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1 **Conservation tillage influences soil structure, earthworm communities and wheat root**
2 **traits in a long-term organic cropping experiment**

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10
11 **Keywords**

12 Tillage gradient, soil profile, soil layers, penetration resistance, soil clods, earthworm
13 populations, roots

14
15 **Abstract**

16 **Background and Aims:** Organic farmers are showing increasing interest in using
17 conservation tillage to improve the biological activities of soils. Here, we assessed whether
18 conservation tillage in organic farming improves earthworm populations, root growth and soil
19 physical quality in a sandy loam after 16 years of experiment.

20 **Methods:** We compared the effect of a tillage gradient, with of two non-ploughed treatments
21 (superficial tillage [ST] at 15 cm; very superficial tillage [VST] at 5–7 cm) and two ploughed
22 treatments (moldboard ploughing [MP] at 30 cm; shallow moldboard ploughing [SMP] at 20
23 cm). Soil clod types, penetration resistance, abundance and activity of earthworms, root traits
24 and biomass were assessed.

25 **Results:** VST decreased soil compaction in topsoil (0 to 10 cm) compared to ploughed
26 treatments (MP and SMP), but led to more compacted soil at 15 to 30 cm. Earthworm

27 biomass (especially anecic) was higher under VST compared to MP and SMP and their
28 galleries were better connected to the soil surface. However, there was no significant
29 difference in the total volume of pores or diameter of galleries between 0 and 30 cm. Soil
30 compaction in the non-ploughed treatments affected root traits, especially under VST, with
31 lower specific root length, higher root diameter, and lower root tip elongation compared to
32 MP and SMP.

33 **Conclusion:** Biological activity did not compensate for the compaction of a sandy soil after
34 16 years without ploughing in organic farming. A more integrated approach (i.e. considering
35 all 5 soil health principles) is needed to sustain soil health and functions, and meet current
36 expectations about “ecological intensification”.

37

38 **Abbreviations**

39 CA: Conservation Agriculture; OF: Organic Farming; MP: Traditional Moldboard Ploughing;
40 SMP: Shallow Moldboard Ploughing; ST: Superficial Tillage; VST: Very Superficial Tillage;
41 SRL: Specific Root Length; RD: Root Diameter

42

43 **Acknowledgments**

44 We thank M. Claude Barber, a French organic farmer, who allowed us to use a field and
45 participated in this study over 16 years.

46

47 1. Introduction

48 Globally, conservation tillage (CT) is being increasingly promoted, and involves no-till,
49 permanent soil cover, and diversified crop rotations (Kassam *et al.*, 2022). CT preserves
50 topsoil fertility (Peigné *et al.*, 2018; Soane *et al.*, 2012), improves soil aggregate stability
51 (Chabert and Sarthou, 2020), and reduces soil degradation (such as slaking and erosion).
52 Reducing soil tillage and stopping soil inversion (due to ploughing) minimizes the impacts on
53 habitats of microorganisms and macro-organisms by avoiding mechanical destruction of
54 galleries and aggregate stability. Cover cropping provides energy for food webs; thus, more
55 macro- and microorganisms inhabit CT compared to other soil systems (Henneron *et al.*,
56 2015). CT is also a technique that can increase soil C storage compared to ploughing
57 (Young *et al.*, 2021). However, this effect is not always confirmed when considering the soil
58 as a whole. Most studies showed an increase in C storage in the top soil, but no effect, or
59 even a loss of storage at greater depths (Bohoussou *et al.*, 2022; Krauss *et al.*, 2022).

60 These benefits of CT have generated interest in this practice by many farmers
61 worldwide (Kassam *et al.*, 2022), including organic farmers in Europe (Casagrande *et al.*,
62 2015). Organic farming is based on several principles. The first of which is the principle of
63 health with the non-use of chemically synthesized pesticides and fertilizers (IFOAM, n.d.).
64 This translates into farming techniques such as the addition of fresh organic matter to supply
65 plants with nutrients, and the use of mechanical tools to regulate bio-aggressors such as
66 weeds and pests. Thus, organic farmers looking into CT practices must overcome technical
67 and agronomical challenges to regulate weeds, bury fresh organic matter and maintain soil
68 porosity with less or without tillage (Peigné *et al.*, 2007). The second principle of organic
69 farming relies on the improvement of soil organisms' abundance through more diversified
70 crop rotations, use of organic fertilizers, and the absence of synthetic pesticides (IFOAM,
71 n.d.). Therefore, organic farming provides a favorable ground to study how organisms can
72 regulate pests and remediate soil structure in the context of tillage reduction (Peigné *et al.*,
73 2007).

74 Different ways to combine OF with CT have been widely explored in the last decade
75 (Krauss *et al.*, 2018) mainly with agronomic trials comparing the effects of ploughed and non-
76 ploughed techniques in OF on different parameters of soil quality and agronomic
77 performances (weed control, crop yields). Benefits from non-ploughed techniques were
78 observed in the first few centimeters of topsoil. In particular, C and nutrients (N, P, K) content
79 were higher (Peigné *et al.*, 2018), microbial activity was greater (Krauss *et al.*, 2020), and soil
80 density was trending lower (Peigné *et al.*, 2018). However, crop yields tend to be lower in the
81 non-ploughed systems of OF (Cooper *et al.*, 2016). Several factors might contribute to this
82 phenomenon, including competition with weeds, which is difficult to mitigate without
83 herbicides and tillage. Increased topsoil compaction below tillage depth has also been
84 reported for different soil types (Cooper *et al.*, 2016) in both OF and conventional farming
85 (Saone *et al.*, 2012). Peigné *et al.* (2018) showed that the topsoil of sandy loam soil was still
86 denser after 10 years of superficial tillage in OF compared to ploughed treatments. Kraus *et*
87 *al.* (2018) also recorded a denser soil layer at 10 and 20 cm depth, below the tilled layer.

88 Without tillage, only abiotic and biotic processes prevent and/or remove soil
89 compaction (Drewry, 2006). In clay soils, successive cycles of wetting and drying of the soil
90 (e.g., dry periods or in winter with frost) lead to the swelling and shrinking of clay, creating
91 porous cracks. Roots and soil organisms, with a large influence from earthworms, also help
92 to maintain and create porosity (Wendel *et al.*, 2022). However, a significant abundance and
93 activity of soil organisms and roots is required to maintain or increase soil porosity. Several
94 studies showed that more soil organisms, such as earthworms are present in CT (Pelosi *et*
95 *al.*, 2014), including under OF (Kuntz *et al.*, 2013). However, these results vary with soil
96 cover, soil type and tillage practices (Peigné *et al.*, 2009).

97 Few field-based studies have considered simultaneously earthworm communities,
98 macropore building and root exploration. A long-term experiment by Capowiez *et al.* (2009)
99 showed that more anecic earthworms were present under reduced tillage compared to
100 ploughing. This increase was correlated with the increase of macropores at greater soil
101 depth, compensating higher bulk density at this soil depth. However, the magnitude of root

102 exploration changes in no-ploughed systems remains unquantified. To enhance the
103 understanding of this phenomenon, root biomass measurements provide a clear indication of
104 the quantity of plant belowground tissues, and provides information on plant nutrient-water
105 acquisition capacity, interactions with soil organisms, and litter inputs to the soil. The vertical
106 distribution of roots also provides evidence of the rooting depth, indicating potential issues
107 with soil structure when roots fail to penetrate a given soil layer. Finally, root morphological
108 traits (e.g., specific root length, root diameter, root tip elongation) are affected by the
109 mechanical resistance of soil to deformation, providing useful information on soil exploration
110 constraints and mechanical stress. In our study, root traits represent 'response traits' which
111 means that their adaptation to the soil is evaluated, rather than their potential effect on it
112 ('effect traits').

113 Thus, determining how no-till affects soil structure and biological activities
114 (earthworms, roots) would provide an opportunity to inform scientists and farmers about
115 practices suitable to combine tillage reduction and organic farming, where nutrient availability
116 is especially important to maintain yields. To address this question, we benefited from a long-
117 term experiment established in 2005, the 'THIL' trial, in which we compared conservation
118 tillage and ploughing techniques under OF conditions. Previous results, after 10 years of
119 experiment under a winter wheat crop, showed that soil fertility was better in the first 15 cm
120 soil depth with very superficial tillage, resulting in more nutrients, C, porosity and roots
121 (Peigné *et al.*, 2018). However, below this depth, soil was more compact, roots became
122 scarcer, and there was no marked effect on the abundance and activity of earthworms
123 (assessed visually). Thus, here, we aimed to explore the results of this field experiment after
124 16 years. Specifically, we sought to confirm whether the results after 10 years still held at 16
125 years. We also conducted an in-depth analysis of earthworm activity (X-ray tomography
126 analysis) and root growth (biomass and morphological traits) in order to assess the changes
127 in soil biological functionalities when ploughing is stopped.

