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► To cite this version:

Esther Guillot, Isabelle Bertrand, Cornelia Rumpel, Claudia Gomez, Didier Arnal, et al.. Spatial heterogeneity of soil quality within a Mediterranean alley cropping agroforestry system: Comparison with a monocropping system. *European Journal of Soil Biology*, 2021, 105, pp.103330. 10.1016/j.ejsobi.2021.103330 . hal-03251274

HAL Id: hal-03251274

<https://hal.inrae.fr/hal-03251274>

Submitted on 13 Jun 2023

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1 **Spatial heterogeneity of soil quality within a Mediterranean alley cropping**
2 **agroforestry system: comparison with a monocropping system**

3

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18

19 **Highlights**

20 - Temperate alley cropping systems induce spatial heterogeneity in soil quality

21 - Soil quality is improved until 2 m beyond the tree row in the interrow

22 - 21 years of agroforestry increased the soil quality by 20% compared with monocrop

23 **Abstract**

24

25 Alley cropping agroforestry systems are complex agroecosystems highlighted for their
26 positive effects on soil quality. However, the potential spatial heterogeneity of soil quality
27 created by tree rows at the plot scale has seldom been studied. The aim of this study was to
28 evaluate soil quality at the plot scale, under tree rows and along transects perpendicular to the
29 tree row and to compare alley cropping systems with monocropping systems. This study was
30 performed on an alley cropping system that combined hybrid walnut trees (21 years old) and
31 peas. Topsoil was sampled at tree rows between 1 and 2, 2 and 4 and 4 and 6.5 m from the
32 tree row in the alley cropping system, as well as in a neighbouring monocropping plot.
33 Physical, chemical and microbiological indicators of soil quality were measured. Tree row
34 implantation induced spatial heterogeneity in the chemical indicators, microbial biomass,
35 activities and community structure at the alley cropping plot scale. Alley cropping not only
36 improved microbiological soil quality indicators within the tree rows but also in the interrows
37 when compared to a monocropping system. These indicators were then integrated into one
38 soil quality index (SQI) built through a statistical approach. The soil quality index was
39 calculated for the monocropping plot and for each position within the alley cropping plot.
40 After 21 years of agroforestry practice, tree rows and permanent grass cover improved the
41 SQI until 2 m in the interrow. Weighted SQIs were calculated relative to the surface area of
42 each location for the entire alley cropping plot (i.e., tree row + interrow positions) and for the
43 entire alley cropping interrow (i.e., removing the tree row surface area). The weighted SQI of
44 the entire alley cropping plot significantly increased compared with that of the monocropping
45 plot.

46

47 **Keywords:** Alley cropping system, spatial heterogeneity, soil quality index, microbial
48 activities, soil organic carbon

49 **1. Introduction**

50

51 Soil quality can be defined as “the continued capacity of the soil to function within
52 ecological and land-use boundaries, to sustain productivity, to promote the quality of air and
53 water, and to maintain plant, animal and human health” [1]. Soil quality indicators are
54 parameters that are sensitive enough to be modified by land use or management practices [2],
55 [3] and should provide key information concerning the composition, structure and function of
56 the soil [4]. In addition, a combination of physical, chemical and biological indicators must be
57 used to correctly interpret soil quality [5], [6].

58 Physical indicators are related to the “inherent quality”, which is influenced by soil
59 age and past climates [7], while most chemical and all biological indicators can be associated
60 with the “dynamic quality”, which is highly sensitive to land use and soil management [8].
61 Inherent and dynamic soil quality indicators refer to changes over long and medium to short
62 terms, respectively [9]. Among physical soil quality indicators, water storage, bulk density,
63 aggregate stability and texture are the most frequently measured [2]. Chemical indicators can
64 be evaluated to characterize soil fertility and nutrient availability (e.g., soil organic carbon,
65 total N, Olsen P, CEC, pH, exchangeable ions, and N mineralization rate) [10]. Biological
66 indicators of soil quality are necessary to connect abiotic soil properties to soil functioning
67 [2], [11]. Soil microbial biomass, abundance, diversity and activity play key roles as the main
68 drivers of soil organic matter decomposition and nutrient cycling [12], [13]. Soil
69 microorganisms can be studied as soil quality indicators through their biomass (e.g., microbial
70 biomass C), activities (e.g., enzyme activities and substrate-induced respiration) and
71 community structure (e.g., PLFA and sequencing) [14]. A minimum dataset of indicators has

