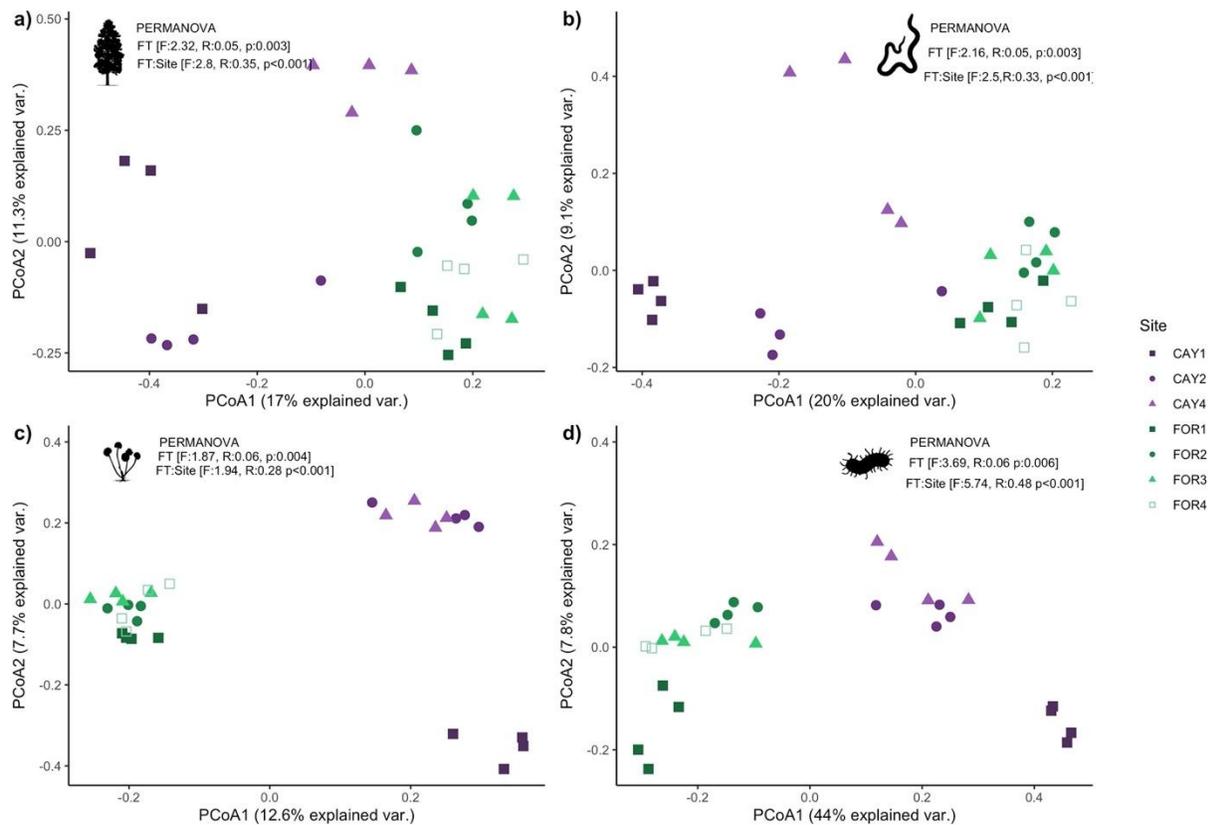
309 extrapolated (dashed) in control (green) and urban (purple) forests for b) plants, c) metazoa, d)
310 fungi and e) bacteria. For all lines, calculated standard errors are $\leq \pm 0.1$.

311

312 3.2 Beta Diversity

313

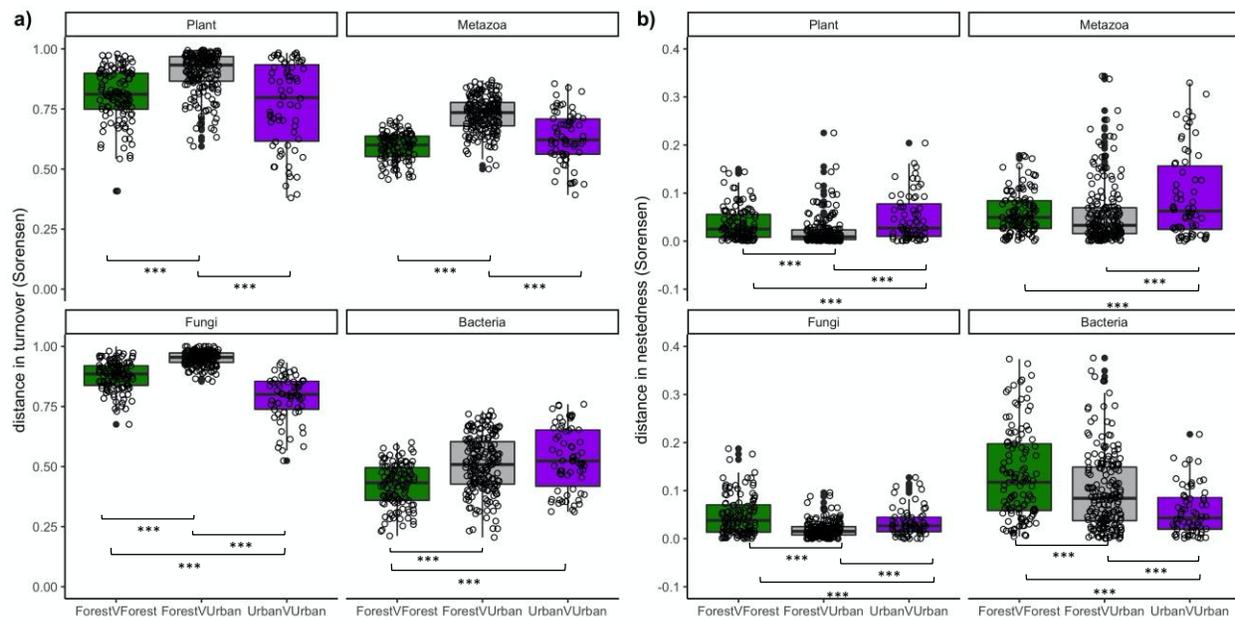
314 For all taxa, the interaction between forest type and site was the strongest effect in explaining
315 turnover in community composition, followed by both forest type, site and soil chemistry
316 (Supplementary 9; Figure 2). Accordingly, the turnover component of beta diversity in inter-
317 forest type comparisons (i.e. CvU) was higher than intra-forest (i.e. CvC, UvU), while
318 nestedness was low across all four clades (Figure 3).