128

129 2. Materials and methods

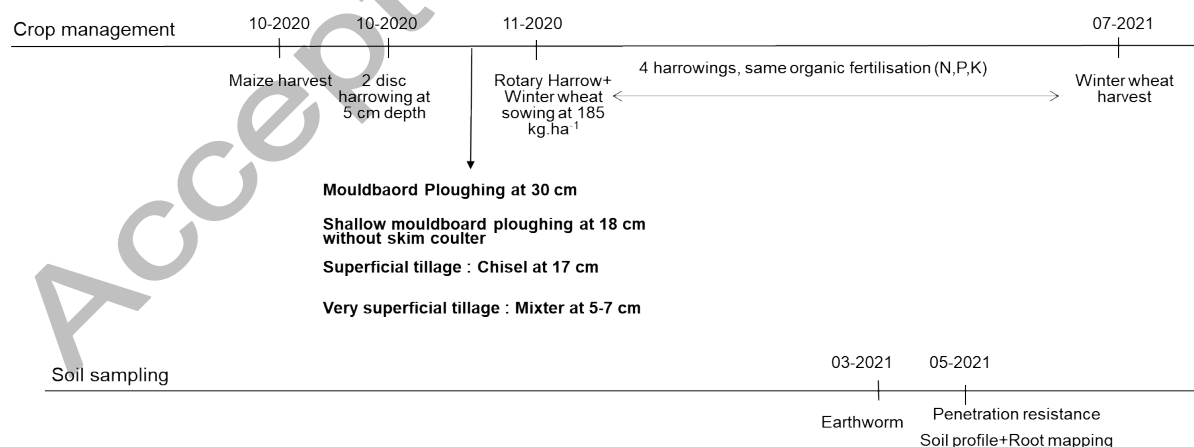
130 2.1. Experimental design

131 The “Thil” trial (45° 49’ 9.44” N and 5° 2’ 2.62” E) was set up in 2004–5 in south-eastern
132 France. The soil type is a calcareous fluvisol developing on a recent alluvium. The soil
133 texture is composed of 53% sand, 32% silt, and 15% clay; therefore a sandy loam soil. The
134 pH is 8.5. Below 60 cm, soil texture is not spatially homogeneous, due to heterogeneity in
135 sand and gravel deposits. The climate is classified as semi-continental with Mediterranean
136 influences. The mean annual temperature is 11.4°C, and cumulative annual rainfall was 825
137 mm in 2021 (20mm below the average annual rainfall for the region). The “Thil” cropping
138 system is an irrigated system with spring crops (maize and soybean), winter wheat, and
139 legumes as a cover crop. This system is representative of the organic stockless grain
140 systems found in this region. Land conversion to organic farming (EU 2092/91) started in
141 1999. Crop rotation is based on Maize (*Zea mays* L.)- Soybean (*Glycine max* L.)- Winter
142 wheat (*Triticum aestivum* L.), with cereal cover crops between maize and soybean, and
143 legume cover crops between winter wheat and maize. Soybean and maize are intensively
144 irrigated each year (around 300 mm), whereas winter wheat is irrigated according to climatic
145 conditions (from 30 mm to 100 mm). Soil measurements were performed between November
146 2004 and March 2005 on 3-year alfalfa (*Medicago sativa*, 2002–2005), just before the
147 beginning of the experiment (with a maize in 2005), to determine an initial point. Soil and root
148 measurements were performed after 10 years of the experiment (Peigné *et al* 2018). Soil and
149 root measurements were performed again after 16 years of the experiment (current study) on
150 winter wheat in 2020–2021 (at the end of the experiment, after four crop rotations).

151 The experimental design consisted of four tillage treatments, representing a tillage
152 gradient, replicated randomly three times. The experimental field of 1.5 ha contained 12
153 experimental plots, each measuring 80 x 12 m² (length x width). The plots were separated by
154 2-m-wide grass strips. All plots were irrigated. The four tillage treatments were selected
155 according to their expected effect on soil biology and soil structure, and represented a tillage
156 depth gradient. Specifically, we implemented two ploughed treatments (moldboard ploughing

157 at 30 cm depth [MP] and shallow ploughing at 20 cm with no skim coulter [SMP]), and two
 158 non-ploughed treatments without soil inversion (superficial tillage at 15 cm with a chisel
 159 plough [ST] and very superficial tillage at 5–7 cm with rotary and/or chisel tools [VST]). In
 160 2005 and 2008, direct sowing under rolled mulch was tested on VST plots (maize on rolled
 161 alfalfa in 2005 and soybean on rolled rye in 2008). The seedbed was prepared with a rotary
 162 harrow in all treatments. Weeds were mechanically destroyed by harrowing and hoeing the
 163 soil in the row crops, with four passes. Weed control was adjusted for each tillage treatment.
 164 The number of weeding passes was adjusted according to the degree of weed infestation in
 165 each treatment.

166 All of the agricultural tools on wheat were 4 m wide. Wheel tracks were located on the
 167 same zones in 2021 as in previous years. However, as the tools used on maize and
 168 soybeans were 4.80 m wide, this, combined with harvesting operations, meant that the entire
 169 plot area might have been compacted by vehicle wheels over the last 16 years. An overview
 170 of the crop management system and sampling in 2021 is provided in Figure 1. In 2021,
 171 winter wheat was irrigated before and after the soil sampling period (35 mm April 17–18, 30
 172 mm on April 25–26, and 35 mm on June 7). Rainfall of 174 mm was recorded in May 2021,
 173 before the sampling period.



174
 175 Figure 1: Crop management and sampling dates. Organic fertilization (feather mill) : 170 kg N/ha 10
 176 kg P/ha, 10 kg K/ha






177

178 2.2. Soil structure

179 2.2.1. Visual assessment of soil structure

180 To understand where and why soil compaction occurs, soil structure was
181 characterized morphologically from the observation face of a pit (1 m deep x 4 m wide),
182 following 'soil profile' methodology (Boizard *et al.*, 2017; Peigné *et al.*, 2012). In each plot of
183 the experimental trial, one pit was dug perpendicular to the wheel tracks of soil cultivation
184 machinery. The face of each pit was described macroscopically (on a vertical plane) in two
185 steps. Three soil profiles per treatment (one per replicate) were performed at the flowering
186 stage of winter wheat in May 2021.

187 First, we located all wheel tracks on the soil surface to indicate lateral stratification of
188 the soil profile. In soil profiles, 3 types of lateral stratification are observed (See figure S1):
189 the area of the profile located under the wheel tracks of field operations done after secondary
190 tillage (visible at soil surface), called L1; the area of the profile located under the wheel tracks
191 of secondary tillage operations, called L2; and part of the profile untouched by wheels since
192 the primary tillage of the growing year, called L3. Then, soil layers delimited from the working
193 depth of successive tillage tools used to prepare the seedbed for the current crop were
194 located in the soil, indicating the vertical stratification of the soil profile. The intersection of
195 vertical and horizontal stratifications naturally defines homogeneous soil compartments for
196 our study (Figure S1).

197 Secondly, we visually assessed the structural quality of every homogeneous
198 compartment previously defined. We classified clods > 2 cm into three classes depending on
199 the proportion of structural porosity visible: (1) delta () clods were clods with no visible
200 structural porosity, with prominent edges and flat surfaces, evidence of severe compaction
201 (Boizard *et al.*, 2017); (2) alpha () clods were moderately compacted clods with visible biological
202 macropores (earthworms, roots) on their flat surfaces; and (3) gamma () clods were clods
203 with a loose structure with clearly visible structural porosity. The clods had distinct physical
204 characteristics. For instance,  clods had higher bulk density compared to  clods, which
205 favor anoxic conditions (Curmi, 1988) with lower biological activity (Vian *et al.*, 2009). To
206 assess structural quality visually, we observed the spatial distribution of the three types of

207 clods in the homogeneous soil compartments (intersection of horizontal and vertical
208 stratification). Then, we calculated the percentage of area occupied by each clod type in the
209 soil (0–30 cm depth).

