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1 **Assessment of the efficiency of three sampling methods for the recovery of soil charcoals in** 2 **tropical anthropogenic sites**

3 Stéphanie C. Bodin^{*1,2}, Thomas Feiss³, Jean-François Molino², and Laurent Bremond^{1,4}

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5 ¹ISEM, Université Montpellier, CNRS, EPHE, IRD, Montpellier, France

6 ²AMAP, IRD, CIRAD, CNRS, INRA, Université Montpellier, Montpellier, France

7 ³Université de Lorraine, AgroParisTech, INRAE, UMR 1434 SILVA, Nancy, France

8 ⁴École Pratique des Hautes Études, PSL University, Paris, France

9

10 Institut des Sciences de l'Évolution (ISEM)

11 UMR 5554 | Université de Montpellier | CNRS | IRD | EPHE

12 Place Eugène Bataillon | Cc 065

13 34095 Montpellier cedex 05 | France

14 s.bodin90@gmail.com

15 [Abstract](#)

16 The recovery and identification of wood charcoals from soil not associated to archaeological
17 excavation is called pedoanthracology. Researchers in this field use different sampling methods, and
18 only one study, in a temperate context, has compared their efficiency so far. In this paper, we
19 compare the efficiency of three sampling methods on charcoal concentration (> 4 mm) and on
20 taxonomic representativeness in a tropical environment for the first time. These methods are pit
21 sampling, auger sampling and opportunistic handpicking of charcoal on tree uprooted mounds or in
22 the soil excavated during the digging of a pit. Our results suggest that the two digging methods (pit
23 and auger) allow to recover similar charcoal concentrations whatever the site and the depth under
24 consideration. But as expected, the charcoal concentration of a site depends on its history (e.g. type
25 of activity). We also show that the estimation of the minimal sampling effort required to obtain
26 taxonomically representative assemblages, in terms of number of charcoals and of auger samplings,
27 varied greatly depending on the site and on the presence of overrepresented taxa. Finally, our results
28 show that auger cores were often monospecific (34 %). Nevertheless, they allowed to recover
29 different taxa from the pit method, with 18 to 21 % of the taxa exclusive to the first method and 30
30 to 40 % of the taxa exclusive to the second one in the sites under consideration. Charcoals from
31 opportunistic handpicking also allowed to improve the taxonomic diversity of the whole assemblage
32 of a site with 5 to 15 % of the taxa being exclusive to this method. The three methods are therefore
33 complementary and we suggest to use the three of them to obtain the best taxonomic diversity in an
34 anthracological assemblage and to overcome the specific biases of each method. With this study, we
35 hope that we will help tropical (pedo)anthracologists to optimize charcoal sampling in anthropogenic
36 sites where the sampling design cannot benefit from archaeological excavations.

37 Key words: Pedoanthracology – Sampling design – Tropical forest – Rarefaction curves –
38 Representativeness

39 1. Introduction

40 Anthracology and pedoanthracology are sister archaeobotanical specialties which are used for the
41 reconstruction of past ligneous vegetation through the identification of wood charcoal. The former is
42 in close relation with archaeology since wood charcoals are sampled in known archaeological
43 contexts (Chabal 1997; Heinz and Thiébault 1998; Dotte-Sarout 2017; Bachelet and Scheel-Ybert
44 2017), whilst the latter deals with soil charcoals not associated to archaeological units (Thinon 1978;
45 Carcaillet and Thinon 1996; Scheel-Ybert et al. 2003; Di Pasquale et al. 2008; Talon 2010; Touflan et
46 al. 2010; Nelle et al. 2013). The reliability of anthracological assemblages to reconstruct past ligneous
47 environments has been demonstrated and the representativeness of these assemblages is the
48 subject of an abundant literature (Badal Garcia 1992; Chabal et al. 1999; Scheel-Ybert 2002; Théry-
49 Parisot et al. 2010; Chrzazvez et al. 2014; Dotte-Sarout et al. 2015). Nevertheless, studies focusing on
50 the efficiency of the sampling protocols are nearly inexistent, although this is a central issue
51 concerning the representativeness of pedoanthracological assemblages. While anthracological
52 sampling protocols 'guided' by the presence of archaeological deposits have been optimized (Chabal
53 et al. 1999), pedoanthracological sampling schemes vary greatly among specialists and, to our
54 knowledge, only one study has addressed this issue (Feiss et al. 2017). Two methods can be used for
55 the systematic sampling of soil charcoals: pit sampling and auger sampling. The first one, which is the
56 most used, consists in sampling soil volumes (usually 10 L or 10 kg) along a soil profile in layers
57 respecting the pedological horizons (Carcaillet and Thinon 1996; Dutoit et al. 2009; Feiss et al. 2017)
58 or in artificial layers of about 10 cm in the tropics where soils horizons (oxisols) are less distinct
59 (Scheel-Ybert et al. 2003; Hubau et al. 2012; Fernandes Caromano et al. 2013; Morin-Rivat et al.
60 2014; 2016; Dotte-Sarout et al. 2015). The dimensions of the pits vary among the studies and can
61 take the form of large trenches of 1 x 2 m wide (Scheel-Ybert et al. 2003; Di Pasquale et al. 2008) or
62 small test-pits of 0.5 m x 0.5 m or 1 x 1 m wide (Dotte-Sarout et al. 2015; Morin-Rivat et al. 2016).
63 The second method, auger sampling, is rarely used alone, i.e. without the pit sampling method
64 (Vleminckx et al. 2014; Feiss et al. 2017). It is often restricted to prospection, i.e. to assess the depth
65 of charcoal layers and the abundance of charcoals, which helps to determine the location for digging
66 a pit (Tardy 1998; Hubau et al. 2012; 2013; Bourland et al. 2015; Dotte-Sarout and Kahn 2017) or to
67 supplement a pit sampling in the deepest layers (Scheel-Ybert et al. 2003). Here again, the number of
68 auger cores and the sampling design differ greatly depending on the environment and the aim of the
69 study. In tropical regions, it is particularly important to develop effective sampling methods because
70 field access is often difficult and environmental constraints are high, therefore sites cannot be easily
71 revisited to supplement an insufficient sampling.

72 In this paper, we compare the efficiency of three sampling methods in a tropical environment for the
73 first time, along the lines of the comparative study by Feiss et al. (2017) in a temperate environment.
74 The three methods are auger sampling, pit sampling (digging methods) and opportunistic
75 handpicking of charcoals during excavations or on uprooted tree mounds. This latter method can be
76 used to prospect sites of interest (Tardy 1998) and as such, it is interesting to estimate its potential in
77 terms of taxonomic coverage.

78 Specifically, we address the following questions:

- 79 1) is one of the two digging methods more efficient than the other for the recovery of soil charcoals?
- 80 2) do the site and sampling depth influence the amount of charcoal retrieved with each digging
81 method?
- 82 3) do any of the three methods yield assemblages with better taxonomic richness?

83 4) do they require the same sampling effort?

84 2. Material and methods

85 2.1. Study area

86 The fieldwork took place in the Nouragues Natural Reserve, central French Guiana, in 12 sites located
87 near the two base camps 'Saut Pararé' (4°02' N - 52°41'W) and 'Inselberg' (4°05' N - 52°41'W). The
88 prospected sites displayed different patterns of past human occupation in terms of vegetation
89 structure and composition and in terms of archaeological evidences (earthworks, potsherds
90 associated with charcoals, dark earths). Sites 1 and 5 are in *Lasiacis* thickets, site 9 is in a liana forest,
91 site 7 is at the limit between a *Lasiacis* thicket and a liana patch and the remaining sites are under
92 forest cover. Site 3 corresponds to a circular anthropogenic ditch circling a hilltop, a type of
93 archaeological earthwork common in French Guiana. Table 1 summarizes the main features of the
94 sites. See also Bodin et al. (2020) for additional information on site location.