319

320 Figure 2: Dissimilarity of a) plants, b) metazoa, c) fungi and d) bacteria across samples. These
321 are visualized with a Principal Coordinates Analysis (PCoA), with PERMANOVA statistics in
322 the insets indicating significant differences between forest types (FT) and its interaction with
323 site. See also Supplementary 9.

324



325
 326 Figure 3: Pairwise beta diversity characterised by a) turnover and b) nestedness for communities
 327 in Forest sites (green), in Urban sites (purple) and between Forest and Urban (grey). Significance
 328 (p-value) of Tukey HSD following ANOVA given by *** < 0.001.

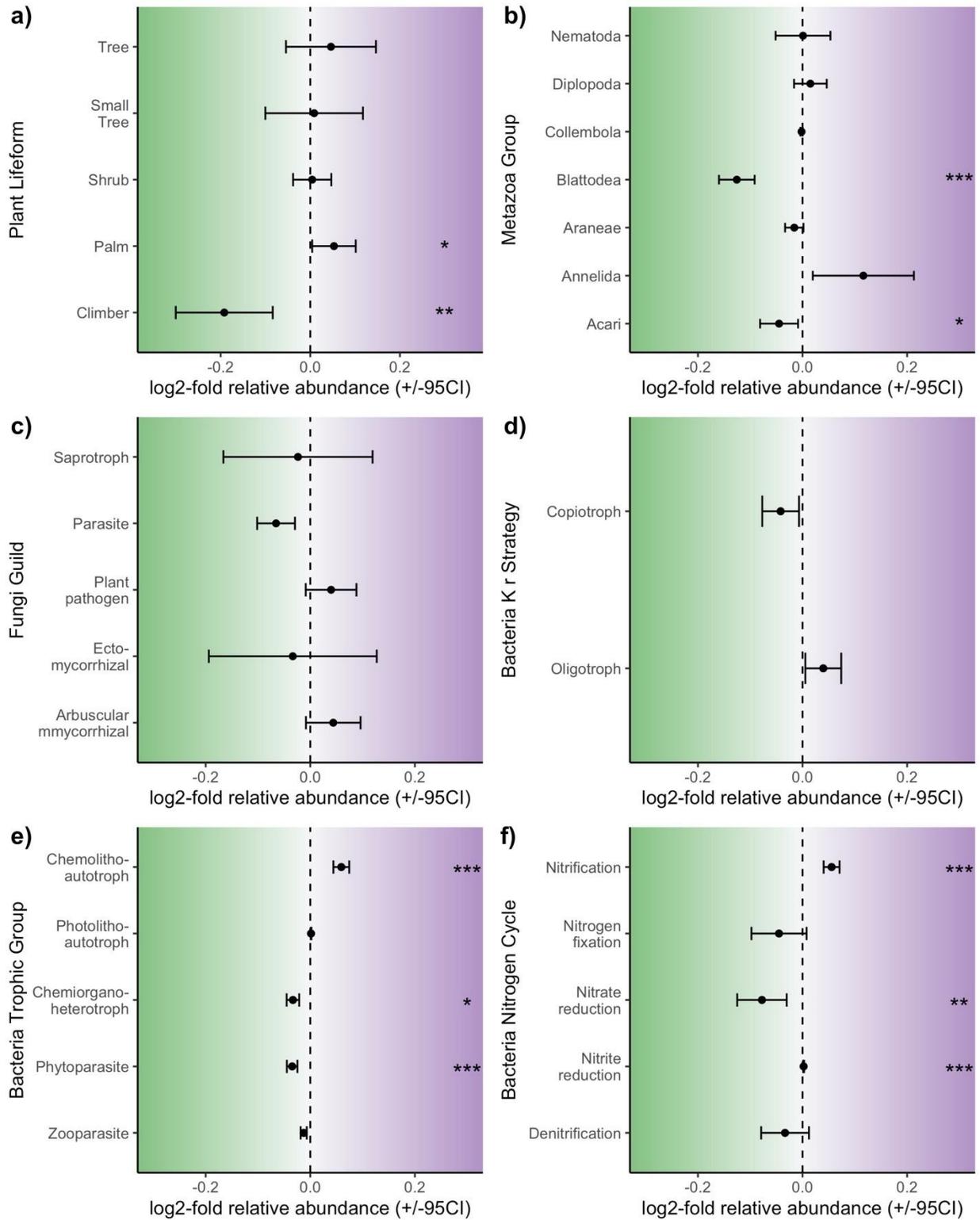
329
 330 Beta diversity within each forest type, as characterised by variation in composition of a sample
 331 relative the forest type centroid, was significantly higher for fungi in urban forests, with a similar
 332 trend, albeit not significant for plants, metazoans, and bacteria (Figure 2, Supplementary 10). In
 333 contrast, the intra-forest type turnover component of beta diversity was equivalent for urban and
 334 control forests for plants and metazoans, higher in control forests for fungi, and higher in urban
 335 forests for bacteria. Nestedness was higher for plants and metazoans in urban forests, and higher
 336 in control forests for fungi and bacteria.

337
 338 Further analysis revealed that bacterial and metazoan community dissimilarity patterns correlated
 339 more with environmental than geographical distances, while the opposite was found for plants
 340 and fungi. (Supplementary 11).