72 to be selected either through expert opinion [15] or through statistical approaches [5], [16] to
73 assess soil quality, avoiding collinearity between indicators. Both methods allow the
74 construction of a soil quality index (SQI), which accounts for a more or less comprehensive
75 set of indicators [10], [17]. The use of aggregated SQIs is currently strongly developed and
76 can improve the comparisons of land use or management effects.

77 Land management is recognized as one of the main drivers affecting soil quality
78 improvement or degradation [18]. Temperate agroforestry systems comprising a combination
79 of trees and crops are examples of agroecosystems that provide a sustainable alternative to
80 conventional cropping systems, in which plant diversity is low; hereafter, these conventional
81 cropping systems are called monocropping systems. A recent review of European agroforestry
82 practices confirmed their positive effect on ecosystem services; however, this effect was
83 context-dependent [19]. One of the most important ecosystem services promoted by
84 agroforestry systems is their potential capacity for carbon (C) storage, both temporarily in tree
85 biomass through photosynthesis [20] and in the long term in soil organic matter [21]. High
86 inputs of above- and belowground litter in the tree row in alley cropping can provide higher
87 amounts of C and nutrient resources and thereby modify soil microbial communities [22].

88 Even though a global positive effect of agroforestry on soil quality has been
89 recognized [23], it should be noted that only a small proportion of studies have considered the
90 spatial heterogeneity induced by tree rows. As highlighted by Cardinael et al. [24], future
91 studies should integrate more spatial dynamics and investigate lateral spatial heterogeneities
92 instead of concentrating on only the tree row. The few publications that have considered the
93 potential spatial heterogeneity of soil quality have not allowed us to claim that agroforestry
94 systems significantly improve soil quality at the plot scale or within the interrow. For
95 instance, [25] did not detect differences between tree rows and interrows in microbial biomass
96 C (MBC) and basal respiration or [26] in bacterial diversity or [27] in enzyme activities.

97 Mungai et al. [28] found significantly more enzyme activities in tree rows than in interrows
98 but no difference within cultivated interrows. The spatial extent to which tree rows and their
99 understorey vegetation strips affect soil quality should be investigated.

100 In the present work, we evaluated the effect of an alley cropping agroforestry system
101 on the physical, chemical and biological components of soil quality compared to a
102 neighbouring, monocropping system managed in the same way as the interrows in the alley
103 cropping system. We first hypothesized that spatial gradients of soil quality occur between the
104 tree row and middle of the interrow, with the greatest values of soil quality indicators on and
105 close to the tree row and decreasing values with increasing distance from the tree row.
106 Second, we hypothesized that the weighted soil quality of alley cropping, integrating its
107 spatial heterogeneity throughout the plot, is higher than that of the neighbouring
108 monocropping plot.

109

110 **2. Materials and methods**

111 *2.1. Site description*

112 The experimental site of Restinclières is located at Prades-le-Lez, 15 km north of
113 Montpellier, southern France (43°42'15 N, 3°51'41 E). This location has a subhumid
114 Mediterranean climate, with an average temperature of 15.4 °C and average annual rainfall of
115 658 mm between 2000 and 2016 (Fréjorgues weather station). The soil is a deep Fluvisol
116 (WRB, 2007) with a silty-clay texture. In February 1995, hybrid walnut trees (*Juglans regia* ×
117 *nigra* cv. NG23) were planted 4 m apart along east-west rows, with an interrow distance of 13
118 m. The initial planting density was 208 trees ha⁻¹, but the plot was thinned in 2004 down to a
119 present density of 110 trees ha⁻¹. Adult walnut trees have not been pruned since 2014. The
120 tree rows (2 m wide) were covered with spontaneous herbaceous vegetation, as they have not
121 been ploughed or treated with herbicides since 1995. Each interrow was ploughed to a 20 cm