95 2.2. Sampling methods

96 The sampling protocol was formerly described in Bodin et al. (2020). Despite the presence of
97 archaeological artefacts (potsherds) and earthworks in several sites, our approach is
98 pedoanthracologic because the recovery of charcoal fragments did not benefit from archaeological
99 excavations. In sites 1 to 9, we dug 80 x 80 cm pits in which we collected 10 kg of soil in each 10-cm
100 depth layer. We also collected charcoal fragments by hand in the soil excavated during the digging
101 process. Second, in sites where charcoals were abundant enough in the pit (at least a dozen of
102 fragments), we sampled with a soil auger along transects centered on the pit in a 1-ha surface (Fig.
103 1), except for site 3 where we sampled at several points in the ditch. The objective with auger cores
104 was to cover a larger area to better catch taxonomic diversity at the stand scale. The number of
105 auger samples depended on site constraints such as vegetation thickness (pseudo-bamboos in site 1
106 and lianas in site 9 are hard to penetrate) and the presence of potential archaeological artifacts
107 detected by magnetic anomalies (site 8). The sampling depth depended on the depth of the bedrock.
108 For lack of time, the pits at sites 10 to 12 were not sampled, nor were sites 11 and 12 with any
109 digging method. Finally, on sites 9 to 12 we looked for charcoals on all uprooted tree mounds
110 present in the 1-ha surface centered on the pit. This last method was chosen because it was a quick
111 way to obtain qualitative information on sites that we did not have time to fully investigate. For sites
112 1 to 8, searching for charcoals in soil excavated during the digging of the pit is considered to be
113 similar as searching on an uprooted tree mound because it is opportunistic. The different sampling
114 methods applied for each site are showed in Table 1.

115 Table 1: history and main vegetation type of the sites investigated and number of samples for each
116 sampling method applied (after Bodin et al. 2020).

Site	History	Vegetation	Number of pit profile samples each 10-cm depth, (max. depth in cm)	Number of auger cores (max. depth in cm)	Number of tree uprooting mounds/Excavated soil investigated for charcoal
1	Anthropized	<i>Lasiacis</i> thicket	6 (60)	20 (80)	0/1
2	Anthropized	Forest	3 (30)	26 (90)	0/1
3	Anthropized	Forest	6 (50)	16 (130)	0/1
4	Non-anthropized	Forest	5 (50)	0	0/0
5	Non-anthropized	<i>Lasiacis</i> thicket	4 (40)	0	0/0
6	Potentially anthropized	Forest	5 (50)	23 (60)	0/1

7	Anthropized	<i>Lasiacis</i> thicket/liana forest	5 (50)	0	0/1
8	Anthropized	Forest	4 (40)	6 (80)	0/1
9	Anthropized	Liana forest	7 (70)	17 (60)	9/0
10	Non-anthropized	Forest	-	16 (60)	11/0
11	Anthropized	Forest	-	-	15/0
12	Non-anthropized	Forest	-	-	3/0

117

118 2.3. Charcoal extraction and identification

119 Soil samples from pit profiles and auger cores were water-sieved in the field to recover charcoal
120 fragments in a 4-mm mesh, a suitable size for charcoal identification, especially in tropical areas
121 (Chabal et al. 1999; Scheel-Ybert 2001). Charcoals were identified with the help of an electronic
122 identification key (Bodin et al. 2019), the InsideWood online database (Wheeler 2011), specialized
123 literature (Détienne et al. 1982; Détienne and Jacquet 1983; Scheel-Ybert and Gonçalves 2017) and
124 reference collections of charred wood from Brazil and French Guiana (Scheel-Ybert 2016).

125 2.4. Testing the effects of sampling method, sampling depth and site on charcoal 126 concentration

127 The sites considered for statistical analysis were those which were sampled with the same methods,
128 i.e. sites 1, 2, 3, 6, 8 and 9. Sites 4 and 5 did not provide any charcoal fragment. We first tested the
129 correlation between the number of taxa and the number of charcoal fragments using a Spearman
130 test. The idea here was to determine whether we could discuss taxonomic richness given the
131 charcoal richness. We then expressed the charcoal concentration as the total number of charcoal
132 pieces per litre of sieved sediment (from pits and augers) to allow for comparison between all sites.

133 We tested the effect of the sampling method (pit vs. auger cores) on charcoal concentration using a
134 Wilcoxon test. We then tested the effect of sampling depth and of site on charcoal concentration
135 using a Kruskal-Wallis test and performed a Conover post hoc test from the PMCMRplus R package
136 (Pohlert 2020) with a holm correction whenever significant.

137 Finally, we tested the effect of depth and of site on each method using Friedman tests: sites 1, 2, 3, 6,
138 7, 8 and 9 were considered for the pit method and sites 1, 2, 3, 6, 8, 9 and 10 were considered for the
139 auger method (Table 1). In each case, comparisons were made for the same sampling depths, i.e.
140 down to the 30 cm level when pits were included and down to the 60 cm level when comparisons
141 were made for the auger method only, as we dug deeper in the soil with the auger (Table 1). We
142 used the exact all-pairs comparisons test for unreplicated blocked data (Eisinga et al. 2017) from the
143 PMCMRplus R package when the Friedman tests were significant.

144 2.5. Determining the minimal sampling effort required to obtain a representative 145 assemblage

146 To determine the minimal number of charcoal fragments and the minimal number of auger cores
147 required to obtain a representative assemblage of the floristic diversity, we followed the procedure
148 described in Chao et al. (2014). We established individual-based rarefaction curves of the richest sites
149 (i.e. 1, 2, 3, 9) for each sampling method and sample-based rarefaction curves using the iNEXT
150 function from the iNEXT package (Hsieh et al. 2016). Individual-based curves represent the number
151 of taxa as a function of the number of identifiable charcoal fragments (i.e. excluding unidentifiable
152 ones) whilst sample-based curves represent the number of taxa as a function of the number of
153 sampling units, here defined as the number of auger cores. The minimal sampling effort is

154 determined as the minimal number of charcoal fragments or auger cores required to reach the
155 asymptote, which corresponds to the predicted diversity. Securely identified taxa were merged with
156 their 'cf.' analogous before building the curves (e.g. 'taxon A' was merged with 'cf. taxon A').

157 Finally, we computed the number of taxa recovered exclusively by each sampling method in these
158 sites to assess the complementarity of the methods.

159 These analyses and the following were performed with R software version 3.5.0 (R Core Team 2018).

160 3. Results

161 Charcoal richness is positively correlated with taxonomic diversity (Spearman test: $\rho = 0.8303$; p-
162 value = 0.0056). The number of charcoals and identifiable taxa retrieved from each site using each
163 sampling methods is given in Supplementary Information (Tables A.1 and A.2).

164 3.1. Effect of sampling method on charcoal concentration

165 Due to high variability in charcoal concentration between sites, the results are hereafter given as
166 median \pm standard error. We found no difference in charcoal concentration between auger cores and
167 pits: 2 ± 1 vs. 2.7 ± 1.52 charcoals/L (Fig. 2; Wilcoxon test: p-value = 0.6461).

168 3.2. Effect of sampling depth

169 We found no significant differences in charcoal concentrations between sampling depths when
170 considering the two sampling methods together (Fig. 3A and and Table A.4, Kruskal-Wallis test: p-
171 value = 0.1029), or separately (Fig. 3B-C and Table A.4, Friedman test: p-values = 0.7716 for the pits
172 and 0.2095 for the augers). Nevertheless, the level "10-20 cm" tends to be richer in charcoals
173 fragments than the other levels with a higher median, whatever the sampling method under
174 consideration.

175 3.3. Effect of site

176 When the results of pit and auger methods are pooled, only sites 2 and 8 appear significantly
177 different, the former having a higher charcoal concentration than the latter (Fig. 4A and Table A.3;
178 Kruskal-Wallis test: p-value = 0.0096). We found a difference between sites 2 and 7 when we
179 compared pits only, and between site 8 and sites 2 and 3 when we compared augers only (Fig. 4B-C
180 and Table A.3; Friedman test: p-values = 0.0247 and 0.0016, respectively). There are no other
181 remarkable differences.