341
 342 **3.3 Functional group responses to habitat**

343 The relative abundance of fungal functional groups did not differ between urban and control
 344 forests (Figure 4). However, plants, metazoans and bacteria did demonstrate shifts in certain
 345 groups (Figure 4; Supplementary 12). For plants, palms were 10% more common in urban

346 forests, whilst climbers were 20% more common in control forests. For metazoans, both
347 blattodea and acarians were more abundant in control forests. We found only a small tendency
348 of higher abundance of copiotrophs in control forests, and of oligotrophic bacteria in urban
349 forests. Chemolithoautotrophs were significantly more abundant in urban forests while in control
350 forests, chemoorganoheterotrophs and phytoparasites were more abundant. Within nitrogen
351 cycle associated bacteria, those involved in nitrification and nitrite reduction were more
352 abundant in urban forests, whilst nitrate reducing bacteria were more abundant in control forests.



353

354 Figure 4: Log₂-fold change in the relative abundance of functional groups for a) plants, b)

355 metazoa, c) fungi, and d-f) bacteria in urban forests relative to undisturbed forests. Legend as in

356 Figure 1, see Supplementary 12 for statistical significance.

357

358 **4. Discussion**

359 ***4.1 Alpha and gamma diversity***

360 Our eDNA-based analysis of whole soil diversity reveals several differences between
361 urban and undisturbed, control forests. First, urban forest fragments harboured lower alpha
362 diversity of metazoans. Reports on soil arthropod responses to urbanization in the tropics are rare
363 and contradicting (see review by Jones & Leather 2013), but the reduction of metazoan diversity
364 observed here could be explained by reductions in resources (Santorufu et al. 2012). Indeed, the
365 urban forests studied here were characterised by soils of lower clay and organic matter content,
366 as observed elsewhere (Lorenz & Lal 2009; Cusack 2013). Plants only tended to exhibit a loss of
367 diversity in urban fragments, as observed elsewhere with traditional survey methods (Aronson et
368 al. 2014). We explain the lack of significance to be due to (i) the fact that soil eDNA does not
369 sample all plants (especially epiphytes, Zinger et al. 2020), and (ii) that the sampling extent for
370 plants was too small (plots of 1ha, with only 3 or 4 sites per forest type). By contrast, we found
371 higher bacterial diversity in urban forests, in agreement with other reports in fragmented or
372 disturbed tropical environments (Rodrigues et al. 2013; Mendes et al. 2015; Navarette et al.
373 2015). Bacteria are likely to respond to higher soil chemical heterogeneity and labile nutrient
374 availability (Vos et al. 2013), whilst macro organisms may instead respond more to declines in
375 soil organic matter as found for termites (e.g. Dahlsjö et al. 2020). Scaling up to the whole study
376 area, we observed declines of diversity for metazoa, fungi and to a lesser extent plants in urban
377 forests. This loss could result from increasing forest fragmentation, dispersal limitation and thus
378 higher local community drift and species extirpation risks (Orrock & Watling 2010). Such a
379 hypothesis is supported by the fact that by contrast, diversity was also higher for bacteria, which
380 have greater dispersal availability and are more sensitivity to small environmental changes. This
381 demonstrates the power of eDNA to reveal both local and more regional scale patterns of habitat
382 dynamics and its relevance for helping establishing conservation priorities.

383 ***4.2 Beta diversity***

384 In addition to differing in alpha and gamma diversity, urban and control forests contained
385 communities distinct from one another across all four taxonomic clades. The response of
386 communities to forest type differed across clades, with bacteria in particular demonstrating the
387 strongest levels of dissimilarity across forest types, likely matching the observed differences in

388 alpha diversity, and their sensitivity to soil chemistry. Urban forest fragments are likely to
389 experience long lasting legacy effects of their previous occupation and management, with greater
390 variation of input and historic disturbance with soil conditions determining community variation
391 across sites, as reported for plant and soil biota community composition in Amazonia and
392 elsewhere (Heckenberger et al. 2007; de Oliveira et al. 2020; Mennicken et al. 2020; Borges et
393 al. 2020).

394 Our study also demonstrates that urban forests communities are not a subset of
395 undisturbed forest, but rather are composed of specific communities. Such a high level of
396 turnover in diversity between control and urban forests demonstrates vulnerability of local native
397 species to extinction, and their replacement by new arrivals of urban-adapted species (McKinney
398 2002), with potential pervasive effects on remaining biodiversity as invasive species (Cadotte et
399 al. 2016). Within forest types, the pairwise comparison of sites demonstrated that turnover
400 contributed a much larger proportion to community dissimilarity than nestedness. The range in
401 pairwise turnover between urban sites was greater for all four taxonomic clades, with this
402 dissimilarity significantly correlating with differences in soil chemistry and geographic distance
403 between sites. This was particularly the case for urban sites, in spite of the smaller distances
404 separating them, again pointing to the effects of fragmentation potentially driving dispersal
405 limitation between sites and promoting community drift and species extirpation risks.

406 Furthermore, we did not observe the expected patterns of homogenization in urban forest
407 fragments relative to controls as found in previous studies of plants and birds (Aronson et al.
408 2014). Instead, the soil biota responded differently, with fungi in fact demonstrating significantly
409 greater heterogeneity in urban forest soils. Fungal diversity and abundance have been shown to
410 decline in an eDNA study of both soil and air along temperate urbanization gradients (Abrego et
411 al. 2020), suggesting potential dispersal limitation in more heavily fragmented urban habitats
412 which could contribute to the increased heterogeneity detected here. Bacteria were similarly
413 homogeneous between forest types in spite of local increases in alpha diversity, contrary to what
414 was observed in Amazonian forest soils converted to agriculture (Rodrigues et al. 2013). Instead,
415 these forest fragments may exhibit more variation in soil physicochemical properties between
416 sites relative to agricultural studies due to more heterogeneous inputs within a city (Byrne 2007).
417 This demonstrates the distinct response of soil biota in urban forest fragments to those in
418 potentially more disturbed agricultural sites, highlighting the need to consider their conservation

419 status differently. In particular, it demonstrates that urban forest fragments under continuous tree
420 cover during extended periods, such as those in Cayenne, contain a soil biota which is unique in
421 composition, with unique forces shaping it.