122 depth every year before the winter crop was sown and was fertilized at an approximate rate of
123 150 kg N ha⁻¹ yr⁻¹ (as NH₄NO₃). Most of the time since 2000, durum wheat has been the
124 major crop in rotation, but pea has been introduced every 4 years since 2010, and barley has
125 been introduced since 2015 [29]. Over the 2014-2016 period, durum wheat (*Triticum*
126 *turgidum durum* cv. Claudio)-barley (*Hordeum vulgare* cv. Augusta)-pea (*Pisum sativum* cv.
127 Igloo) rotation was practised on this 4.6-ha alley cropping plot and on the adjacent 1.4-ha
128 monocropping plot, which has been managed in the same way as the cultivated interrows in
129 the alley cropping since the experimental site was established. All crops were sown between
130 the end of October and the beginning of December and harvested by the end of June/early
131 July.

132

133 2.2. Soil sampling

134 Soil sampling was conducted in April 2016, when pea was growing in the
135 monocropping plot and in the interrows of the alley cropping plot. At this stage, the walnut
136 trees had no leaves yet. In the 1.4 ha monocropping plot, five sampling areas of 1 m² each, at
137 least 15 m apart, were identified for soil sampling. In each 1 m² surface area, one composite
138 soil sample, based on 4 soil cores (8 cm in diameter) from each angle of the area, was
139 collected from the 0-15 cm layer of topsoil using a root auger. For the alley cropping plot,
140 five transects at least 40 m apart were sampled from the tree row to the middle of the interrow
141 on both the north and south sides of the tree row. Each north-south transect corresponded to 7
142 positions (35 soil samples in total) (**Fig. A.1**). Soils were sampled in the tree row, with the
143 centre of the 2-m² sampling area located on the axis of the tree row, 1 m from the nearest tree.
144 Other sampling areas were located in the interrow between 1 and 2 m from the middle of the
145 tree row, between 2 and 4 m and between 4 and 6.5 m. Soil samples were collected from the
146 0-15 cm layer of topsoil using a root auger at each angle of a centred subsurface (representing

147 half of the total surface area of the location) (**Fig. A.1**). Areas sampled on the north side are
148 indicated by “N”, and those on the south side are indicated by “s”. Once collected, the fresh
149 soil samples were passed through a 2-mm sieve, and subsamples were stored at 4 °C for
150 microbial biomass and MicroResp® analyses, which were performed within two weeks after
151 sampling and at -20 °C prior to freeze-drying for PLFA analyses.

152

153 *2.3. Physical and chemical analyses*

154 Physical and chemical indicators were measured on the north side of the trees
155 (corresponding to 4 positions per transect) and in the monocropping system, i.e., for a total of
156 25 samples. The moisture content was determined for all samples after drying for 48 h at 105
157 °C. Bulk density was determined by dividing the soil dry mass by the volume of the root
158 auger. Additional analyses were performed by the national routine soil testing lab of INRAE
159 at Arras (LAS, France). Soil texture was determined after CaCO₃ dissolution, and pH was
160 determined in a water extract. The cation exchange capacity (CEC) was determined using the
161 cobalt hexamine chloride method and analysed using inductively coupled plasma-atomic
162 emission spectrometry (ICP-AES) [30]. Soil organic C (SOC) and total N were determined by
163 dry combustion. The available P content was determined colorimetrically after extraction
164 using the Olsen method [31]. Roots were collected from each soil core (15 cm height × 8 cm
165 diameter) and pooled into one composite sample (4 cores) for each area. They were separated
166 into three size classes as follows: 0-2 mm (fine roots), 2-5 mm (medium roots) and > 5 mm
167 (large roots).