182 3.4. Estimating the minimal sampling effort using rarefaction curves

183 Observed and predicted diversity, and sampling effort required to reach the predicted diversity for
184 the richest sites 1, 2, 3 and 9 are summarized in Table 3. Individual- and sample-based rarefaction
185 curves are presented in Fig. 5. The percentage of the total number of taxa exclusively recovered by
186 each sampling method is presented in Fig. 6.

187 Table 3: outputs from individual- and sample-based rarefaction curves in each site, for each of the
188 three sampling methods and when pooled ('All'). 'Exc. soil' refers to the excavated soil investigated
189 for charcoals with the naked eye.

Site	Method	Individual-based					Sample-based				
		Observed diversity	Estimated asymptote	s. e.	Predicted diversity	Sampling effort (nb of charcoals)*	Observed diversity	Estimated asymptote	s. e.	Predicted diversity	Sampling effort (nb of auger cores)*
1	Exc. soil	17	41.615	23.873	41	657	—	—	—	—	—
	Pit	26	36.062	8.965	36	1073	—	—	—	—	—
	Augers	13	17.327	4.636	17	76	13	18.819	5.799	18	54
	All	39	52.944	9.863	52	926	—	—	—	—	—
2	Exc. soil	5	5.992	2.183	5	131	—	—	—	—	—
	Pit	10	30.942	17.198	30	3733	—	—	—	—	—
	Augers	16	40.173	30.699	40	1356	16	50.615	32.142	50	331
	All	26	123.828	111.3666	123	19544	—	—	—	—	—
3	Exc. soil	10	12.213	3.345	12	165	—	—	—	—	—
	Pit	20	30.057	8.961	30	1017	—	—	—	—	—
	Augers	14	18.127	4.840	18	409	14	37.438	22.756	37	168
	All	30	38.615	6.798	38	968	—	—	—	—	—
9	Mounds	8	11.765	5.002	11	44	8	—	—	—	—
	Pit	25	55.055	28.464	55	2826	—	—	—	—	—
	Augers	14	21.875	7.999	21	99	14	25.765	10.086	25	74
	All	43	68.657	16.071	68	1192	—	—	—	—	—

*based on the integer part of the estimated asymptote, i.e. the predicted diversity.

190

191 Overall, the pit method tends to recover more taxa (81) than the auger method (57). The excavated
 192 soil/mound method (thereafter exc. soil/mound method) recovered less taxa (42), but with a higher
 193 variability among sites.

194 At sites 1, 3 and 9 augers presented a lower observed diversity than pits (13 vs 26, 14 vs 20 and 14 vs
 195 25, respectively). Only at site 2 did the augers have a greater observed diversity than the pit (16 vs
 196 10).

197 At sites 2, 3 and 9, the exc. soil/mound method showed a lower observed diversity (5, 10 and 8,
 198 respectively) than other methods. At site 1, the observed diversity from excavated soil was lower
 199 than in the pit (17 vs 26) but higher than in augers (17 vs 13).

200 The sampling effort estimated to reach a plateau varied greatly depending on site and sampling
 201 method. We only reached a plateau with the excavated soil from site 2 with a very low diversity of 5
 202 taxa for a sampling effort of 131 charcoals. The estimated sampling effort varied from 1017 to 3733
 203 charcoals with the pit method, from 76 to 1356 charcoals with the auger method and from 44 to 657
 204 with the exc. soil/mound method.

205 Even the pooling of all methods, i.e. the use of as many charcoals as possible, did not allow to reach
 206 an asymptote.

207 Sampled-based curves sometimes gave a much higher predicted diversity than individual-based
 208 curves: 50 vs 40 for site 2 and 37 vs 18 for site 3 with the auger method. Consequently, this led to a
 209 very high estimated sampling effort of 331 auger cores for site 2 and 168 auger cores for site 3.

210 Sampled-based curves of sites 1 and 9 presented a predicted diversity closer to that of individual-
211 based curves: 18 vs 17 for site 1 and 25 vs 21 for site 9. The estimated sampling effort for these two
212 sites was consequently much lower than for sites 2 and 3 with 54 auger cores for site 1 and 74 auger
213 cores for site 9.

214 3.5. Percentage of taxa found exclusively in one or another method

215 At each site, between 5 and 15 % of the taxa were only found through the exc. soil/mounds method
216 (Fig. 6 and Table A.5). In sites 1, 3 and 9, 30 to 40% of the taxa were only found in the pit and 18 to
217 21% were only found in the auger cores. Site 2 shows a different pattern with 50% of the taxa found
218 only in auger cores and 23% found only in the pit.

219 4. Discussion

220 4.1. Complementarity of sampling methods

221 The observed diversity was lower with the auger method than with the pit method at three sites out
222 of four. The low number of taxa in most auger cores could explain this difference. Indeed, we noticed
223 during charcoal identification that many auger cores contained either a single charcoal and
224 consequently a single taxon or a majority of charcoals belonging to the same taxon: nearly 34 % of
225 the auger cores containing charcoals were monospecific and 25 % contained only one charcoal
226 fragment. This is probably due to the small soil surface covered by an auger core (ca. 40 to 60 cm²
227 depending on devices), which increases the probability of sampling a single individual (e.g. a piece of
228 trunk). Therefore, despite a better spatial coverage on a 1 ha area, the fact that we get few taxa in
229 each auger core did not help to have a higher taxonomic richness than with pits. Only the augers
230 from site 2 allowed to recover a better observed diversity. At this site, the pit assemblage was
231 dominated by two taxa counting together for 97% of all charcoal pieces. In this case, the spatially
232 extended coverage of augers allowed to better catch taxonomic diversity: 50% of the taxa found at
233 this site were exclusive to augers (Fig. 6). In the other sites, the auger method also allowed to recover
234 new taxa that we had not found in pits. In sites 1, 3 and 9, between 18 and 21 % of the taxa were
235 found exclusively in augers. There is a need for other methodologic studies to compare our results.
236 Indeed, the auger method is rarely used in pedoanthracology (Feiss et al. 2017) and particularly in
237 the tropics where we did not find any studies using this method, except for prospection (Tardy 1998;
238 Hubau et al. 2012; Bourland et al. 2015; Dotte-Sarout and Kahn 2017). The exc. soil/mound method
239 also allowed to discover new taxa that we did not recover with the two other methods. This
240 opportunistic method is not intended to replace the two others because of the bias induced by a
241 naked eye collect. However, our results show that it can be used additively to obtain a
242 supplementary qualitative information. The variability of the observed diversity obtained with this
243 method depends on the presence of monospecific lenses. At site 2, we reached an asymptote
244 because of a high amount of charcoal fragments from the same individual (probably a piece of trunk
245 or branch which was broken during digging). At site 9, we obtained a lower observed diversity than
246 with the excavated soils from site 1 and 3 despite a better spatial coverage (7 sampled mounds)
247 because of the overrepresentation of some taxa.

248 These results suggest that these three methods are complementary for the recovery of charcoal > 4
249 mm. With the pit method, which is a very local sampling point, there is a risk to dig at the location of
250 a monospecific lens of charcoal which can correspond to a single individual (cf. site 2). A similar issue
251 has been pointed by Scheel-Ybert et al (2003), in a site at the ecotone between *cerrado* and semi-
252 deciduous forest in Brazil. In trenches of 1.0 m x 2.0 m large and up to 2.4 m depth, a maximum of 15
253 taxa were identified. One can be surprised by such poor taxonomic diversity in a highly diverse
254 floristic environment, but the authors point out that trees burn were they stand, especially during a
255 natural fire, explaining the feeble amount of taxa discovered in a single sampling point. This could

256 explain what we observed at site 2. In this case, a broader spatial sampling is required to overcome
257 the overrepresentation issue. Even if auger cores are often monospecific and contain few charcoals,
258 almost every new core brings in a new taxon. At site 1, 54 % of the taxa recovered in augers cores
259 were found only once (i.e. in only one core) and this figure reaches 75 % at site 2 and 71 % at sites 3
260 and 9. At sites 2 and 3, characterized by many rare taxa, this resulted in a high predicted diversity
261 and a high estimated sampling effort (sample-based curves) because each new auger core is likely to
262 contain a new taxon. Collecting charcoals on mounds can also allow to increase the taxonomic
263 diversity of a charcoal assemblage of a site (see Tardy 1998), but one has to be careful not to pick
264 only the largest charcoal fragments which can come from the same burnt individual.