422 ***4.3 Functional Groups***

423 Replacement in species between urban and control forests may result in a loss of
424 ecosystem function, except if urban-related species are functionally similar. Here, we found that
425 whilst plant communities differ strikingly in diversity and composition between control and
426 urban fragments, their life forms are largely consistent in their relative abundance for both forest
427 types. However, palms were found to be greater in their relative abundance in urban sites, which
428 could correspond to either their cultivation as crops in the past, or their preference for nutrient
429 poor, sand rich soils (de Castilho et al. 2006). By contrast, we found greater numbers of climbers
430 in control forests, contradicting our initial hypothesis and current global trends (Laurance et al.
431 2001; DeWalt et al. 2010). In a study of a lowland tropical forest national park adjacent to a city
432 in Malaysia, liana abundance and species richness declined in areas of secondary forest which
433 displayed increased human disturbance (Addo-Forjour et al. 2012). This could explain our
434 results, since human disturbance in Cayenne's forest fragments is more likely, with these sites
435 providing a much-frequented recreational space for city residents.

436 Within the Metazoa, only blattodea were less abundant in urban forests. The majority of
437 OTUs in this group were identified as termites, a group known to be sensitive to forest
438 disturbance and resulting desiccation (Woon et al. 2019). They also rely on the availability of
439 organic matter as detritivores (Eggleton et al. 2002; Davis 2002; Jones et al. 2003; Dahlsjö et al.
440 2020), likely to be lower in urban forest fragments which exhibited lower total C and N. Acarian
441 mites, which represent a range of ecological strategies from fungal feeding to predation (Orgiazzi
442 et al. 2016), were also found to be lower in urban soils, which may again reflect reductions in
443 organic matter and leaf litter availability (Erdman et al. 2012).

444 For fungi, functional groups were largely equivalent between forest types due to a high
445 intra-forest type variability. Nevertheless, we found that AM fungi tended towards being more
446 abundant in urban forest soils, in agreement with previous reports in undisturbed and disturbed
447 forests in Brazil, where AM fungi were more abundant in disturbed habitats (Carrenho et al.,
448 2011). In urban or disturbed forests, host tree plants are likely to depend to a greater extent on
449 AM associations to survive under reduced nutrient conditions. The compositional shifts in plant

450 taxa detected between forest types may support this hypothesis, since palms, found to be more
451 abundant in urban forests, are reported as being associated with AM fungi (Núñez-Castillo &
452 Álvarez-Sánchez 2003; Dreyer et al. 2009). Still, the lack of strong shifts in functional groups
453 between urban and control forests is surprising given the substantial shifts in fungal taxonomic
454 turnover observed here and elsewhere (Abrego et al. 2020). This indicates that in spite of their
455 isolation within the urban environment, and the high levels of species turnover experienced, that
456 urban fragments are able to maintain a fungal community which functions in an equivalent
457 manner.

458 Unlike fungi, bacterial functional groups differed between forest types, with greater
459 abundance of chemolithoautotrophs in urban forests, and a greater abundance of
460 chemoorganoheterotrophs in control forests. This is consistent with differences in C and N
461 availability and potentially that of organic matter between forest types. Likewise, nitrifying and
462 nitrite reducing bacteria were more abundant in urban forests, consistent with previous
463 observations of higher nitrifying enzyme activity in these conditions (Reisinger et al. 2016).
464 This may be caused by greater fertilizer use in the urban surroundings or in the past, or
465 alternatively, to the higher oxygenation of the soils (Enloe et al. 2015), supported by our
466 detection of a higher abundance of earthworms in urban forests. In control forests, we detected a
467 greater relative abundance of phytobionts, counter to a recent finding showing greater
468 pathogen richness in disturbed forests (Makiola et al. 2019). Instead, our result may reflect a
469 wider niche space for plant pathogens in undisturbed rainforest where plant species
470 alpha-diversity is higher.

471 ***4.4 Limitations***

472 Although this study identifies several broad patterns, some caveats must be
473 acknowledged. First, our study relies on a limited number of replicate sites, and we therefore
474 lack statistical power to detect differences. Second, the DNA markers we used to identify soil
475 organisms provide limited taxonomic resolution or may lack of records in taxonomic reference
476 databases, preventing taxonomic assignment of certain OTUs. Markers can also bias detection
477 towards certain taxa, although such biases should be limited with the primers selected here
478 (Taberlet et al. 2018). Third, our approach of classifying functional groups depends on functional
479 databases, where tropical soil organisms, and microorganisms in particular, are likely to be
480 under-represented (Truong et al. 2014; Orgiazzi et al. 2015). In addition, our definition of

481 functional groups was coarse scale and may not detect fine differences between urban and
482 control forests.

483 **4.5 Conclusion**

484 Isolated forest fragments within an urban matrix contain assemblages of soil organisms
485 distinct from those of undisturbed continuous forest, a finding hitherto unobtainable using more
486 common ecological inventory approaches. From a conservation perspective, this suggests that
487 forest fragments play a limited role in sheltering native soil organism diversity in the face of
488 urbanization. However, from a functional point of view, urban forest remnants harbour
489 assemblages that do not deeply differ from those of pristine forests. Although we document
490 differences, urban forest fragments may maintain a baseline level of functional diversity, a
491 conservation argument that adds to the other services these ecosystems provide for human
492 wellbeing (FAO 2016). Ultimately, eDNA metabarcoding is confirmed as a powerful method for
493 determining multi-taxa patterns in soil biodiversity, with justified potential for future deployment
494 in biodiversity monitoring.

495

496 **Author contributions**

497 **Julian Donald:** Conceptualization; Data curation; Formal analysis; Investigation; Validation;
498 Writing - original draft.

499 **Jérôme Murienne:** Funding acquisition; Project administration; Conceptualization;
500 Investigation; Resources; Methodology; Writing - review & editing.

501 **Jérôme Chave:** Conceptualization; Investigation; Methodology; Resources; Writing - review &
502 editing.