168

169 *2.3.1. Density and particle size fractionation*

170 Density fractionation was performed on the north side of the trees and in the
171 monocropping system, i.e., for a total of 25 soil samples. The fractionation procedure was

172 derived from Roscoe et al. [32]. Briefly, 25 g of soil was shaken with 100 ml of a 1.6 Mg m⁻³
173 dense sodium polytungstate solution (NaPT) and then centrifuged for 30 min at 6800 g. The
174 suspended material was termed the free light fraction (f-LF < 1.6 Mg m⁻³) and was separated
175 from the supernatant by filtration using a 0.7 µm glass fibre filter. The material recovered on
176 the filter paper was washed with distilled water to remove residual NaPT. New NaPT (density
177 1.6 Mg m⁻³) was added to the remaining material. The new suspension was subjected to
178 ultrasonic dispersion (25 J ml) for 3 min. Afterwards, the suspension was centrifuged at 6800
179 g for 30 min, and the supernatant was filtered to recover the occluded light fraction (o-LF <
180 1.6 Mg m⁻³) (procedure see above). The remaining material was then separated into two
181 particle-size fractions by wet sieving: 50-2000 µm (sand) and < 50 µm (silt and clay). Before
182 C and N measurements, each fraction was decarbonated by acid fumigation using 12 M HCl
183 in a desiccator for 6 h [33]. After removing calcium carbonate, C and N contents in the total
184 soil, the f-LF o-LF and 50-2000 µm and < 50 µm fractions were analysed by dry combustion
185 with an elemental analyser (Elemental Analyser Vario Pyro Cube). This density fractionation
186 procedure allowed the separation of free soil organic matter (f-LF), soil organic matter
187 associated with minerals (o-LF and 50-2000 µm) and physically protected soil organic matter
188 (< 50 µm).

189

190 2.3.2. *Non-cellulosic neutral sugar determination*

191 Rhamnose, fucose, mannose, galactose, glucose, ribose, arabinose and xylose were
192 quantified in the total soil, f-LF, o-LF and < 50 µm fractions following the protocol of
193 Rumpel and Dignac [34] modified through the addition of 0.9 ml of 2 M EDTA after
194 hydrolysis with trifluoroacetic acid (TFA) [35]. Briefly, 500 mg for total soil, between 60 and
195 70 mg for f-LF and o-LF and 600 mg for the < 50 µm size fraction were added to 10 ml of
196 TFA and hydrolysed at 105 °C for 4 h. After hydrolysis, myoinositol was added as an internal

197 standard. Samples were filtered through a glass fibre filter (0.7 μm), and TFA was eliminated
198 by evaporation before derivatization. Aldoses were reduced to their corresponding alditols
199 after the addition of 1 ml of NaBH_4 dissolved in dimethyl sulfoxide (DMSO). Acetylation
200 was performed by adding 2 ml of acetic anhydride and 2 ml of glacial acetic acid using
201 methylimidazole (2 ml) as the catalyst. The reaction was stopped after 10 min by adding 7 ml
202 ice-cold deionized water. The derivatised sugar monomers were extracted with 1 ml of
203 dichloromethane. The analyses were performed using a gas chromatograph (HP 6890 GC-
204 FID) equipped with an SGE BPX-70 column (60 m \times 0.32 mm internal diameter, 0.25 μm
205 film thickness). The gas chromatography oven temperature program was 200 $^\circ\text{C}$ to 250 $^\circ\text{C}$ at
206 8 $^\circ\text{C min}^{-1}$ and isothermal at 250 $^\circ\text{C}$ for 15 min with helium as the carrier gas.