265 4.2. Obtain a representative assemblage in a species-rich area

266 The minimal sampling effort estimated from our data is very high, exceeding 1000 charcoals with the
267 pit method to reach 30 taxa (site 3) and even 3000 when there is an overrepresentation of some taxa
268 in the charcoal assemblage (site 2). With the auger method, 76 to more than 1300 charcoal pieces
269 and 54 to more than 300 auger cores would be required to reach a hypothetical plateau of 17 to 30
270 taxa, depending on taxonomic richness and monospecific concentrations of charcoals. In a temperate
271 environment, where the floristic diversity is weaker, Feiss et al. (2017) estimated the minimal
272 sampling effort to 500 charcoals with the pit method and to 600 charcoals with the auger method. If
273 these figures can be achieved in temperate milieus, most of our estimated minimal sampling efforts
274 are however not realistic both in terms of number of charcoals and number of auger cores, because
275 of the time required to drill and sieve large amounts of soil. Even when considering the three
276 methods together, which can be considered as a greater sampling effort because it multiplies the
277 number of charcoals, the curves did not reach an asymptote (Fig. 5). As a matter of fact, constantly
278 increasing the sampling effort would lead to the collection of more and more taxa and would only
279 push back the hypothetical asymptote (predicted diversity).

280 With an estimated tree diversity of 1,700 native species in French Guiana (Molino et al. 2009), it
281 seems unrealistic to try to get a stabilization of the rarefaction curves, whatever the sampling
282 method under consideration. The sampling effort should simply aim to maximize the number of
283 charcoals collected, which should in turn mechanically maximize taxonomic diversity. This can be
284 achieved by combining pit and auger sampling methods, thereby overcoming their respective biases
285 (monospecific auger cores, spatially limited pits). Now, if it is not possible to estimate a minimum
286 sampling effort based on the extrapolation of rarefaction curves, then how to proceed?

287 The incapacity to reach a plateau with anthracologic data has already been observed in Brazilian
288 coastal environments (Scheel-Ybert 2002) and in tropical forests of New Caledonia (Dotte-Sarout et
289 al. 2015). In these cases, the construction of a Gini-Lorenz curve, which represents the cumulative
290 frequencies of taxa as a function of the relative taxa rank, is an alternative to assess the
291 representativeness of an anthracological assemblage (Chabal et al. 1999; Scheel-Ybert 2002). The
292 intersection between the curve and the second diagonal gives the Gini-Lorenz index which is typically
293 comprised between 28:72 and 22:78 in tropical environments (estimations based on floristic surveys,
294 Scheel-Ybert 2002). An index of 22:78 means that 22 % of the taxa are represented in 78 % of the
295 sampled individuals. When the Gini-Lorenz index of an anthracological assemblage falls within this
296 range, this assemblage can be considered as representative of the past floristic diversity, even if no
297 conclusive plateau was reached with rarefaction or accumulation curves. Therefore, the Gini-Lorenz
298 index may be a good way to assess the representativeness of anthracological assemblages from
299 French Guianan forests as well, and the minimal sampling effort may be estimated as the one
300 required to obtain typical tropical values of the Gini-Lorenz index. According to Scheel-Ybert (2002),

301 this minimal sampling effort would be of 200-300 charcoal fragments per sampling level in a pit or a
302 trench.

303 4.3. Proposal for an efficient sampling scheme

304 How can the sampling protocol be optimized to get the best taxonomic diversity? According to our
305 results, the charcoal concentration is equivalent between the pit and the auger sampling methods for
306 a same volume of sampled soil (Wilcoxon test). Therefore, both are equally efficient for the recovery
307 of soil charcoals. Nevertheless, our results also show that the two methods should be used together
308 to overcome their respective bias and to get the best picture of the past diversity on a study site.

309 The number of pits or auger cores can be adjusted according to the charcoal richness of a site.
310 Among the 6 sites compared on the basis of their charcoal concentration, whatever the sampling
311 method we saw that some are significantly richer. In these sites, it is worthy to increase the sampling
312 effort to get high diversity assemblages, but in the poorest sites, the effort should be adapted to not
313 lose too much time digging for feeble amounts of charcoals. It is very helpful to have an idea of the
314 charcoal richness of a site before starting systematic digging methods, bearing in mind that,
315 according to our experience in the Nouragues forest, drilling 20 auger cores of 60 cm-depth in a 1-ha
316 area takes about 10 hours, whereas digging and sampling an 80 x 80 pit of the same depth takes *ca.* 4
317 hours with two people. To quickly assess the charcoal richness of a site before starting any
318 excavation, prospections can be made thanks to small test-pits (Morin-Rivat et al. 2016), auger cores
319 (Hubau et al. 2012; Kahn et al. 2015; Feiss et al. 2017) and investigation of uprooted tree mounds
320 (Tardy 1998) if they are abundant in the site of interest. In French Guiana, Riéra et al. (1989)
321 estimated the windfall frequency at 0.75/ha/yr, 33% of which accounting for uprooted trees. In old-
322 growth temperate forests of Czech Republic, for example, the uprooting frequency vary from 0.6
323 (highlands) to 1.9 (mountains) (Šamonil et al. 2017). Investigation of uprooted tree mounds is the
324 fastest method to have a glimpse on charcoal distribution: we estimated that about 3 hours are
325 needed to prospect about ten uprooted mounds in a 1-ha area.

326 We found no statistical evidence to differentiate charcoal richness between sampling levels, but this
327 might be due to a low number of replicates. However, our results show that the soil layer between
328 10 and 20 cm-depth tends to be the richest one. Our charcoal assemblages were dated to the late
329 Holocene (Bodin et al. 2020); in other charcoal studies, the highest charcoal concentration
330 concerning Holocene-dated fragments is often observed in the first 50 cm. Peaks were recorded
331 between 0 and 40 cm depth in temperate zones (Carcaillet 2001; Touflan et al. 2010; Robin et al.
332 2013; Feiss et al. 2017) and between 0 and 50 cm depth in tropical ones (Piperno and Becker 1996;
333 Hubau et al. 2013; Fernandes Caromano et al. 2013; Vleminckx et al. 2014). Variations may depend
334 on the type of soil and on the intensity of soil disturbances or bioturbation. The activity of the
335 pedofauna plays a key role in the accumulation of charcoal and organic matter in the first layers of
336 the soil. In the tropics, termites, ants and earthworms mix old and recent organic matter in soil and
337 contribute to the upward reworking of the buried material (Lee and Wood 1971; Lavelle 1997; Gabet
338 et al. 2003; Topoliantz et al. 2006) which can explain the concentration of charcoal in the upper soil
339 layers. Burrows of larger animals, tree uprooting and tree root activity are also important processes
340 in the mixing of organic matter in soil (Lavelle et al. 1997; Šamonil et al. 2015). To assess the burial
341 depth of charcoal fragments, a quick prospection with auger cores can be done before a systematic
342 sampling. In our case, focusing on these charcoal-rich levels during sampling and not digging too
343 deep would have saved time to make some additional auger cores at the most interesting sampling
344 depths. In our case, most of the information given by anthracological data on past human activity
345 was contained in the first 30 cm of soil (Bodin et al. 2020). Therefore, selecting richest depths for
346 sampling can be enough informative.