503 **Amaia Iribar:** Investigation; Methodology; Resources;

504 **Eliane Louisanna:** Investigation; Resources;

505 **Sophie Manzi:** Investigation; Resources;

506 **Melanie Roy:** Investigation; Writing - review & editing.

507 **Shengli Tao:** Visualization.

508 **Jérôme Orivel:** Funding acquisition; Project administration; Conceptualization; Writing -
509 review & editing.

510 **Heidy Schimann:** Conceptualization; Investigation; Writing - review & editing.

511 **Lucie Zinger:** Conceptualization; Investigation; Methodology; Software; Supervision; Writing -
512 review & editing.

513

514 **Acknowledgements:**

515 The work has benefitted from “Investissement d’Avenir” grants managed by Agence Nationale
516 de la Recherche (CEBA: ANR-10-LABX-25-01; TULIP: ANR-10-LABX-0041), as well as from
517 the GlobNet project (ANR-16-CE02-0009). We also thank the Genotoul bioinformatics platform
518 of Toulouse Midi-Pyrenees (Bioinfo Genotoul) for providing computing and storage resources.

519

520 **Data availability**

521 Raw sequencing data and processed data used for analysis will be uploaded to the Zenodo
522 depository upon acceptance of manuscript, prior to publication

523 <https://zenodo.org/deposit/5069405>

524

525 **References**

526

527 Abrego, N. *et al.* (2020) ‘Fungal communities decline with urbanization—more in air than in
528 soil’, *The ISME Journal*, pp. 1–10.

529 Addo-Fordjour, P., Rahmad, Z. B. and Shahrul, A. M. S. (2012) ‘Effects of human disturbance
530 on liana community diversity and structure in a tropical rainforest, Malaysia: implication for
531 conservation’, *Journal of Plant Ecology*, 5(4), pp. 391–399.

532 Aerts, R. *et al.* (2016) ‘Conservation of the Ethiopian church forests: threats, opportunities and
533 implications for their management’, *Science of the Total Environment*, 551, pp. 404–414.

534 Anderson, M. J. (2017) ‘Permutational Multivariate Analysis of Variance (PERMANOVA)’, in
535 *Wiley StatsRef: Statistics Reference Online*. American Cancer Society, pp. 1–15.

536 Arnfield, A. J. (2003) ‘Two decades of urban climate research: a review of turbulence,
537 exchanges of energy and water, and the urban heat island’, *International Journal of Climatology*,
538 23(1), pp. 1–26.

539 Aronson, M. F. J. *et al.* (2014) ‘A global analysis of the impacts of urbanization on bird and
540 plant diversity reveals key anthropogenic drivers’, *Proceedings of the Royal Society B:
541 Biological Sciences*, 281(1780), p. 20133330.

542 Bardgett, R. D. and van der Putten, W. H. (2014) ‘Belowground biodiversity and ecosystem
543 functioning’, *Nature*, 515(7528), pp. 505–511.

544 Barlow, J. *et al.* (2018) ‘The future of hyperdiverse tropical ecosystems’, *Nature*, 559(7715), pp.
545 517–526.

546 Barnes, A. D. *et al.* (2017) ‘Direct and cascading impacts of tropical land-use change on multi-
547 trophic biodiversity’, *Nature Ecology & Evolution*, 1(10), pp. 1511–1519.

548 Baselga, A. (2013) ‘Separating the two components of abundance-based dissimilarity: balanced
549 changes in abundance vs. abundance gradients’, *Methods in Ecology and Evolution*, 4(6), pp.
550 552–557.

551 Baselga, A., Jiménez-Valverde, A. and Niccolini, G. (2007) ‘A multiple-site similarity measure
552 independent of richness’, *Biology Letters*, 3(6), pp. 642–645.

553 Baselga, A. and Orme, C. D. L. (2012) ‘betapart: an R package for the study of beta diversity’,
554 *Methods in Ecology and Evolution*, 3(5), pp. 808–812.

555 Bates, D. *et al.* (2007) ‘The lme4 package’, *R package version*, 2(1), p. 74.

556 Borges, E. R. *et al.* (2020) ‘The evolutionary diversity of urban forests depends on their land-use
557 history’, *Urban Ecosystems*, 23(3), pp. 631–643.

558 Boyer, F. *et al.* (2016) ‘obitools: a unix-inspired software package for DNA metabarcoding’,
559 *Molecular Ecology Resources*, 16(1), pp. 176–182.

560 Burns, R. G. *et al.* (2013) ‘Soil enzymes in a changing environment: Current knowledge and
561 future directions’, *Soil Biology and Biochemistry*, 58, pp. 216–234.

562 Byrne, L. B. (2007) ‘Habitat structure: A fundamental concept and framework for urban soil
563 ecology’, *Urban Ecosystems*, 10(3), pp. 255–274.

564 Cadotte, M. W. *et al.* (2017) ‘Are urban systems beneficial, detrimental, or indifferent for
565 biological invasion?’, *Biological Invasions*, 19(12), pp. 3489–3503.

566 Calderón-Sanou, I. *et al.* (2020) ‘From environmental DNA sequences to ecological conclusions:
567 How strong is the influence of methodological choices?’, *Journal of Biogeography*, 47(1), pp.
568 193–206.

569 Campbell, M. J. *et al.* (2018) ‘Edge disturbance drives liana abundance increase and alteration of
570 liana–host tree interactions in tropical forest fragments’, *Ecology and Evolution*, 8(8), pp. 4237–
571 4251.

572 Canale, G. R. *et al.* (2012) ‘Pervasive Defaunation of Forest Remnants in a Tropical Biodiversity
573 Hotspot’, *PLOS ONE*, 7(8), p. e41671.

574 Cardoso, D. *et al.* (2017) ‘Amazon plant diversity revealed by a taxonomically verified species
575 list’, *Proceedings of the National Academy of Sciences*, 114(40), pp. 10695–10700.