210 *2.2.2. Soil penetration resistance*

211 The soil penetration resistance was used to assess the state of the soil structure at
212 different depths. It was measured in zones with no evidence of wheel marks (L3 zones; axis
213 of the seeder). Soil penetration resistance was measured in May 2021 (Figure 1) at the
214 wheat flowering stage with a manual penetrometer “EIJKELKAMP”. After choosing the most
215 appropriate cone diameter (according to compaction level), the penetrometer was pushed
216 vertically into the soil at an approximate constant rate of 2 cm per second for each handle.
217 Soil resistance pressure was measured every 5 cm to 45 cm depth. The recorded resistance
218 was standardized, taking into account the cross-section area of the cone. Penetration
219 resistance was expressed in MPa. Ten randomly distributed measurements of soil
220 penetration resistance were implemented per plot, only in areas with no recent wheel tracks.
221 In total 30 measurements of soil penetration were obtained per tillage treatment (3 plots X 10
222 measurements). Gravimetric soil water content (% mass basis) was measured for every sub-
223 replicate at five soil depths (0-5, 5-15, 15-20, 20-30, and 30-50 cm) to verify that soil
224 moisture was similar between sampling zones. Moisture content averaged 14% over the 0-50
225 cm soil layer, varying from 14.5% in the first centimeters (0-5 cm) to 12.2% in the 30-50 cm
226 soil layer.

227

228 **2.3. Root growth and soil exploration**

229 *2.3.1. Root density*

230 Roots were mapped by counting roots during soil profile observations. On the face of
231 the soil profile, soil fragments were cut off with a knife to a thickness of 1 cm to refresh the
232 face and highlight the roots. A grid (70 cm wide, 1 m long) was attached to the face of the
233 soil profile (Pagès *et al.*, 1999; Pierret *et al.*, 2007). The number of roots present in each cell
234 (2 * 2 cm²) of the grid was counted and recorded, providing information on the number of

235 roots present throughout the soil profile. Root density was calculated every 2 cm, from the
236 soil surface to 70 cm soil depth, by summing the number of roots in the 35 cells of the 2-cm
237 line of the grid.

238 Root density was counted twice for three soil profiles (*i.e.*, 6 measurements per
239 treatment) at the flowering stage of winter wheat in May 2021. Grids were located on zones
240 in the soil profile without wheel tracks (*i.e.*, L3; Figure 1).

241

242 2.3.2. Root biomass and morphological traits

243 Three cores of a depth of 45 cm were collected in each plot (*i.e.* 9 samples per
244 treatment) in areas without recent wheel traffic (*i.e.*, L3, same areas where penetrometry was
245 measured). Each soil core with a diameter of 8 cm was divided into four sections to
246 distinguish between the 0–5, 5–15, 15–30, and 30–45 cm soil layers. Soil samples were
247 stored at -20 °C until further processing. Soil samples were soaked in buckets of water for 15
248 min. Then, the soil was washed off of the roots under running water over two sieves of 1 and
249 0.2 mm mesh-size (Freschet *et al.*, 2021). After the soil was removed, all roots were
250 collected from the sieves manually. Roots were then transferred and spread on a flat tray
251 filled with water to remove any remaining impurities. Roots were not sorted between live and
252 dead roots, and were all considered as absorptive roots (“fine roots”) since root diameter was
253 consistently <1mm.

254 Sub-samples of roots were taken after washing to document root morphology
255 measurements. Roots were cut and spread on a flat transparent tray to avoid any roots
256 overlapping. They were then digitized at 600 dpi with a flatbed scanner (Epson V800 Pro)
257 and analyzed using a digital image analysis system (RhizoVision Explorer, Seethepalli *et al.*,
258 2021) to obtain root length and root diameter. Root dry mass was obtained after oven-drying
259 scanned roots for 48 h at 60 °C. From these estimates, we calculated the average root
260 diameter (RD, mm) and specific root length (SRL, cm. g⁻¹).

261 For each sample, the roots not used in the sub-sample were over dried for 48h at 60°C
262 and weighed. The dry mass obtained was added to the one of sub-sample in order to get

263 total root biomass. Root length density ($\text{cm}\cdot\text{cm}^{-3}$) was obtained by multiplying root biomass in
264 the soil core section by its estimated specific root length, and dividing it by the volume of the
265 soil core section.

266 To assess root tip elongation, three undisturbed soil blocks were taken per tillage
267 treatment (one per replicate). Each soil block measured 25 cm x 20 cm on the soil surface,
268 centered on one crop row, and was 30 cm deep. These blocks were carefully washed and
269 progressively opened to extract the whole root system. Six aliquots were collected that
270 corresponded to entire branched roots from the soil surface to 30 cm depth. Each aliquot
271 was divided into three sub-aliquots to distinguish 0–5, 5–15, and 15–30 cm rooting depth.
272 Contrary to segmented roots scanned for assessing root diameter and length, the roots
273 within each sub-aliquot were maintained intact. Each sub-aliquot was spread in a flat
274 transparent tray, and was digitized with a flatbed scanner (Epson V800 Pro). Digital image
275 analysis was used to estimate the number of root tips per root centimeter from the whole and
276 branched roots that were scanned. Root tip is here defined as the root apical zone, from the
277 apex to first lateral roots. Root endings created by cutting the roots during the division of sub-
278 aliquots were identified and not considered as root tips. This provided a good overview of the
279 importance of root tip elongation and spread of root branches.

280

281 **2.4. Earthworm community and activity**

282 *2.4.1. Biomass, abundance, and density of earthworms*

283 Earthworms were sampled in plots in areas without recent wheel traffic (i.e., L3, same
284 areas where penetrometry was measured). In 2004, when the experiment was initiated,
285 earthworm biomass was sampled using the formaldehyde method, and the results were
286 published by Peigné *et al.* (2009). As this method presents a health risk to living organisms,
287 and is not in accordance with organic farming principle, earthworm biomass was sampled
288 using a hand sorting method after 2010 (Peigné *et al.*, 2018). The earthworm population was
289 sampled for the winter wheat crop in March 2021, three months before the wheat flowering
290 stage (Figure 1) when earthworms are very active. Six samples were assessed in each plot

291 (i.e. 18 samples per treatment)., on a surface of 30 cm X 30 cm and to a depth of 30 cm. To
292 sort earthworms, we simply dug the soil with a spade and worked fast enough to limit the
293 number of anecic species that escaped. We poured the soil into a container, and then
294 inspected the sides and bottom of the hole to detect any escaping earthworms. After
295 carefully sorting the soil to capture all the earthworms *in situ*, we placed them in a bowl filled
296 with fine soil. Jars containing the earthworms were placed in the shade (where it was cool)
297 and were transported to the laboratory. There, the earthworms were cleaned and fixed with
298 4% of formaldehyde, classified by ecological categories (anecic, endogeic, and epigeic) and
299 species. They were then counted and weighed (formaldehyde weight). Brown-headed anecic
300 species (*Aporrectodea giardi*, *A. longa*, and *A. nocturna*) were grouped together because
301 they occurred at relatively low abundances, it was not possible to separate the juveniles of
302 each species, and they are assumed to have more similar functional roles compared to
303 anecic *Lumbricus* species (Hoeffner *et al.* .2022).

304

305 2.4.2. Soil cores and earthworm burrow systems

306 Three cores (16 cm diameter, 25 cm depth) were collected in each plot (i.e. 9
307 samples per treatment) following the protocol described by Capowiez *et al.* (2021) with 3
308 cores in each experimental plot. In sum, soil cores were manually collected using a PVC tube
309 placed vertically on the soil. The soil around the tube was carefully excavated to a depth of a
310 few centimeters using a small hand-spade. Then, only the soil just below the PVC tube was
311 removed using a knife vertically at 1 cm intervals. After each centimeter, the tube was gently
312 inserted into the soil using a hammer on top of the PVC tube. The process continued until the
313 depth of the soil core in the PVC was 25 cm. Then, the soil below the core was horizontally
314 cut with a knife, and the core was put in a plastic bag. The macrofauna inside each core was
315 killed to prevent further burrowing by adding 3 mL chloroform.

316 The macroporosity of the soil cores was analyzed by X-ray tomography using a
317 medical scanner (BrightSpeed Exel 4, General Electric) at the INRAE Nancy Research
318 Centre. The following settings were used: 50 kV, 130 mA, and 1.25 mm between images.

319 The final image resolution was 0.38 mm per pixel. Images were first transformed into 8-bit
320 images (using 1000 and 2000 HU as minimum and maximum grey-level values,
321 respectively). The images were then binarized using a unique and fixed threshold for all
322 cores, because the separation between peaks (void and soil matrix) in the grey-level
323 histograms was easy.