347 Depending on the time available for fieldwork and site accessibility, a sampling scheme could be to
348 dig 3 to 5 pits in a site. One pit can be large enough to allow pedological description (e.g. 80 x 80 cm)
349 and the others can be smaller (e.g. 30 x 30 cm). Multiplying the number of pits would allow to
350 overcome spatial heterogeneity issues, as several studies have shown that charcoals deposit
351 heterogeneously in tropical (Vleminckx et al. 2014; Morin-Rivat et al. 2016), boreal (Ohlson and
352 Tryterud 2000) and temperate environments (Eckmeier et al. 2007; Touflan and Talon 2009). If
353 logistical limitations prevent the digging of several pits, then each profile of a same pit can be
354 searched for charcoals to increase the number of samples. Then the sampling can be supplemented
355 by auger cores on a grid superimposed on pits, the spatial coverage of which may depend on the size
356 of the site and its topography, or could be adapted to match that of an existing floristic survey
357 (generally 1 ha for tree inventory plots). As recommended by Feiss et al. (2017) in temperate forests,
358 the auger cores should be sufficiently spaced, i.e. a few meters, to avoid spatial autocorrelation. The
359 number of auger cores can be adapted to vegetation constraints and accessibility (especially in
360 tropical forests). It may also be adjusted according to the volume of soil collected from pits for
361 comparison purposes, as both methods are equally efficient for charcoal recovery.

362 5. Conclusion

363 Our study showed that the two digging methods that we compared – pit and auger sampling – each
364 have their biases, so it can be problematic to choose only one, at least in highly diverse tropical
365 environments. Digging a single pit can be risky, because it may contain an assemblage in which a few
366 taxa are over-represented, an issue than can only be detected after the identification phase in the
367 lab. However, we found that auger cores contained fewer taxa than pits despite a higher sampling
368 coverage, as many cores were monospecific. Nevertheless, our results showed that the two methods
369 are complementary because they allowed to collect different taxa. We therefore suggest combining
370 the two methods to get as diverse anthracological assemblages as possible, by digging several pits to
371 overcome spatial heterogeneity issues and by drilling cores to get a better representativeness of the
372 taxonomic diversity in the tropical forest. The opportunistic harvesting of charcoals during digging or
373 on uprooted tree mounds can also be used to increase the pool of taxa in the anthracological record.
374 This is also a very quick method to get an insight into the charcoal richness of a site, which can be
375 helpful to adjust the sampling effort with the two digging methods.

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538 [Supplementary material](#)

539 Table A.1: charcoal concentration and number of identifiable taxa recovered in each site investigated
540 with the pit and the auger sampling methods, with respect to the sampling depths and to the volume
541 of soil sampled

	Pits	Auger cores
--	------	-------------

Site	Depth (cm)	Vol. (L)	Charcoal concentration (nb _{tot} /L)	Nb of identifiable taxa	Vol. (L)	Charcoal concentration (nb _{tot} /L)	Nb of identifiable taxa
site1	10	10	2,8	10	4,2	1,67	2
	20	10	12,8	22	4,2	2,62	6
	30	10	1,3	9	4,05	0,99	4
	40	10	0,6	5	3,9	0,51	1
	50	10	1,1	4	2,85	0,35	0
	60	10	0,4	2	1,65	4,85	4
site2	10	10	26,2	3	5,1	2,94	3
	20	10	5,1	8	5,1	5,69	7
	30	10	8	2	5,1	2,75	5
	40	-	-	-	4,95	4,44	7
	50	-	-	-	2,85	3,51	5
	60	-	-	-	0,6	8,33	1
site3	10	10	0,6	4	3,75	1,87	1
	20	10	6,3	12	3,75	16,27	4
	30	10	7,3	10	3,75	2,13	4
	40	10	2,4	4	3,75	2,67	5
	50	10	0,3	3	3,45	1,74	4
	60	-	-	-	3,45	2,03	2
site6	10	10	0	0	4,95	0,81	2
	20	10	0,6	3	4,95	11,92	6
	30	10	0,5	3	4,95	1,41	2
	40	10	0	0	4,05	0	0
	50	10	0	0	3,75	0,8	1
	60	-	-	-	1,5	1,33	1
site7	10	10	0	0	-	-	-
	20	10	0	0	-	-	-
	30	10	0,3	3	-	-	-
	40	10	0	0	-	-	-
	50	10	0,4	2	-	-	-
site8	10	10	0,5	3	0,9	1,11	1
	20	10	2,6	7	0,9	0	0
	30	10	0	0	0,9	0	0
	40	10	0,1	1	0,9	0	0
	50	-	-	-	0,9	0	0
	60	-	-	-	0,75	0	0
site9	10	10	8,9	11	5,1	0,78	2
	20	10	4	12	5,1	3,14	4
	30	10	2,4	7	5,1	3,14	9
	40	10	2,1	5	5,1	0,78	2
	50	10	1	5	5,1	0,20	1
	60	10	0,3	2	4,2	0,24	0

site10	10	-	-	-	4,8	1,46	1
	20	-	-	-	4,8	1,25	6
	30	-	-	-	4,8	2,08	6
	40	-	-	-	4,8	2,92	3
	50	-	-	-	4,8	1,67	2
	60	-	-	-	4,8	1,04	2

542

543 Table A.2: number of charcoals and number of identifiable taxa recovered in excavated pit soils and
544 tree uprooting mounds

Site	Type of sampled feature	Total nb of charcoals	Nb of identifiable taxa
Site 1	Excavated soil	67	18
Site 2	Excavated soil	163	5
Site 3	Excavated soil	67	10
Site 6	Excavated soil	7	1
Site 7	Excavated soil	1	1
Site 8	Excavated soil	11	5
Site 9	Mounds (x7)	23	8
Site 10	Mounds (x10)	63	7
Site 11	Mounds (x14)	60	15
Site 12	Mounds (x3)	52	3

545

546 Table A.3: charcoal concentrations (median \pm se) considering the pit and auger sampling methods
547 together and separately. The letters indicate the significant differences according to the Conover
548 post-hoc test for the pit + augers comparison and to the exact all-pairs comparisons test for the pit
549 and augers comparisons ($p < 0.05$)

Site	Pit and augers	Pit	Augers
1	2.14 \pm 1.84 ^{ab}	2.80 \pm 3.61 ^{ab}	1.33 \pm 0.69 ^{ab}
2	5.39 \pm 3.64 ^a	8.00 \pm 6.60 ^a	3.98 \pm 0.87 ^a
3	4.22 \pm 2.37 ^{ab}	6.30 \pm 2.09 ^{ab}	2.08 \pm 2.37 ^a
6	0.70 \pm 1.89 ^{ab}	0.50 \pm 0.19 ^{ab}	1.07 \pm 1.85 ^{ab}
7	--	0.00 \pm 0.10 ^b	--
8	0.25 \pm 0.42 ^b	0.50 \pm 0.80 ^{ab}	0.00 \pm 0.19 ^b
9	3.14 \pm 1.12 ^{ab}	4.00 \pm 1.96 ^{ab}	0.78 \pm 0.57 ^{ab}
10	--	--	1.56 \pm 0.28 ^{ab}

550

551 Table A.4: charcoal concentrations (median \pm se) of sites 1, 2, 3, 6, 8 and 9 in each sampling depth
 552 considering the pit and auger sampling methods together and separately