576 Carrenho, R. and Gomes-da-Costa, S. M. (2011) ‘Environmental degradation impact on native
577 communities of arbuscular mycorrhizal fungi in an urban fragment of semideciduous plateau
578 forest’, *Acta Botanica Brasilica*, 25(2), pp. 373–379.

579 Carvalho, T. S. de *et al.* (2016) ‘Land use intensification in the humid tropics increased both
580 alpha and beta diversity of soil bacteria’, *Ecology*, 97(10), pp. 2760–2771.

581 de Castilho, C. V. *et al.* (2006) ‘Variation in aboveground tree live biomass in a central
582 Amazonian Forest: Effects of soil and topography’, *Forest Ecology and Management*, 234(1),
583 pp. 85–96.

584 Centre, E. C. J. R. (2016) *Global Soil Biodiversity Atlas*. Publications Office of the European
585 Union.

586 Cordier, T. *et al.* (2021) ‘Ecosystems monitoring powered by environmental genomics: A review
587 of current strategies with an implementation roadmap’, *Molecular Ecology*

588 Cusack, D. F. (2013) ‘Soil nitrogen levels are linked to decomposition enzyme activities along
589 an urban-remote tropical forest gradient’, *Soil Biology and Biochemistry*, 57, pp. 192–203.

590 Dahlsjö, C. A. L. *et al.* (2020) ‘The local impact of macrofauna and land-use intensity on soil
591 nutrient concentration and exchangeability in lowland tropical Peru’, *Biotropica*, 52(2), pp. 242–
592 251.

593 Davies, R. G. (2002) ‘Feeding group responses of a Neotropical termite assemblage to rain forest
594 fragmentation’, *Oecologia*, 133(2), pp. 233–242.

595 De Boer, W. and Kowalchuk, G. A. (2001) ‘Nitrification in acid soils: micro-organisms and
596 mechanisms’, *Soil Biology and Biochemistry*, 33(7), pp. 853–866.

597 Decaëns, T. *et al.* (2018) ‘Biodiversity loss along a gradient of deforestation in Amazonian
598 agricultural landscapes’, *Conservation Biology*, 32(6), pp. 1380–1391.

599 DeFries, R. S. *et al.* (2010) ‘Deforestation driven by urban population growth and agricultural
600 trade in the twenty-first century’, *Nature Geoscience*, 3(3), pp. 178–181.

601 DeWalt, S. J. *et al.* (2010) ‘Annual Rainfall and Seasonality Predict Pan-tropical Patterns of
602 Liana Density and Basal Area’, *Biotropica*, 42(3), pp. 309–317.

603 Dreyer, B. *et al.* (2010) ‘Comparative study of mycorrhizal susceptibility and anatomy of four
604 palm species’, *Mycorrhiza*, 20(2), pp. 103–115.

605 Eggleton, P. *et al.* (2002) ‘Termite diversity across an anthropogenic disturbance gradient in the
606 humid forest zone of West Africa’, *Agriculture, Ecosystems & Environment*, 90(2), pp. 189–202.

607 Enloe, H. A. *et al.* (2015) ‘Urbanization effects on soil nitrogen transformations and microbial
608 biomass in the subtropics’, *Urban Ecosystems*, 18(3), pp. 963–976.

609 Erdmann, G., Scheu, S. and Maraun, M. (2012) ‘Regional factors rather than forest type drive the
610 community structure of soil living oribatid mites (Acari, Oribatida)’, *Experimental and Applied
611 Acarology*, 57(2), pp. 157–169.

612 Ewers, R. M. and Didham, R. K. (2006) ‘Confounding factors in the detection of species
613 responses to habitat fragmentation’, *Biological Reviews*, 81(1), pp. 117–142.

614 Fahrig, L. *et al.* (2019) ‘Is habitat fragmentation bad for biodiversity?’, *Biological Conservation*,
615 230, pp. 179–186.

616 Ficetola, G. F. *et al.* (2010) ‘An In silico approach for the evaluation of DNA barcodes’, *BMC
617 Genomics*, 11(1), p. 434.

618 Fountain, M. T. and Hopkin, S. P. (2004) ‘Biodiversity of Collembola in Urban Soils and the
619 Use of *Folsomia candida* to Assess Soil “Quality”’, *Ecotoxicology*, 13(6), pp. 555–572.

620 Franco, A. L. C. *et al.* (2019) ‘Amazonian deforestation and soil biodiversity’, *Conservation
621 Biology*, 33(3), pp. 590–600.

622 Geisen, S. *et al.* (2019) ‘A methodological framework to embrace soil biodiversity’, *Soil Biology
623 and Biochemistry*, 136, p. 107536.

624 Heckenberger, M. J. *et al.* (2007) ‘The legacy of cultural landscapes in the Brazilian Amazon:
625 implications for biodiversity’, *Philosophical Transactions of the Royal Society B: Biological
626 Sciences*, 362(1478), pp. 197–208.

627 Heijden, G. M. F. van der, Powers, J. S. and Schnitzer, S. A. (2015) ‘Lianas reduce carbon
628 accumulation and storage in tropical forests’, *Proceedings of the National Academy of Sciences*,
629 112(43), pp. 13267–13271.

630 Hendriksen, N. B. *et al.* (2016) ‘Soil exo-enzyme activities across Europe—The influence of
631 climate, land-use and soil properties’, *Applied Soil Ecology*, 97, pp. 44–48.

632 Ho, A., Lonardo, D. P. D. and Bodelier, P. L. E. (2017) ‘Revisiting life strategy concepts in
633 environmental microbial ecology’, *FEMS Microbiology Ecology*, p. fix006.

634 Hsieh, T. C., Ma, K. H. and Chao, A. (2016) ‘iNEXT: an R package for rarefaction and
635 extrapolation of species diversity (Hill numbers)’, *Methods in Ecology and Evolution*, 7(12), pp.
636 1451–1456.

637 Jim, C. Y. (1998) ‘Physical and chemical properties of a Hong Kong roadside soil in relation to
638 urban tree growth’, *Urban Ecosystems*, 2(2), pp. 171–181.