324 Macroporosity was characterized using a variety of approaches. Volume was
325 calculated as the sum of the volumes of burrows. The number of burrows larger and smaller
326 than 2 cm³ were differentiated. Estimated diameter was computed as the equivalent circular
327 diameter for only the most circular 2D pores (i.e., for which circularity, computed in ImageJ,
328 was higher than 0.8). Vertical barycenter was computed as the mean of the vertical center of
329 mass of each macropore taking into account their respective volume. Vertical continuity was
330 assessed by counting the number of burrows that had a vertical extension larger than 30% of
331 the length of soil cores (i.e., 7.5 cm). The volume of macroporosity that was connected to the
332 soil surface was quantified. All computations were done using ImageJ (Schindelin *et al.*,
333 2012) and adapted macros.

334

335

336

337 **2.5. Statistical analyses**

338 The normal distribution and homoscedasticity of each variable was verified. The
339 earthworm data did not follow a normal distribution, so a Kruskal Wallis test (non-parametric
340 test) was performed (alpha = 0.05) followed by a Dunn test (alpha=0.05) to perform pairwise
341 comparisons if the Kruskal-Walis test is statically significant. For the same reason, analysis
342 of the percentages of , b and clods observed in the soil profiles was also performed
343 with a Kruskal Wallis test followed by a Dunn test. The composition of earthworm
344 communities was analyzed using a Linear Discriminant Analysis (i.e. a projection of the data
345 so that the differences between treatments are maximized). Data for burrow systems were

346 analyzed with ANOVA (after log-transformation when required). The characteristics of the
347 burrow systems were also analyzed using Linear Discriminant Analysis.

348 To analyze root density, biomass and traits of roots, along with penetration
349 resistance, two statistical analyses were performed. First, linear mixed models with
350 autoregressive errors were adjusted to account for the dependence of values with respect to
351 soil depth. As fixed effects, the interaction of tillage treatment with soil depth used a
352 polynomial function of 4 degrees (penetration resistance model) or 2 degrees (root trait
353 models, root density models). An autoregressive (order 1 covariance) structure using soil
354 depth was used. The random effects corresponded to the number of replicates. Post-hoc
355 analysis were performed with tukey's multiple comparison test ($\alpha = 0.05$). All statistical
356 analyses were performed with R software (R Core Team, 2016).

357 3. Results

358 3.1. Changes in soil compaction and root exploration across the soil profile

359 In the 0–45 cm soil layer, penetration resistance, root density, average diameter and
360 specific root length were all significantly influenced by tillage practices in interaction with soil
361 depth (Table 1). Root biomass was only affected by soil depth (Table 1).
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	Soil Penetration resistance	Root density	Root Biomass	Root average Diameter	Specific root length	Root Tip elongation
Depth	<0.0001(***)	<0.0001(***)	<0.0001(***)	<0.0001(***)	<0.0001(***)	<0.0001(***)
Tillage Treatment	0.9712	0.6305	0.6864	0.0015(**)	0.0006(***)	0.0738
Depth*Tillage Treatment	<0.0001(***)	0.0097(**)	0.2489	0.0004(***)	0.0387(*)	0.0003(***)

365 Table 1: P-values obtained from the different models testing the influence of tillage treatments and soil
366 depth on soil penetration resistance, root density, biomass, average diameter, specific root length and
367 root tip elongation.

368
369 The topsoil (0-15 cm) was characterized by the highest root biomass, regardless of
370 the tillage treatment (Figure 2a). All root traits were also similar with the exception of root
371 density which was high with VST in the first 10 centimeters but lower compared to the other
372 treatment if integrated on the depth range of 0-20 cm (Figure 3). Significant differences in
373 penetration resistance among the four tillage treatments were recorded in the surface 10 cm,
374 with higher resistance for MP compared to ST and VST (Figure 4). This result was explained
375 by the presence of smoothing at 10 cm during the passage of the rotary harrow at sowing,
376 which smoothed the soil deeply in plots that were very crumbled by ploughing.

377 Below the top soil (15-30 cm), root biomass decreased for all situations (Figure 2a).
378 Root average diameter was higher when no tillage occurred at this depth (ST, VST; Figure
379 2b). In the case of VST, specific root length was lower, as well as root tip elongation (higher
380 number of tips per root cm; Figure 2c,d) and root density (Figure 3). ST and VST also had
381 higher resistance compared to the tilled situations at 20 cm deep (MP and SMP), with VST
382 having the highest resistance (Figure 4). At 30 cm depth, MP treatment was the only
383 treatment being tilled at this depth, and remained with lower resistance values compared to
384 the other treatments (SMP, ST, VST).

385 Higher soil compaction with less tillage was also strengthened by the qualitative
386 observations of clods in the soil profiles. The main difference between soil profiles was the
387 percentage of porous clods (☐) being higher in MP (61%) compared to VST (29%; Figure 5).
388 In comparison, SMP and ST had intermediate percentages (40 and 41%, respectively).

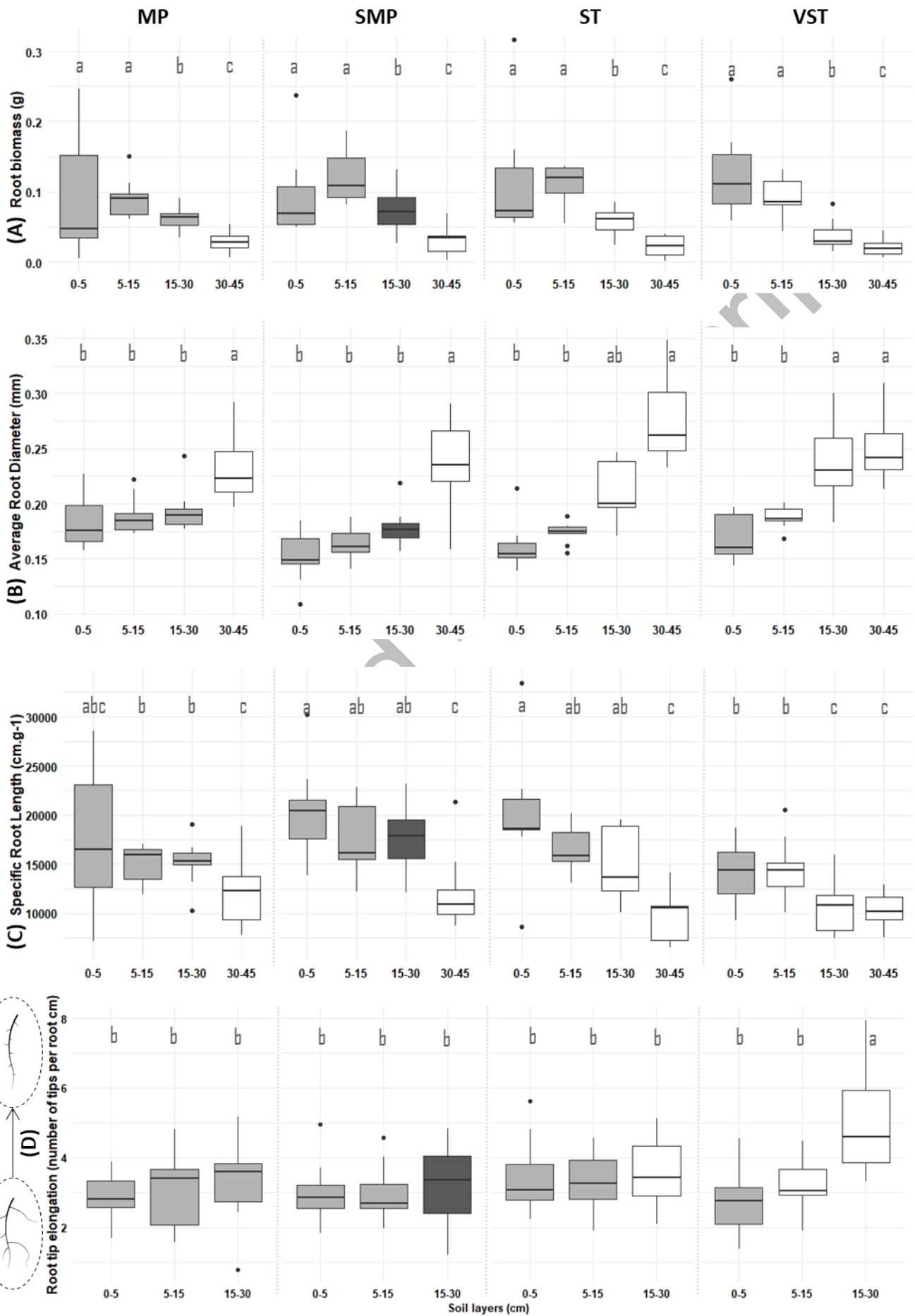
389 Below 30 cm deep, no tillage operations were performed for any plots. Root biomass was the
390 lowest and root traits were similar for all situations (Figure 6a,b,c). However, VST exhibited
391 lower resistance to penetration at 40 cm deep compared to MP (Figure 4), and showed a