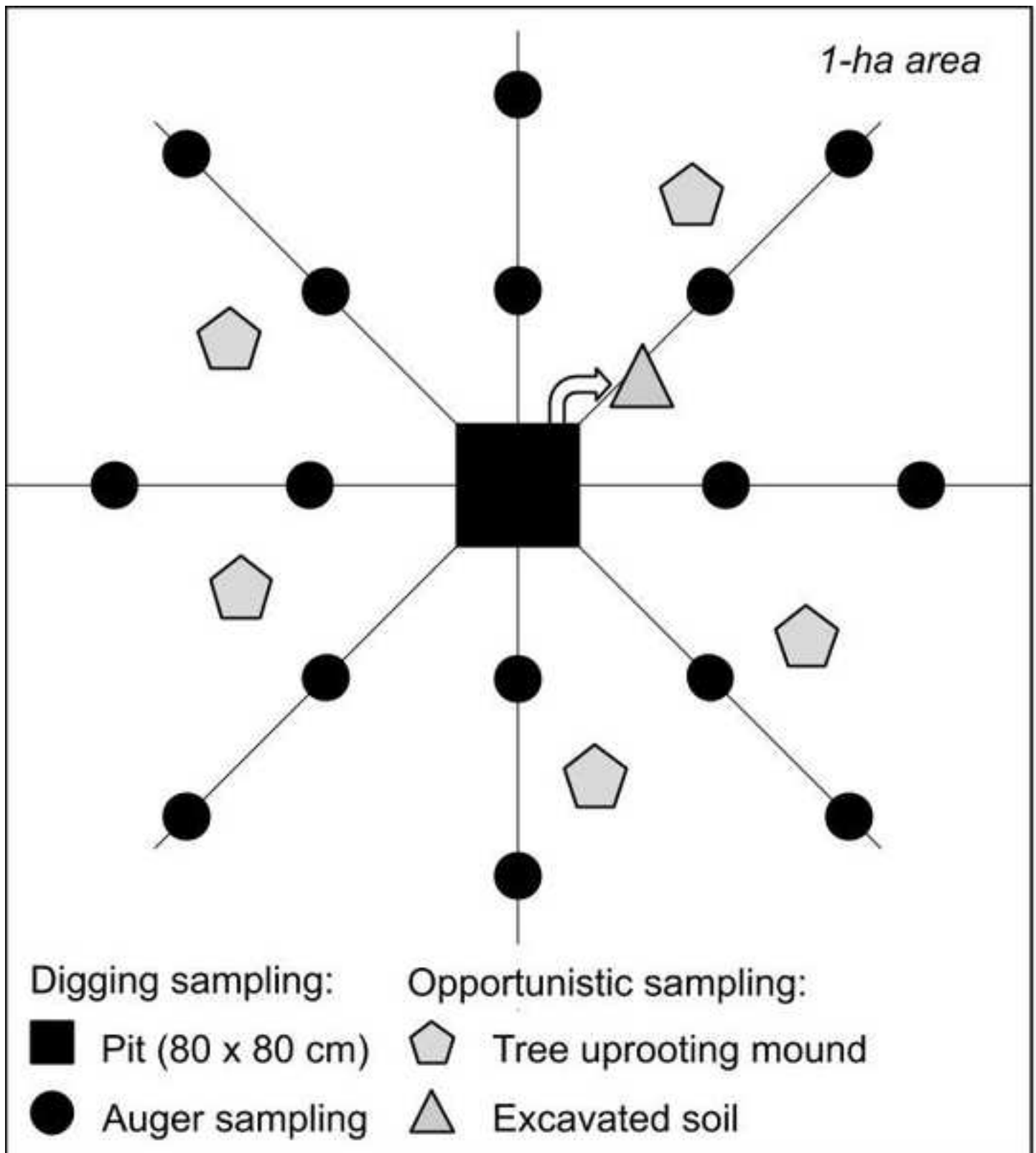
Sampling depth (cm)	Pit and augers	Pit	Augers
0-10	1.39 \pm 2.13	0.60 \pm 3.64	1.46 \pm 0.28
10-20	4.55 \pm 1.48	4.00 \pm 1.63	3.14 \pm 2.28
20-30	1.77 \pm 0.76	1.30 \pm 1.28	2.08 \pm 0.41
30-40	--	--	0.78 \pm 0.65
40-50	--	--	0.80 \pm 0.47
50-60	--	--	1.33 \pm 1.14

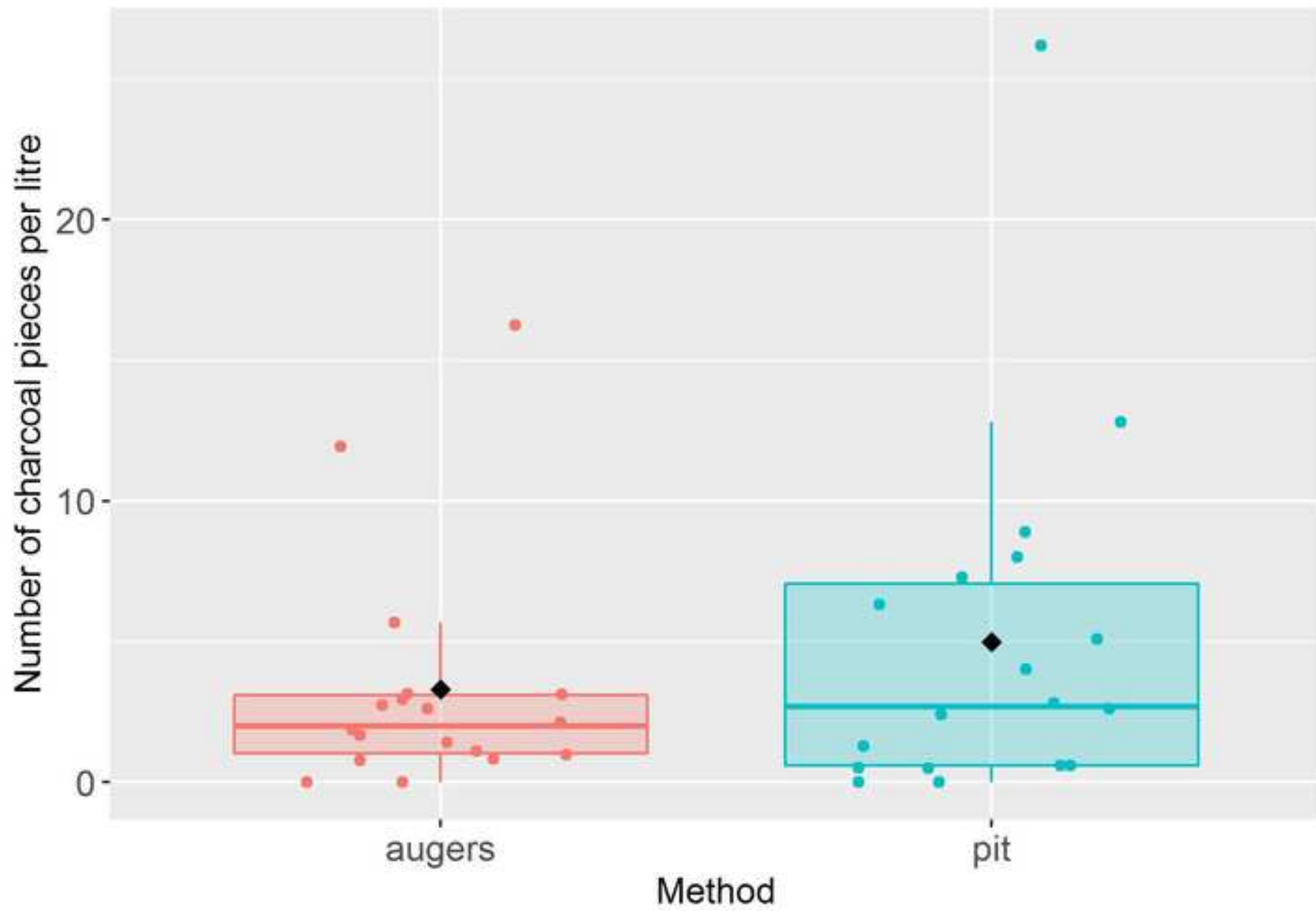
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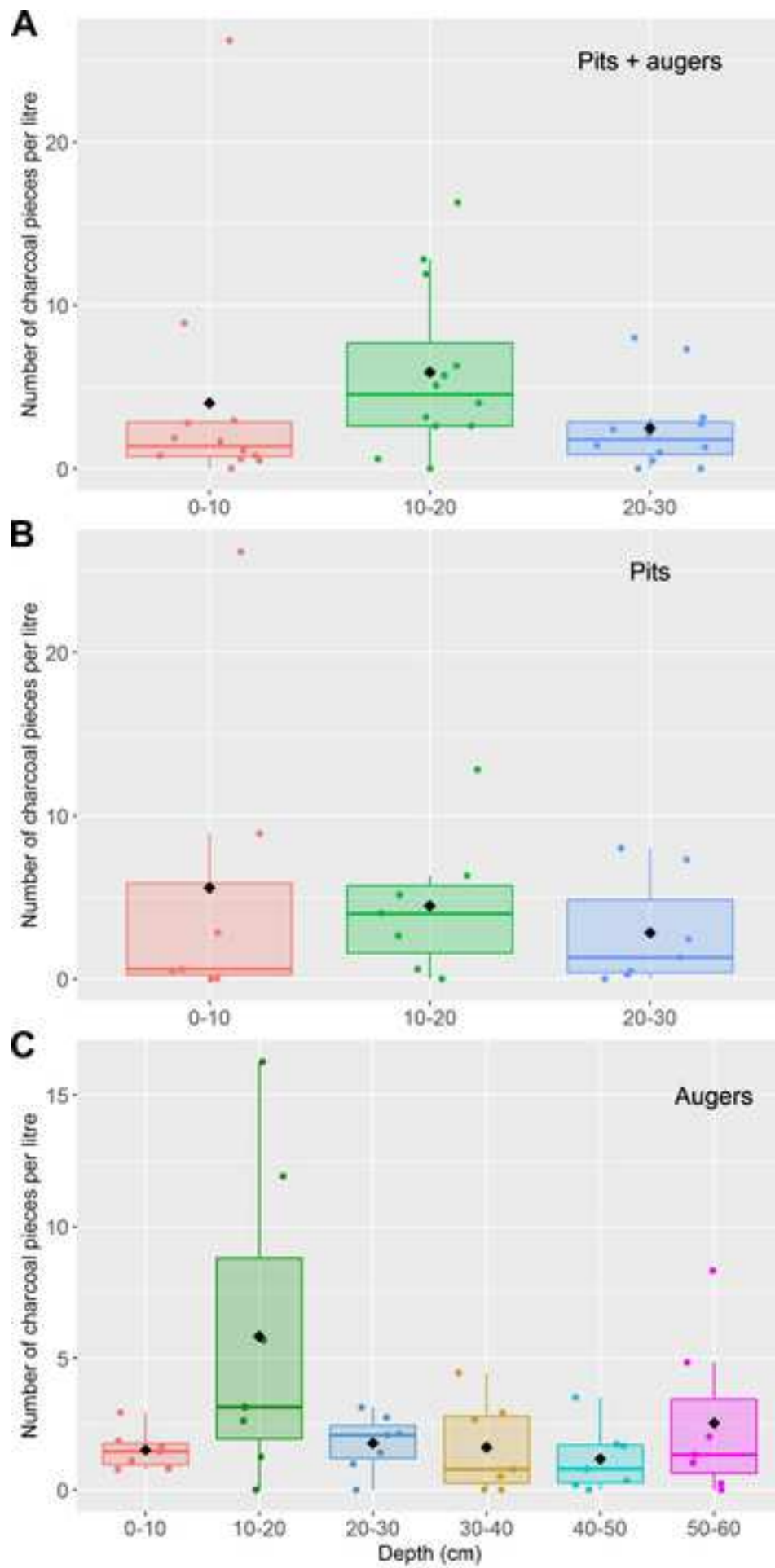
554 Table A.5: number of exclusive taxa recovered with each sampling method for sites 1, 2, 3 and 9