639 Jones, D. T. *et al.* (2003) ‘Termite assemblage collapse along a land-use intensification gradient
640 in lowland central Sumatra, Indonesia’, *Journal of Applied Ecology*, 40(2), pp. 380–391.

641 Jones, E. L. and Leather, S. R. (2013) ‘Invertebrates in urban areas: A review’, *EJE*, 109(4), pp.
642 463–478.

643 Laurance, W. F. *et al.* (2001) ‘Rain Forest Fragmentation and the Structure of Amazonian Liana
644 Communities’, *Ecology*, 82(1), pp. 105–116.

645 Laurance, W. F. *et al.* (2002) ‘Ecosystem Decay of Amazonian Forest Fragments: a 22-Year
646 Investigation’, *Conservation Biology*, 16(3), pp. 605–618.

647 Legendre, P. and Legendre, L. (2012) *Numerical Ecology*. Elsevier.

648 de Lima, R. A. F. *et al.* (2020) ‘The erosion of biodiversity and biomass in the Atlantic Forest
649 biodiversity hotspot’, *Nature Communications*, 11(1), p. 6347.

650 Lorenz, K. and Lal, R. (2009) ‘Biogeochemical C and N cycles in urban soils’, *Environment
651 International*, 35(1), pp. 1–8.

652 Louca, S., Parfrey, L. W. and Doebeli, M. (2016) ‘Decoupling function and taxonomy in the
653 global ocean microbiome’, *Science*, 353(6305), pp. 1272–1277.

654 Madin, J. S. *et al.* (2020) ‘A synthesis of bacterial and archaeal phenotypic trait data’, *Scientific
655 Data*, 7(1), p. 170.

656 Magrach, A. *et al.* (2016) ‘Selective logging in tropical forests decreases the robustness of liana–
657 tree interaction networks to the loss of host tree species’, *Proceedings of the Royal Society B:
658 Biological Sciences*, 283(1826), p. 20153008.

659 Makiola, A. *et al.* (2019) ‘Land use is a determinant of plant pathogen alpha- but not beta-
660 diversity’, *Molecular Ecology*, 28(16), pp. 3786–3798.

661 McDonald, R. I. *et al.* (2020) ‘Research gaps in knowledge of the impact of urban growth on
662 biodiversity’, *Nature Sustainability*, 3(1), pp. 16–24.

663 McDonald, R. I., Kareiva, P. and Forman, R. T. T. (2008) ‘The implications of current and future
664 urbanization for global protected areas and biodiversity conservation’, *Biological Conservation*,
665 141(6), pp. 1695–1703.

666 McGee, K. M. *et al.* (2019) ‘Determinants of Soil Bacterial and Fungal Community Composition
667 Toward Carbon-Use Efficiency Across Primary and Secondary Forests in a Costa Rican
668 Conservation Area’, *Microbial Ecology*, 77(1), pp. 148–167.

669 McKinney, M. L. (2002) ‘Urbanization, Biodiversity, and Conservation The impacts of
670 urbanization on native species are poorly studied, but educating a highly urbanized human
671 population about these impacts can greatly improve species conservation in all ecosystems’,
672 *BioScience*, 52(10), pp. 883–890.

673 Mendes, L. W. *et al.* (2015) ‘Land-use system shapes soil bacterial communities in Southeastern
674 Amazon region’, *Applied Soil Ecology*, 95, pp. 151–160.

675 Mennicken, S. *et al.* (2020) ‘Effects of Past and Present-Day Landscape Structure on Forest Soil
676 Microorganisms’, *Frontiers in Ecology and Evolution*, 8, p. 118.

677 Mercier, C. *et al.* (2013) ‘SUMATRA and SUMACLUST: fast and exact comparison and
678 clustering of sequences’, in *Programs and Abstracts of the SeqBio 2013 workshop. Abstract.*
679 Citeseer, pp. 27–29.

680 Navarrete, A. A. *et al.* (2015) ‘Soil microbiome responses to the short-term effects of Amazonian
681 deforestation’, *Molecular Ecology*, 24(10), pp. 2433–2448.

682 Nelson, M. B., Martiny, A. C. and Martiny, J. B. H. (2016) ‘Global biogeography of microbial
683 nitrogen-cycling traits in soil’, *Proceedings of the National Academy of Sciences*, 113(29), pp.
684 8033–8040.

685 Newbound, M., Mccarthy, M. A. and Lebel, T. (2010) ‘Fungi and the urban environment: A
686 review’, *Landscape and Urban Planning*, 96(3), pp. 138–145.

687 Nguyen, N. H. *et al.* (2016) ‘FUNGuild: An open annotation tool for parsing fungal community
688 datasets by ecological guild’, *Fungal Ecology*, 20, pp. 241–248.

689 Núñez-Castillo, O. and Alvarez-Sánchez, F. J. (2003) ‘Arbuscular mycorrhizae of the palm
690 *Astrocaryum mexicanum* in disturbed and undisturbed stands of a Mexican tropical forest’,
691 *Mycorrhiza*, 13(5), pp. 271–276.

692 Nychka, D. *et al.* (2005) ‘fields: Tools for spatial data’, *National Center for Atmospheric
693 Research*.

694 Oksanen, J. *et al.* (2013) ‘Package “vegan”’, *Community ecology package, version*, 2(9), pp. 1–
695 295.

696 Oliveira, E. A. de *et al.* (2020) ‘Legacy of Amazonian Dark Earth soils on forest structure and
697 species composition’, *Global Ecology and Biogeography*, 29(9), pp. 1458–1473.

698 Orgiazzi, A. *et al.* (2015) ‘Soil biodiversity and DNA barcodes: opportunities and challenges’,
699 *Soil Biology and Biochemistry*, 80, pp. 244–250.

700 Orrock, J. L. and Watling, J. I. (2010) ‘Local community size mediates ecological drift and
701 competition in metacommunities’, *Proceedings of the Royal Society B: Biological Sciences*,
702 277(1691), pp. 2185–2191.