207 The sum of extracted monosaccharides from plant-derived hemicellulose and
208 microbial products [36] is hereafter termed “sugars”. The proportion of microorganism-
209 derived sugars in relation to plant-derived sugars can be estimated from the ratio of
210 hexose:pentose sugars: (galactose + mannose):(arabinose + xylose), hereafter called GM:AX
211 [37]. GM:AX ratios < 0.5 and > 2 are representative of carbohydrates predominantly derived
212 from plants and microorganisms, respectively [37].

213

214 2.4. Soil microbiological indicators

215 The study plot had an east-west tree row orientation, and we wanted to test whether
216 this orientation could modify soil microbiological parameters, which are sensitive to changes
217 in climatic conditions at the local scale. Accordingly, we measured microbiological
218 parameters on both the north and south sides of the tree row., i.e., for a total of 40 samples.

219 Microbial biomass C (MBC), N (MBN) and P (MBP) contents were quantified using
220 the chloroform fumigation-extraction technique [38]. Briefly, 10 g (for MBC and MBN) or 2
221 g (for MBP) of equivalent dry soil was exposed to chloroform vapor for 24 h and then

222 extracted with 40 ml of 0.025 M K₂SO₄ (for MBC and MBN) or with 40 ml 0.5 M NaHCO₃
223 (for MBP), shaken for 45 min, centrifuged (10 min at 2683 g) and then filtered through 0.22-
224 µm PTFE filters. Soil organic C and N concentrations in the extracts were measured by a
225 TOC/TON analyser (OI-Analytical, Aurora 1030, College Station, USA). Inorganic P
226 concentrations in the extracts were quantified colorimetrically using the malachite green
227 method [39]. Microbial biomass C, N, and P (MBC, MBN and MBP) contents were
228 calculated from the difference between the chloroform-fumigated and non-fumigated samples.
229 We applied a conversion factor of 0.45 for MBC [40], 0.54 for MBN [41] and a conversion
230 factor of 0.4 for MBP [40]. Data are expressed in mg C, N or P kg soil⁻¹. The total C of
231 nonfumigated samples was used to represent dissolved organic carbon (DOC).

232 Basal and substrate-induced respiration was measured using the MicroResp™
233 approach [42]. The soil water holding capacity (WHC) was determined using Richard's
234 membrane press at pF 2.5 [43]. Sieved soils were adjusted to 40% of their WHC and were
235 preincubated for 7 days at a temperature of 23 °C ± 2 °C in the dark following the protocol of
236 Bérard et al. [44]. We tested three different soluble C substrates: glucose, trehalose and
237 alanine [44]. In a 96-deep-well microplate, 350 mg of soil sample was distributed, and then 25
238 µl of each substrate was dispensed in each of the wells at 10 µg substrate mg⁻¹ soil. The 96-
239 deep-well microplate was then sealed with a CO₂-trap gel and incubated for 6 h in dark
240 conditions at 23 ± 2 °C. The optical density of each well of the CO₂-trap gel was measured on
241 a fluorometric microplate reader (Victor 3, Perkin Elmer) at 570 nm.

242 The microbial community structure was assessed using the phospholipid fatty acid
243 (PLFA) method described by [45]. All analyses of PLFAs were performed by Microbial iD,
244 Inc. (Newark, USA). Briefly, lipids were extracted from 5 g of freeze-dried soil by using a
245 modified Bligh-Dyer extraction with 19 ml of extractant. Lipids were separated on a solid-
246 phase extraction column, and phospholipids were eluted with 5 ml of methanol. After