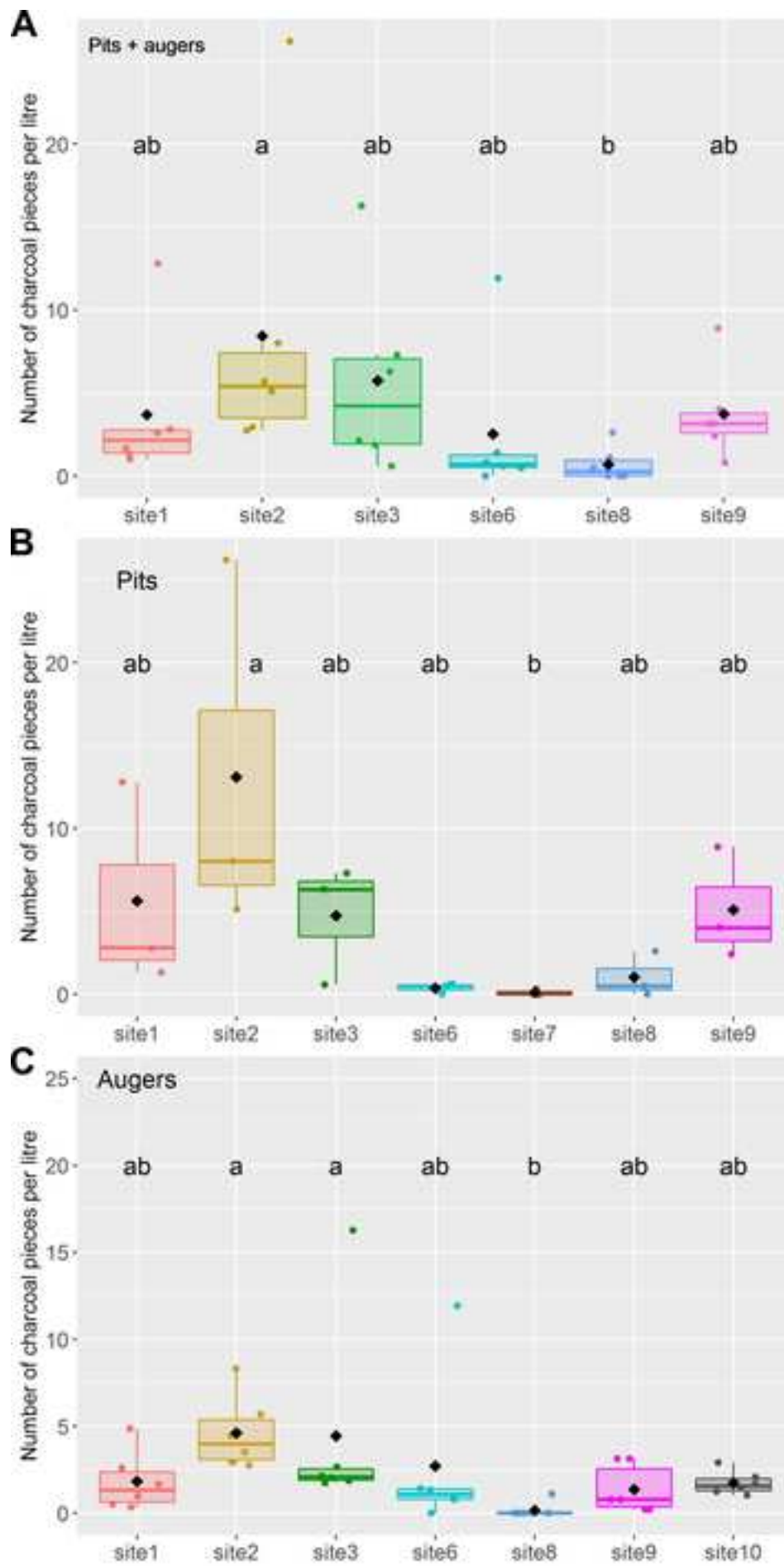
	Site 1	Site 2	Site 3	Site 9
Exc.soil/mounds	6	3	4	2
Pit	12	6	9	18
Augers	7	13	6	9
Total nb. of taxa	39	26	30	43