392 tendency (not significant) to have higher root density compared to the more intensive tillage
393 treatments (MP and SMP; Figure 3)

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Tillage NO Transition YES

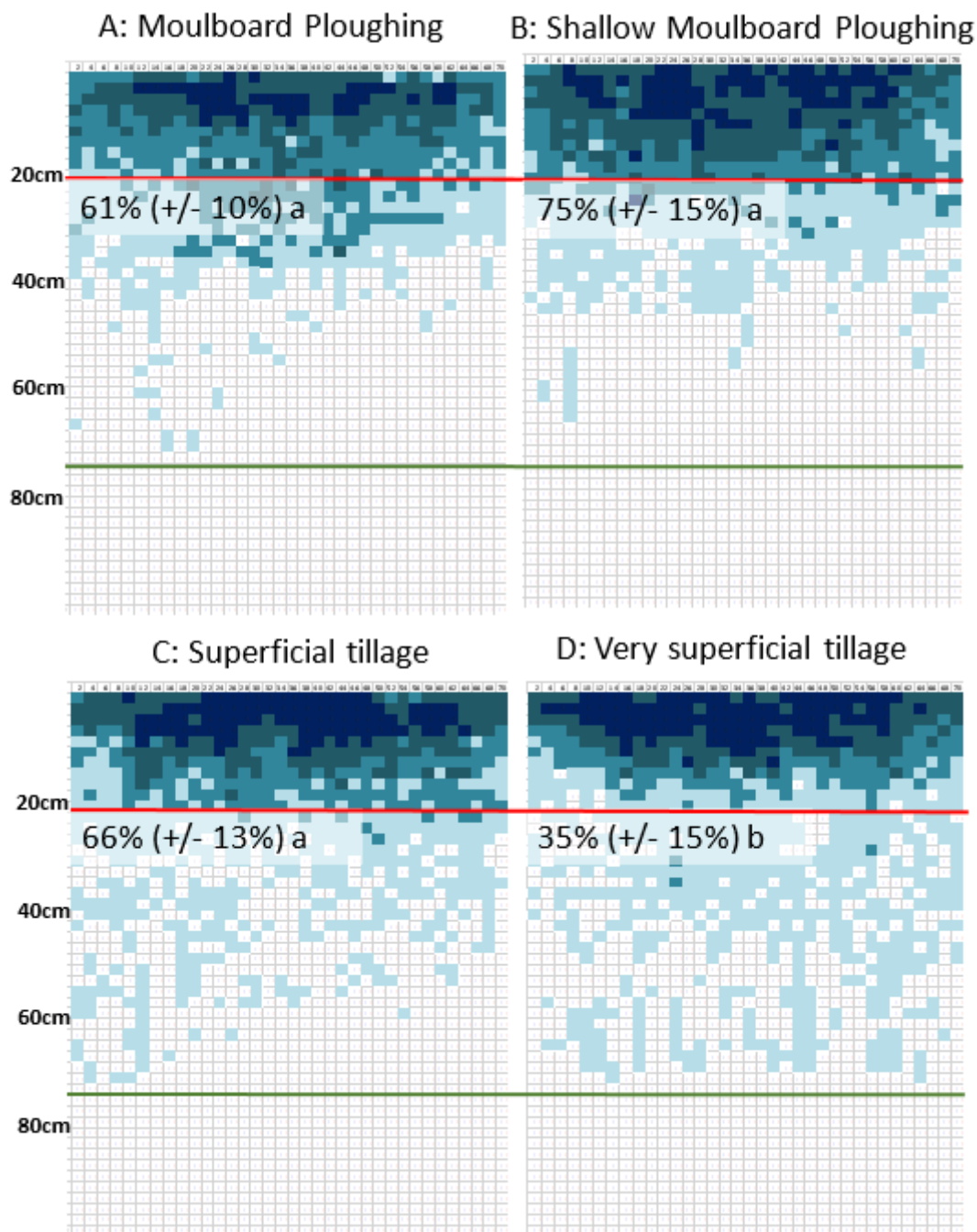


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397 Figure 2. (a) Root biomass, (b) average root diameter, (c) specific root length and (d) root tip
398 elongation (number of root tips per root cm) depending on tillage treatments (MP= moldboard
399 ploughing; SMP= shallow moldboard ploughing; ST = superficial tillage, VST = very superficial tillage)
400 and soil layers. For (d) root tip elongation, higher values mean less tip elongation. Lowercase letters
401 indicate statistical group differences between boxplots (tuckey's multiple comparison, $\alpha = 0.05$).
402 White boxplots ("NO") indicate situations with no mechanical disturbance from tillage practices, light
403 grey boxplots ("YES") indicate situations where mechanical disturbance is applied by tillage practices,
404 and dark grey boxplots ("Transition") indicates situations where mechanical disturbance does not
405 concern the whole sample, but only the upper part. This case is only found for the SMP treatment,
406 where ploughing is done until 18-20 cm deep.