247 evaporation, phospholipids were transesterified to fatty acid methyl esters, extracted in 4 ml of
248 hexan, evaporated again and then analysed using a gas chromatograph (Agilent 6890
249 Technologies, Wilmington, USA) [45]. PLFA peaks were identified using the MIDI PLFAD1
250 calibration mix and naming table (MIDI, Inc., Newark, USA). Individual PLFA markers were
251 used to determine the number of peaks identified per position (tree row, 1-2 m_{N/S}, 2-4 m_{N/S},
252 4-6.5 m_{N/S} for alley cropping and monocropping). Gram-positive (GP) bacteria were
253 identified by 13:0 iso, 14:0 iso, 15:1 iso w6c, 15:1 anteiso w9c, 15:0 iso, 15:0 anteiso, 16:0
254 iso, 16:0 anteiso, 17:1 iso w9c, 17:1 anteiso w9c, 17:1 anteiso w7c, 17:0 iso, 17:0 anteiso,
255 18:0 iso, and 20:0 iso; gram-negative (GN) bacteria were identified by 13:f1 w5c, 13:1 w4c,
256 14:1 w5c, 15:1 w6c, 16:1 w9c, 16:1 w7c, 17:1 w8c, 17:0 cyclo w7c, 18:1 w7c, 18:1 w5c, 19:1
257 w8c, 19:0 cyclo w7c, 20:1 w9c, 20:1 w6c, 21:1 w8c, 21:1 w3c and 22:1 w3c (reference table
258 from MIDI, Inc., Newark, DE). The peaks associated with 12:0, 14:0, 15:0, 16:0, 17:0, 18:0,
259 20:0, 22:0, 23:0 and 24:0 were considered general indicators [46], and they were not
260 integrated in analyses and were classified as “non-identified peaks” in **Table A.1**. The GP:GN
261 ratio was calculated based on the previously described peaks. A relative measure of the
262 fungi/bacteria (F:B) ratio was calculated by dividing the fungal PLFA marker: 18:2 w6c, 18:1
263 w9c [46], [47], [48] and 16:1 w5c, the last one representing the arbuscular mycorrhizal fungi
264 (AMF) by the sum of GN and GP bacteria. Actinobacteria were identified by 16:0 10-methyl,
265 17:1 w7c 10-methyl, 17:0 10-methyl, 18:1 w7c 10-methyl, 18:0 10-methyl and 19:1 w7c 10-
266 methyl. Other unknown peaks were associated with the “others” category and were
267 considered only in the calculation of the relative abundance of each PLFA group. All PLFA
268 markers are listed in **Table A.1**.

269

270 *2.5. Calculation of the soil quality index (SQI)*

271 We followed the method proposed by Obriot et al. [10] to build an aggregated index of
272 soil quality. This method is based on 4 steps and starts with a dataset from different soil
273 variables and leads to a unitless single score. The first step (step 1, [10]) consisted of the
274 inventory of soil parameters. The second step (step 2, [10]) aimed to produce a minimum
275 dataset (MDS) for each category. Only those parameters that significantly discriminated the
276 various locations within the agroforestry plot (mixed model analysis, $P < 0.05$) were
277 considered relevant [10]. Then, a Pearson correlation analysis was performed to identify
278 correlated parameters (> 0.8) and to avoid redundant information in the aggregated index. For
279 the third step (step 3, [10]), each indicator was normalized between 0 and 1 using the “more is
280 better”, “less is better” or “optimum” response curves. For each parameter, the type of
281 response curve was determined according to the literature [10], [49], [50]. For all parameters,
282 we used the “more is better” response curves except for bulk density and Olsen P, for which
283 the “optimum” response curve was chosen [51], [52]. Selected indicators from the MDS were
284 then computed in a principal component analysis (PCA), and the contribution of each
285 indicator to the dimensions of the PCA was calculated. Finally, the SQI was calculated
286 according to Equation 1 and Equation 2 (step 4, [10]), with a weighting of the transformed
287 variables using the PCA eigenvectors and the percentages of total variability explained by
288 each principal component:

289

290 Equation 1: $W_i = \sum_{j=1}^p \lambda_j \times f_j$

291

292 Equation 2: $SQI = \sum_{i=1}^n S_i \times W_i$

293

294 where f_j = relative percentage of total variability attributed to each principal component, λ_j =
295 sum of squared coordinates on each eigenvector, S_i = normalized indicator scores and W_i =

296 weighted factors. One SQI was calculated for each position within the alley cropping plot, and
297 one for the entire alley cropping (hereafter called weighted alley cropping), weighting each
298 surface area on the north side for the SQI. Another SQI was calculated for the alley cropping
299 interrow excluding the tree row area in the weighted calculation (hereafter called weighted
300 interrow). The last SQI was calculated for the monocropping system.