703 Paula, F. S. *et al.* (2014) ‘Land use change alters functional gene diversity, composition and
704 abundance in Amazon forest soil microbial communities’, *Molecular Ecology*, 23(12), pp. 2988–
705 2999.

706 Qian, H., Ricklefs, R. E. and White, P. S. (2005) ‘Beta diversity of angiosperms in temperate
707 floras of eastern Asia and eastern North America’, *Ecology Letters*, 8(1), pp. 15–22.

708 Quast, C. *et al.* (2013) ‘The SILVA ribosomal RNA gene database project: improved data
709 processing and web-based tools’, *Nucleic Acids Research*, 41(D1), pp. D590–D596.

710 Ramirez, K. S. *et al.* (2014) ‘Biogeographic patterns in below-ground diversity in New York
711 City’s Central Park are similar to those observed globally’, *Proceedings of the Royal Society B:
712 Biological Sciences*, 281(1795), p. 20141988.

713 Reisinger, A. J., Groffman, P. M. and Rosi-Marshall, E. J. (2016) ‘Nitrogen-cycling process rates
714 across urban ecosystems’, *FEMS Microbiology Ecology*, 92(fiw198).

715 Ritter, C. D. *et al.* (2019) ‘Locality or habitat? Exploring predictors of biodiversity in
716 Amazonia’, *Ecography*, 42(2), pp. 321–333.

717 Rodrigues, J. L. M. *et al.* (2013) ‘Conversion of the Amazon rainforest to agriculture results in
718 biotic homogenization of soil bacterial communities’, *Proceedings of the National Academy of
719 Sciences*, 110(3), pp. 988–993.

720 Santorufo, L. *et al.* (2012) ‘Soil invertebrates as bioindicators of urban soil quality’,
721 *Environmental Pollution*, 161, pp. 57–63.

722 Saunders, D. A., Hobbs, R. J. and Margules, C. R. (1991) ‘Biological Consequences of
723 Ecosystem Fragmentation: A Review’, *Conservation Biology*, 5(1), pp. 18–32.

724 Seto, K. C., Güneralp, B. and Hutyra, L. R. (2012) ‘Global forecasts of urban expansion to 2030
725 and direct impacts on biodiversity and carbon pools’, *Proceedings of the National Academy of
726 Sciences*, 109(40), pp. 16083–16088.

727 Shen, G. *et al.* (2019) ‘Spatial patterns and estimates of global forest litterfall’, *Ecosphere*, 10(2),
728 p. e02587.

729 Simberloff, D. and Abele, L. G. (1982) ‘Refuge Design and Island Biogeographic Theory:
730 Effects of Fragmentation’, *The American Naturalist*, 120(1), pp. 41–50.

731 Sinsabaugh, R. L. (2010) ‘Phenol oxidase, peroxidase and organic matter dynamics of soil’, *Soil*
732 *Biology and Biochemistry*, 42(3), pp. 391–404. d
733 Sritongchuay, T. *et al.* (2019) ‘Forest proximity and lowland mosaic increase robustness of
734 tropical pollination networks in mixed fruit orchards’, *Landscape and Urban Planning*, 192, p.
735 103646.
736 Stein, L. Y. and Klotz, M. G. (2016) ‘The nitrogen cycle’, *Current Biology*, 26(3), pp. R94–R98.
737 Strous, M. *et al.* (1999) ‘Missing lithotroph identified as new planctomycete’, *Nature*,
738 400(6743), pp. 446–449.
739 Taberlet, P. *et al.* (2012) ‘Environmental DNA’, *Molecular Ecology*, 21(8), pp. 1789–1793.
740 Taberlet, P. *et al.* (2018) *Environmental DNA: For Biodiversity Research and Monitoring*.
741 Oxford University Press.
742 Team, R. C. (2013) *R: A language and environment for statistical computing*. Vienna, Austria.
743 Truong, C. *et al.* (2017) ‘How to know the fungi: combining field inventories and DNA-
744 barcoding to document fungal diversity’, *New Phytologist*, 214(3), pp. 913–919.
745 Tschartke, T. *et al.* (2012) ‘Landscape moderation of biodiversity patterns and processes - eight
746 hypotheses’, *Biological Reviews*, 87(3), pp. 661–685.
747 Vieira, J. *et al.* (2018) ‘Green spaces are not all the same for the provision of air purification and
748 climate regulation services: The case of urban parks’, *Environmental Research*, 160, pp. 306–
749 313.
750 Vleminckx, J. *et al.* (2019) ‘Coordinated community structure among trees, fungi and
751 invertebrate groups in Amazonian rainforests’, *Scientific Reports*, 9(1), p. 11337.
752 Vos, M. *et al.* (2013) ‘Micro-scale determinants of bacterial diversity in soil’, *FEMS*
753 *Microbiology Reviews*, 37(6), pp. 936–954
754 Wardeh, M. *et al.* (2015) ‘Database of host-pathogen and related species interactions, and their
755 global distribution’, *Scientific Data*, 2(1), p. 150049.
756 Zinger, L. *et al.* (2016) ‘Extracellular DNA extraction is a fast, cheap and reliable alternative for
757 multi-taxa surveys based on soil DNA’, *Soil Biology and Biochemistry*, 96, pp. 16–19.
758 Zinger, L. *et al.* (2019) ‘Body size determines soil community assembly in a tropical forest’,
759 *Molecular Ecology*, 28(3), pp. 528–543.
760 Zinger, L. *et al.* (2020) ‘Chapter Nine - Advances and prospects of environmental DNA in
761 neotropical rainforests’, in Dumbrell, A. J., Turner, E. C., and Fayle, T. M. (eds) *Advances in*
762 *Ecological Research*. Academic Press (Tropical Ecosystems in the 21st Century), pp. 331–373.
763 Zinger, L. *et al.* (2021) ‘metabaR: An r package for the evaluation and improvement of DNA
764 metabarcoding data quality’, *Methods in Ecology and Evolution*