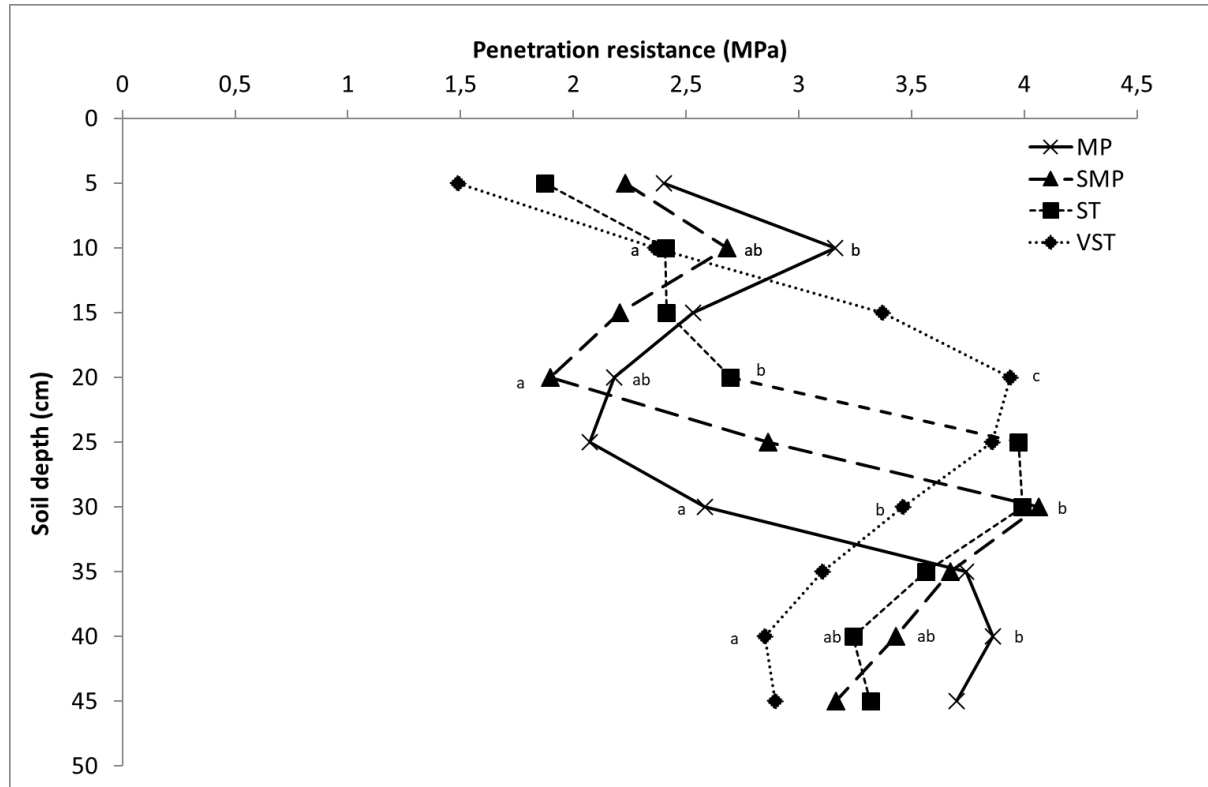
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 409 Figure 3 (A-B-C-D): Winter wheat root (mean values) maps obtained in moldboard ploughing (A),
 410 Shallow Moldboard Ploughing (B), Superficial Tillage (C) and Very Superficial Tillage (D) treatments –
 411 at the flowering stage of winter wheat in 2021. From light blue (1 root) to dark blue (more than 4 roots).
 412 Six individual values are used to calculate mean percentage (given above). Red lines indicate the
 413 depth of 20 cm where interaction between tillage treatment and depth was significant (p-value
 414 <0.0125). Lowercase letters indicate statistical group differences considering the percentage of cells
 415 between 0 and 20 cm with at least one root observation. No statistical differences were found at
 416 deeper depths. Green line the end of the rooting at 72 cm depth.
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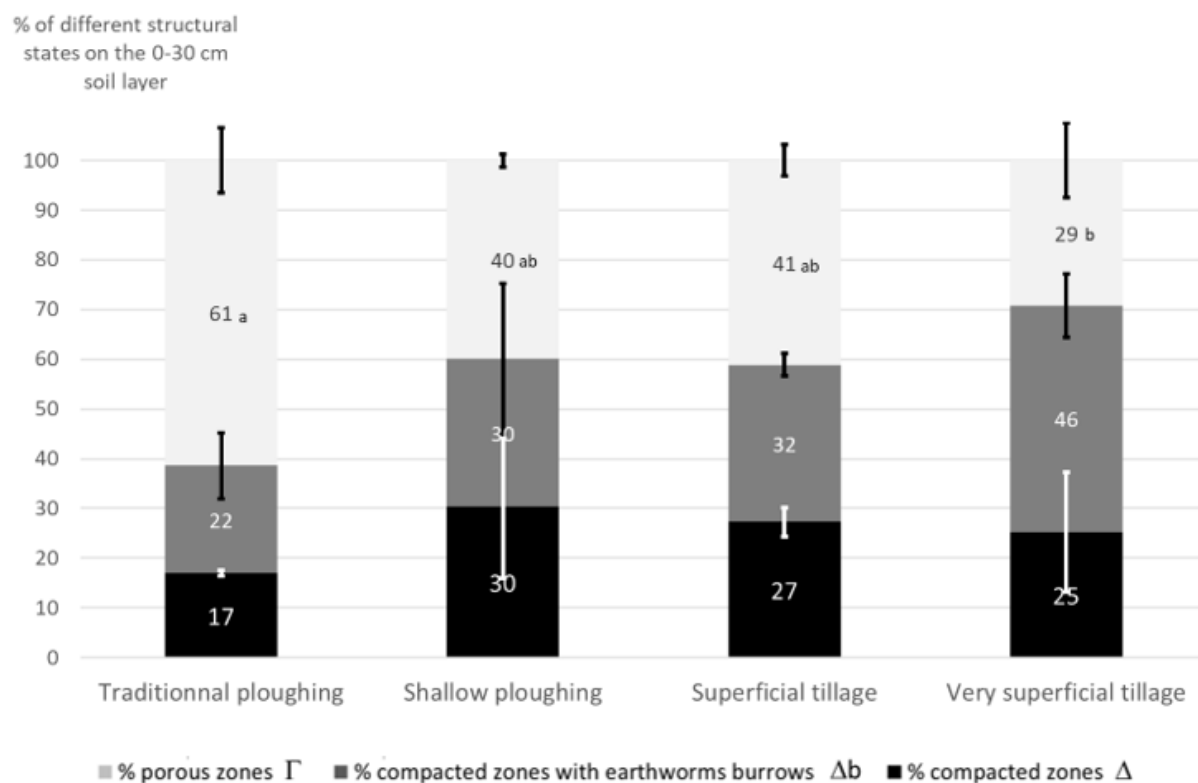
422 Figure 4: Penetration resistance in MPa of the 4 tillage treatments from 5 cm to 45 cm depth in May
 423 2021 under zones without wheel tracks. Thirty individual values are used to calculate the mean. At 10,
 424 20, 30 and 40 cm different lowercase letters indicate statistical group differences according to tuckey's
 425 multiple comparison test ($\alpha=0.05$). MP: Moldboard ploughing; SMP: shallow moldboard ploughing;
 426 ST: superficial tillage; VST: very superficial tillage.

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 432 Figure 5: Percentage of porous zones (Γ), compacted zones with earthworm burrows (Δb) and
 433 compacted zones (Δ) of the 4 tillage treatments observed in May 2021 in a soil profile (on the 0-30
 434 cm soil layer in zones without wheel track). Three individual values are used to calculate the mean
 435 and standard error. MP: Moldboard ploughing; SMP: Shallow moldboard ploughing; ST: superficial
 436 tillage; VSP: very superficial tillage. No significant difference was found with the Kruskal-Wallis test
 437 (alpha = 0.05) for compacted zones (Δ) (p.value=0,4), nor for compacted zones with earthworm
 438 burrows (Δb) (p.value=0,07). Significant differences were found with the Kruskal-Wallis test (alpha =
 439 0.05) for porous zones (Γ) (p.value=0.025), and lowercase letters indicate statistical group differences
 440 (post-hoc Dunn's test) between tillage treatments.

441 3.2. Earthworm community and activity

442 Ten species of earthworms were identified, including one epigeic species, four anecic
 443 species with three brown-headed species, and six endogeic species with one unspecified
 444 endogeic species (Table S1). The communities were different between MP and SMP on one
 445 hand and ST on the other hand (Figure S2), with the first group characterized by a higher
 446 contribution of *A. icterica* and ST characterized by a higher contribution of *A. antipai* (Table
 447 S1). Our results showed that earthworm biomass was higher for VST (67.7 g.m⁻²) compared
 448 to MP (34.9 g.m⁻²) and SMP (38 g.m⁻²) (Table 3, Kruskal Wallis p-value < 0.001), which was
 449
 450

451 consistent with more compacted clods with visible earthworm macropores (Fig. 5b) found under
 452 VST (46%) compared to MP (22%) (Figure 5). The difference in earthworm biomass for VST
 453 was attributed to greater anecic biomass (Kruskal Wallis p-value < 0.001) and total adult
 454 biomass (Kruskal Wallis p-value < 0.01). The only significant difference observed for
 455 abundance was the density of total adults, with significantly higher values in VST (26.5
 456 individuals.m⁻²) compared to MP (13.6 individuals.m⁻²) and SMP (11.7 individuals.m⁻²)
 457 (Kruskal Wallis p-value < 0.05).

458

Species/adult or juvenil	Moldboard Ploughing		Shallow Moldboard Ploughing		Superficial Tillage		Very Superficial Tillage		Test Kruskal.Wallis: p.values	
	Ind.m ⁻²	BM g.m ⁻²	Ind.m ⁻²	BM g.m ⁻²	Ind.m ⁻²	BM g.m ⁻²	Ind.m ⁻²	BM g.m ⁻²	Ind.m ⁻²	BM g.m ⁻²
Total epigeic	4.9	1.3	0.6	0.1	3.1	1.4	3.1	1.1	0.368	0.322
Total epigeic adults	0.6	0.4	0.0	0.0	1.9	0.9	0.6	0.5	0.543	0.563
Total anecic	44.4	13.9a	43.2	18.7a	53.7	29.8ab	56.8	45.1b	0.231	>0.001
Total anecic adults	1.2	2.4	1.9	3.9	3.7	5.8	7.4	15.2	0.256	0.119
Total endogeic	72.2	17.6	70.4	17.4	65.4	20.4	59.3	20.9	0.526	0.737
Total endogeic adults	10.5	6.8	9.3	7.2	15.4	6.9	17.9	10.6	0.122	0.293
Total	125.9	34.9a	122.2	38.0a	122.8	52.6ab	120.4	67.7b	0.967	>0.001
Total adult	13.6A	9.1a	11.7A	10.4a	21.6AB	14.1a	26.5B	27.8b	0.011	0.008

459 Table 2: Earthworm biomass (BM.g⁻²) and abundance (Ind.m⁻²) in March 2021 (n=18) per main
 460 ecological category. Data per species are in supplementary information. A,B ; a,b corresponds to
 461 significant difference between treatments with the Kruskal-Wallis test (α=0.05) followed by post-Hoc
 462 Dunn's (α=0.05)