301

302

303 2.6. *Statistical analyses*

304 Mixed models with distance to the tree row as a fixed effect and transect number as a
305 random effect were created for all soil parameters. Normal distribution of residuals and
306 homogeneity of variance were tested by using the Shapiro and Bartlett tests. When necessary,
307 data were Box-Cox transformed (MASS R package). If significant, a Tukey post hoc test was
308 used for pairwise multiple comparisons ($P < 0.05$). Differences between monocropping and
309 each position from the spatial gradient were tested by a one sample t-test ($P < 0.05$)
310 considering the monocropping system as a reference.

311 A Pearson correlation matrix was calculated to assess relationships between
312 environmental parameters and the abundance of soil microbial functional groups. Redundancy
313 analysis (RDA) was performed to assess the relationship between the soil microbial
314 community composition, i.e., relative abundance of different microbial groups and
315 environmental parameters. Most discriminating variables were selected using a forward
316 procedure, and significance was also tested (100,000 permutations) (vegan R package). All
317 statistical analyses were performed with R software v.3.2.3 (R development Core Team,
318 2015).

319

320 **3. Results**

321 *3.1. Physical and chemical soil quality*

322 The soil was calcareous with more than 50% calcium carbonate, which explained the
323 alkaline pH (**Table 1**). Levels of Olsen P were systematically low regardless of the position in
324 the alley cropping or monocropping plots. The tree row showed significantly higher values
325 than the interrow positions for the following variables: soil moisture, SOC, DOC, N_{tot},
326 mineral N, Olsen P, CEC and exchangeable cations.

327 The largest C content was systematically found in the smallest fraction, i.e., < 50 µm
328 fraction, contributing to more than 50% of the total soil C (**Table 2**). The highest C content
329 was found in the tree rows regardless of the fraction, e.g., within the 50-2000 µm fraction, 3.6
330 g C kg⁻¹ soil was measured in the tree rows versus 2.0 - 2.3 g C kg⁻¹ soil within the interrows
331 and 1.5 g C kg⁻¹ soil in the monocropping plots. The C:N ratios of f-LF and o-LF were
332 significantly lower under the tree row (18.7 and 16.5, respectively) than under the middle of
333 the interrow (22.8 and 18.7, respectively) (**Table A.2**).

334 The highest sugar-C concentration was also found in the < 50 µm fraction (1.87 - 2.36 mg
335 sugar C g⁻¹ soil) and did not significantly differ among positions in the alley cropping plot or
336 compared with the monocropping plot (**Table 3**). The sugar-C concentration in f-LF under the
337 tree row was significantly (two-fold) higher than that in the middle of the interrow or in the
338 monocropping plot. In the same fraction, the proportion of microorganism-derived sugars in
339 relation to plant-derived sugars (GM:AX ratio) decreased from 0.85 in the tree row down to
340 0.67 in 4-6.5 m_N and was significantly higher than that in the monocropping plot (0.62).

341 In the tree row, the total root biomass was more than 2-fold higher than anywhere
342 within the interrow or compared with the monocropping plot (**Fig. A.2**). Significant
343 differences were noticeable for fine- and medium-class roots.

344

345 *3.2. Biological soil quality indicators*

346 We observed a soil MBC close to 2-fold higher in the tree row (427 mg C kg⁻¹) than in
347 the middle of the interrow (219 mg C kg⁻¹ soil) and in the monocropping plot (199 mg C kg⁻¹
348 soil) (**Fig. 1**). The same difference was observed for MBN and MBP. Compared with
349 monocropping, we systematically found a significant increase in MBC, MBN and MBP in the
350 tree row and in 1-2 m_{N/S}.