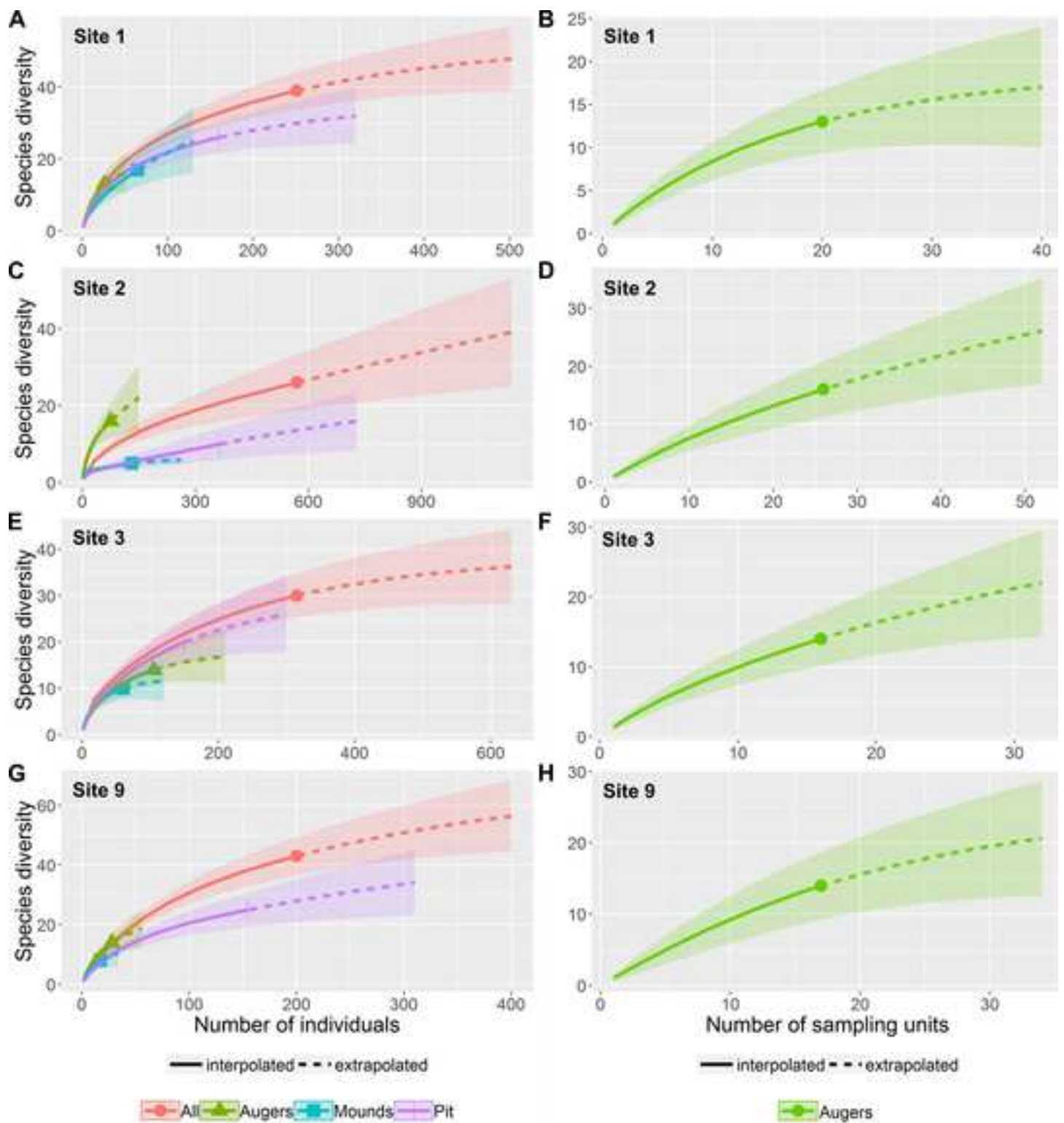
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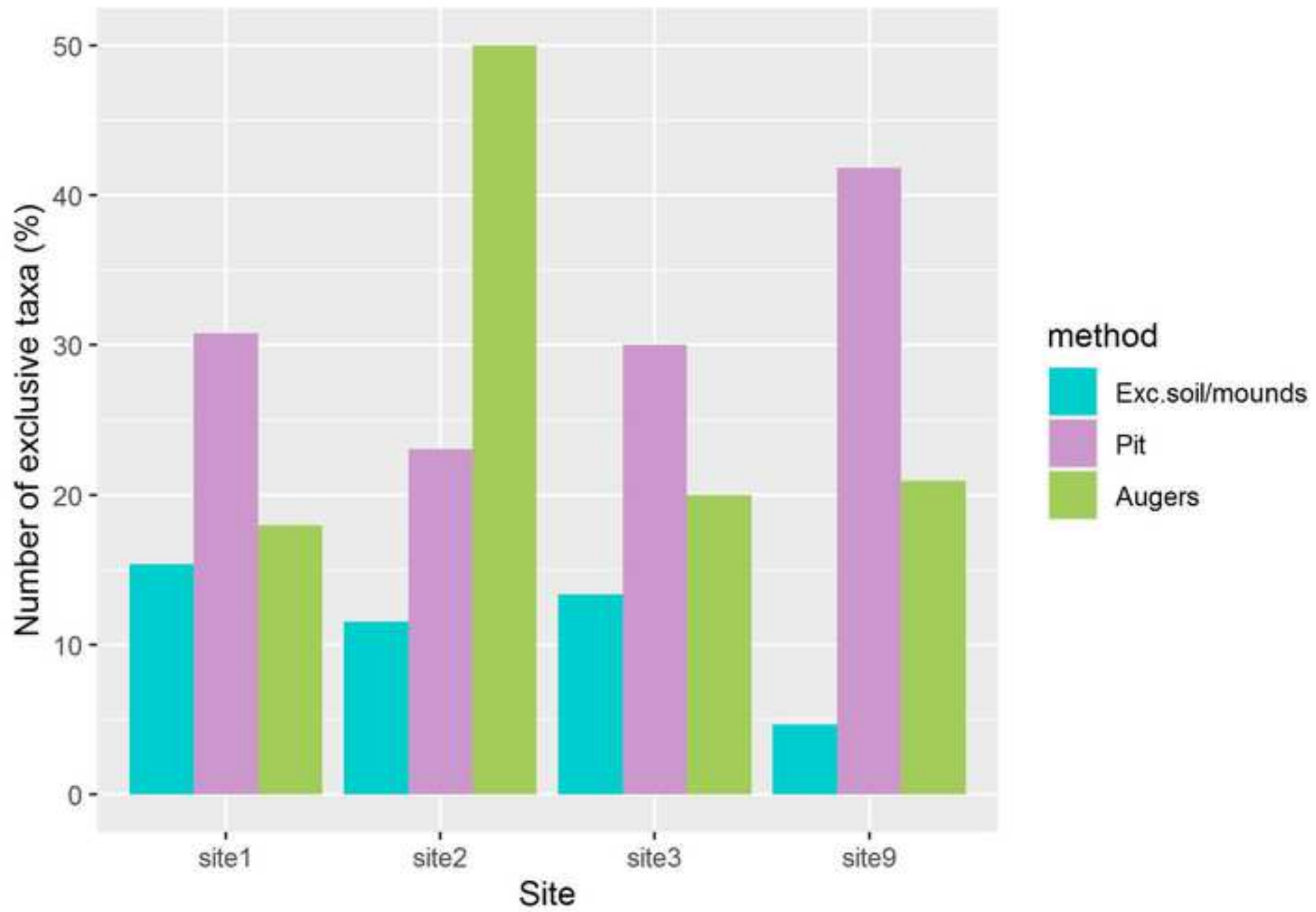












Captions

Figure 1: Sampling design representing the different sampling methods used for the recovery of charcoal in this study. Distance between auger samplings differed between sites depending on vegetation and archaeological limitations. The spacing between samples was managed to cover a 1-hectare area. The soil excavated during the digging of the pit and tree uprooting mounds were searched for charcoal fragments in some sites. As charcoals were searched with the naked eye with these two methods, they are considered opportunistic.

Figure 2: Charcoal concentrations with pit and auger sampling methods. Black diamonds indicate the means.

Figure 3: Charcoal concentrations in different sampling levels, for pit and auger sampling methods together (**A**); pit sampling alone (**B**); auger method alone (**C**). Black diamonds indicate the means.

Figure 4: Charcoal concentrations in sites investigated with pit and auger sampling methods (**A**); pit method only (**B**) and auger method only (**C**). Letters in diagrams indicate significant differences between sites according to the Conover test (**A**) and to the exact all-pairs comparisons test (**B** and **C**). Black diamonds indicate the means.

Figure 5: Rarefaction curves with 95% confidence intervals for the most charcoal-rich sites 1, 2, 3, and 9 according to each sampling method and to all methods together ("All"). Left panel: individual-based rarefaction curves; right panel: sample-based rarefaction curves (sampling unit = one auger core). **A** and **B**: site 1; **C** and **D**: site 2; **E** and **F**: site 3; **G** and **H**: site 9.

Figure 6: Percentage of the total number of taxa recovered exclusively by a sampling method at sites 1, 2, 3 and 9.