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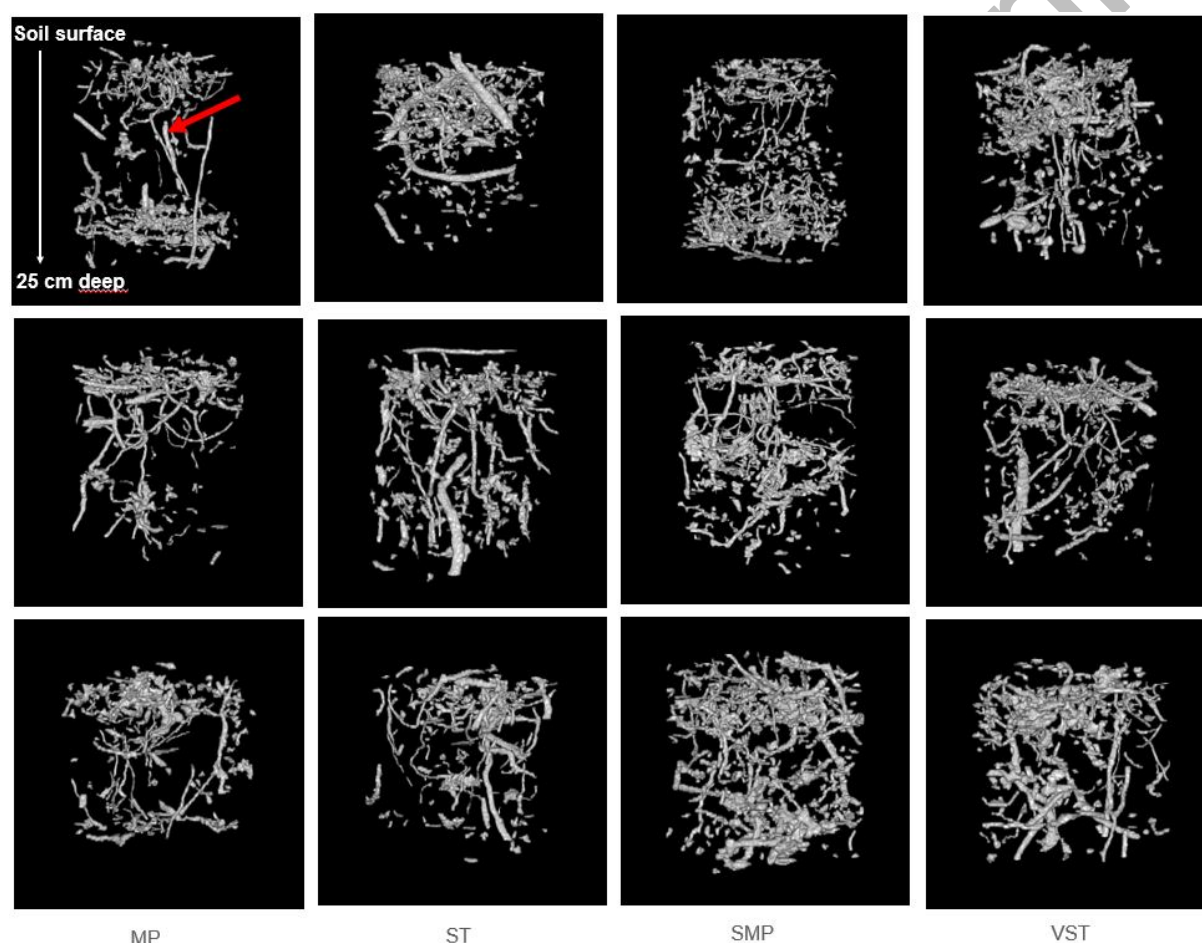
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465 Some representative burrow systems are presented in Figure 6, showing many tubular
 466 macropores, assumed to be earthworm burrows, that were mainly vertically-oriented.

467 Significant difference was observed for the total volume of burrows between SMP on one
 468 side and MP and ST on the other side, while VST had intermediate values. No significant
 469 difference was revealed for diameter, or continuity (Table 3). The vertical barycenter was
 470 significantly higher for the two treatments MP and SMP, compared to ST and VST treatments
 471 which had shallower tillage and no soil inversion. SMP was characterized by a higher

472 number of macropores (below and above 2 cm³) compared to VST, ST, and MP, but a
 473 greater volume of burrows was connected to the surface under VST and ST treatments.
 474 Linear Discriminant Analysis (Figure S2) showed that our tillage depth gradient is mainly
 475 separating VST and ST from MP plots along the first axis, and from SMP plots along the
 476 second axis. Situations with reduced tillage and no soil inversion (VST and ST) were mostly
 477 alike and were related to higher burrow diameter and higher volume of burrow connected to
 478 the surface.

479



480

481 Figure 6: Examples of burrows systems per plots and treatments. The red arrow indicates an example
 482 of tubular macropore. MP: Moldboard ploughing; SMP: Shallow moldboard ploughing; ST: superficial
 483 tillage; VSP: very superficial tillage)

3D burrow characteristics	Moldboard Ploughing	Shallow Moldboard Ploughing	Superficial Tillage	Very Superficial Tillage	Anova test: p.values
Volume of burrows (cm ³)	28.85b	55.89a	36.29ab	46.11ab	0.01
Diameter (mm)	7.18	7.33	7.50	7.56	0.13

Vertical barycenter (cm)	77.61a	88.60a	45.13b	53.45b	<0.001
Continuity#	5.88	4.67	6.00	6.12	0.59
Number of 2D macropores whose area was lower than 2 cm ³	120.2b	227.3a	157.2b	159.3b	<0.001
Number of 2D macropores whose area was larger than 2 cm ³	50.89b	84.8a	50.44b	59.89ab	0.0064
Macroporosity connected to the surface (cm ³)	4.90b	8.61b	11.81a	14.78a	0.004

484

485

486 Table 3. Main characteristics of the burrow systems observed in the sampled soil cores (diameter = 16
487 cm and depth= 25 cm.

488 4. Discussion

489 4.2. Soil stratification and compaction with long-term tillage reduction

490 Reichert *et al.* (2016) suggested a conceptual framework on the evolution of soil
491 structure under no-till with 4 phases. In the first three phases, after 1.5 years (initial phase),
492 3.5 years (intermediate), and 5 to 14 years (transitional phase), respectively, soil structure
493 gradually improved due to its re-aggregation through biological activities in the 0–15 cm soil
494 layer, before reaching the fourth phase (after 14 years) where the whole cultivated soil layer
495 becomes stabilized and homogenous through the complete restoration of biological activity.
496 While the transitional phase was indeed observed in our experiment after 10 years (Peigné
497 *et al.*, 2018), the final and complete restoration stage was not yet observed after 16 years. In
498 our context, with a sensitive sandy loam soil, the stratification of the soil structure remained.

499 For the topsoil (0-15 cm) where tillage disturbance is involved for the whole soil layer
500 in all treatments but VST, the most intensive tillage situation (MP) caused soil compaction at
501 a depth of 10 cm due to the smoothing effect of the rotary harrow at sowing. It was not
502 observed as a factor preventing root development. In the contrary, similar root biomass was
503 observed across tillage treatments, and root density was slightly lower under VST at 20 cm,
504 with more concentrate root density near the soil surface (Figure 3).

505 Deeper, between 15 and 30 cm, non-ploughed treatments (ST and VST) had more
506 compacted soil with higher penetration resistance and less porous clods (Figure 2a). Root
507 biomass was not significantly altered, although reduced root morphological traits changes
508 suggest mechanical stress from compaction, impeding resource uptake and use efficiency.
509 Root average diameter was found to be particularly linked to the tillage gradient though
510 higher diameter when less tillage (Figure 2b). Differences in root diameter for a given root
511 order are associated with specific adaptations to environmental conditions (Ryser, 1998).
512 Morphological changes occur as a result of mechanical stress, with the thickening of root
513 diameter representing one well-identified consequences in literature (Atwell, 1990; Colombi
514 *et al.*, 2017; Schmidt *et al.*, 2013), although it remains unclear whether these changes are
515 the consequence of mechanical stress or an adaptation that favors root penetration capacity
516 (Freschet *et al.* 2020).


517 Furthermore, lower root density, tip elongation, and specific root length were found
518 under VST between 15 and 30 cm. These results underlined again the limitation effect of
519 compacted soil layers on root cell elongation, and showed that the less tilled treatment (VST)
520 was impacted the most in terms of root growth. Specific root length responses to resource
521 limitation noticeably differ across publications (Freschet *et al.*, 2015); however, this trait
522 represents a key feature for evaluating the efficiency of resource acquisition by roots, where
523 high specific root length indicates large absorptive surface with low cell mass, i.e. low
524 structure and development costs. Furthermore, decrease in root tip elongation could impact
525 root function in various ways. The root tip is a hotspot of soil-root exchange, through
526 exudation, respiration, and water-nutrients uptake processes (Bidel *et al.*, 2000; Laporte *et*
527 *al.*, 2013; Nguyen, 2009).

528 As a consequence of compaction increase and root growth limitations, the reduction
529 of tillage could also lead to a stratification of the root-soil exchanges, where most flux
530 exchanges are concentrated in the first centimeters of soil. The deeper soil layers could then
531 be mainly providing extra water pools and being less favorable for nutrient acquisition
532 (nutrients mineralization decrease and immobilization increase). This pattern is supported in

533 many no-till studies, with nutrients content and porosity improved in the first centimeters of
534 no-till soil, while deeper centimeters are more compacted and less furnished with nutrients
535 (Martínez et al., 2016; Krauss *et al.*, 2018). Thus, tillage is a major factor shaping soil
536 features and influencing soil-root interactions. In the past decades, productive grain systems
537 were helped by ploughing operations because it temporarily creates a significant soil depth
538 (20-30 cm deep) that allows fine roots to grow rapidly and homogeneously, and fast growing
539 cultivars were improved simultaneously and can quickly acquire mineralized nutrients (Isaac
540 et al., 2021; Roucou et al., 2018). The development of cropping systems with less tillage
541 might require new research on crops and cultivars with relevant root trait adaptations.

542

543 **4.3. Earthworm community and activity improvements with long-term tillage** 544 **reduction**

545 The earthworm community was different between the two ploughed treatments (MP
546 and SMP) and the ST treatment. However, the biomass of earthworms was higher in VST
547 compared to MP and SMP, which was attributed to the higher body mass of adults and
548 anecic earthworms. Those are known for being more prevalent in lightly tilled soils (Pelosi *et*
549 *al.*, 2016) and our results are consistent with having slightly more  clods under VST
550 (Figure 5). These results support those obtained in other organic (Krauss *et al.*, 2022) and
551 conventional (Pelosi *et al.*, 2014) cropping trials. . Low soil disturbance allows the
552 persistence of anecic earthworms, as their vertical galleries are very sensitive to the
553 destructive actions of soil inversion. These galleries are real burrows that contribute to the
554 survival and reproduction of these earthworms. However, the biomass recorded in our study
555 (34.9 g.m⁻² (MP) to 67.0 g.m⁻² (VST)) was lower than that recorded in other experiments in
556 France (Pelosi *et al.*, 2014). This difference might be explained by the sandy soil and
557 intensive organic cropping system at our site. Spring crops were intensively weeded, with low
558 vegetation cover during crop rotation. The biomass measured in 2021 was similar to that in
559 2015, indicating that the population did not grow over the six years (Peigné *et al.*, 2018).
560 While this is purely speculative, we can imagine that more favorable conditions for

561 earthworm activity improve the effect of reduced tillage with a stronger biological leverage to
562 recreate functional soil porosity.

563 Earthworm biomass (mainly due to anecic worms) was higher in SMP compared to
564 the other treatments in 2015. SMP might have presented the best compromise at that time to
565 preserve earthworm biomass, with lower soil disturbance than traditional ploughing, but with
566 less soil compaction than the non-ploughed treatments (27% of μ clods with SMP compared
567 to 45% and 49% for ST and VST respectively in 2015, Peigné et al., 2018). However in 2021,
568 compaction in the non-ploughed treatments was half that of 2015 in 2021 (in % of μ clods,
569 same methodology). This compaction decrease is likely an effect of the duration of the
570 experiment, gradually allowing earthworms to evolve and penetrate the compacted areas, as
571 shown by the better proportion of clods containing visible earthworms macropores (μ clods
572 clods) under ST and VST in this study compared to the previous one, 6 years ago (Peigné et
573 al., 2018) This gradual decrease in the negative effect of compaction can explain why the
574 earthworms biomass was finally higher under VST treatment in 2021 compared to the
575 ploughed treatments.

576 However, tomography observations of the burrow systems in 2021 were limited to the 0-
577 20 cm depth, a layer where no clear differences were observed regarding soil compaction.
578 Nevertheless, more burrows were connected to the soil surface in the non-ploughed
579 treatments (ST and VST). This may be partly due to the increase of anecic earthworms
580 activity (including epi-anecic ones), as VST was the only treatment that statistically indicated
581 such anecic abundance increase. As these earthworms mainly feed on fresh organic matter
582 at the soil surface, they had vertical burrows that are directly connected to the surface in
583 order to access litter for their daily consumption (Capowiez *et al.* 2015). The connection of
584 the macropores to the surface of the soil can support major positive functional change such
585 as water infiltration and retention (Hangen et al., 2002; Shipitalo *et al.*, 1990). However, we
586 did not observe a larger total volume of pores nor galleries with larger diameters in non-
587 ploughed treatments, linked to the higher presence of anecic earthworms and adults. The
588 overall low earthworm biomass, regardless of treatment, prevented us from detecting any

589 differences. Moreover, although the galleries of anecic earthworms are larger, they are
590 generally less numerous in the soil, especially for epi-anecic earthworms compared to other
591 earthworm categories (Capowiez *et al.* 2021). Another reason for this lack of difference might
592 be linked to the higher soil penetration resistance below 15 cm in the non-ploughed
593 treatments. Soil bulk density negatively affects the burrowing behavior of earthworms
594 (Capowiez *et al.* 2021; Rushton 1986). Thus, after 16 years of very superficial tillage, the
595 activity of earthworms likely failed to increase soil macroporosity compared to ploughed soils
596 in our pedoclimatic and agronomic contexts.

597 Finally, the greater abundance of anecic earthworms might explain the decrease in
598 penetration resistance at greater depth (40 cm) under VST since these earthworms are the
599 only ones to burrow so deep (Capowiez *et al.*, 2012). Unfortunately, no information regarding
600 burrow systems was available due to the difficulty in sampling soil cores at these depths.
601 Anecic and epi-anecic earthworms build deep vertical burrows that improve macroporosity,
602 potentially enhancing root growth at depth. Capowiez *et al.* (2009) showed the presence of
603 larger macropores in reduced tillage compared to ploughing at 30–40 cm depth in loamy
604 soils, supporting this possible improvement in depth. Other studies also identified this
605 beneficial role of deep vertical burrows made by anecic earthworm species or roots, and their
606 associations with greater oxygenation and water infiltration at depth (Wendel *et al.*, 2022;
607 Zhang and Pend, 2021).

608 **5. Conclusions**

609 After 16 years of organic conservation tillage in a sandy loam soil, our study suggests that
610 the first centimeters of soil are crucial to observe the main benefits of CT practices in terms
611 of soil functioning. Without ploughing (ST, VST), better connection of the galleries to the soil
612 surface are key features to sustain water infiltration and nutrients concentration (Peigné *et*
613 *al.*, 2018). But in spite of increased earthworm's abundance, the absence of ploughing was
614 still associated with a substantial stratification of soil and root features, i.e. differences
615 between soil layers. Notably under VST and below 15 cm, the soil became denser, and

616 altered the morphological traits of roots, with higher root diameter and lower specific root
617 suggesting less efficient resource uptake by roots. Further analysis on microorganism
618 activities and nutrients fluxes should be performed to determine the nature and magnitude of
619 plant-soil interaction changes.

620 Our results raise questions on current recommendations for tillage techniques in such
621 sensitive sandy loam soil. In sandy soils, intermittent or strategic tillage with shallow
622 mouldboard ploughing might avoid compaction problems, which are, ultimately, poorly
623 compensated for by biological activity in the long term under conservation tillage. Of
624 importance, a diversified but relatively short and intensive, crop rotation with only temporary
625 cover crops was used in this study. Our study is therefore strengthening that soil health goals
626 cannot be targeted solely through the reduction of tillage and the minimization of soil
627 disturbance. Within an integrated approach, all other soil health principles must be
628 considered too (soil armor, plant diversity, living roots, and livestock integration; Natural
629 Resources Conservation Service, USDA, n.d.). New and more disruptive practices such as
630 temporary grasslands, agroforestry, or permanent cover crops, could be integrated in no-till
631 systems to sustain soil health and functions, meet current expectations about “ecological
632 intensification”.

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811 Author Contributions

812 All authors contributed to the study conception and design. Material preparation, data
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814 Cadiergues for roots study, Yvan Capowiez and Vincent Ducasse for earthworm study, Jean-
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819 Data Availability

820 The datasets generated and analysed during the current study are available from the
821 corresponding author on reasonable request.

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