351 The MB-C:N ratio significantly increased with increasing distance from the tree row,
352 from 6.0 in the tree row to 7.4 in 4-6.5 m_N (**Table A.3**). The MB-C:P ratio was significantly
353 lower under the tree row (24.5) than in 4-6.5 m_N (41.4). Values of MB-C:N and values of
354 MB-C:P were similar in the monocropping plot and close to those in the tree rows.

355 Basal respiration was significantly increased only in the 1-2 m_S position compared
356 with all others (**Table 4**). Compared with monocropping, glucose-induced respiration was
357 significantly enhanced at all positions in the alley cropping plot except in 4-6.5 m_N. Alanine-
358 and trehalose-induced respiration was significantly higher in the tree row and close to it in the
359 alley cropping plot than in the monocropping plot. Alanine-induced respiration was more than
360 2-fold higher under the tree row than in the middle of the interrow. The metabolic quotient
361 (qCO₂) was similar across all positions ($P > 0.05$, **Table 4**).

362 The PLFA diversity, i.e., the number of detected peaks, decreased with increasing
363 distance from the tree row (**Fig. A.3**). Actinobacteria abundance was significantly highest in
364 the tree row (2.58 μg PLFAs g⁻¹ soil) and similar for the interrow positions and for the
365 monocropping plot (**Fig. 2a**). For GP and GN bacteria, AMF and other fungi, soils under the
366 tree row and in 1-2 m_S showed significantly higher abundance than those in the middle of the
367 interrow or in the monocropping plot (**Fig. 2a, b**). The F:B ratio did not show significant
368 differences between positions within the alley cropping plot or compared with the
369 monocropping plot: all values were approximately 0.30 ± 0.1 (data not shown). The GP:GN

370 ratio was significantly lower in and close to the tree row compared with the interrow or
371 monocropping plot (**Fig. 2a**).

372

373 *3.3 Soil quality index*

374 Eight indicators, i.e., bulk density, SOC, N_{tot}, CEC, Olsen P, basal respiration, and
375 glucose- and alanine-induced respiration ultimately contributed to the construction of the SQI
376 (**Fig. 3**).

377 We observed a gradual decrease in the SQI with increasing distance from the tree row (0.76 to
378 0.53). The monocropping plot had an SQI similar (0.52) to that found in the middle of the
379 interrow. The contribution of each individual indicator to SQI construction was approximately
380 12%, except Olsen P, which contributed less than 3%. Individual contributions varied
381 according to the position in the alley cropping plot following a same spatial pattern that was
382 similar to the SQI. The weighted SQI of the entire alley cropping plot (0.64) was significantly
383 20% higher than that of the monocropping plot (0.52), while an increase of 10% of the
384 weighted SQI for the alley cropping interrow (0.57) was not significantly different from that
385 of the monocropping system ($P = 0.08$) (**Fig. 3**).

386

387 *3.4. Relationships between environmental parameters and microbial community structure*

388 To understand the link between soil environmental parameters and soil microbial
389 community structure, we i) calculated a Pearson correlation matrix between different soil
390 characteristics and diversity, and total PLFA biomass and abundance of each microbial group
391 (**Table A.4**) and ii) performed RDA to identify the determinants of the relative abundance of
392 PLFA markers (**Fig. 4**).

393 The two first axes explained 45 and 10% of the total variation, respectively (**Fig. 4**).

394 The first axis clearly distinguished the relative abundance of actinobacteria and other GP

395 bacteria, which were positively correlated with silt content, and the relative abundance of
396 AMF, GN bacteria and fungi. The AMF and GN bacteria were positively correlated with
397 sugar content and GM:AX ratio in the f-FL fraction, and fungi were positively correlated with
398 sugar content in the o-LF fraction and negatively correlated with clay content.

399

400

401

402 **4. Discussion**

403 *4.1. Soil quality index in agroforestry and monocropping plots*

404 In the present work, the selected indicators for the minimum dataset were in line with
405 expert opinion [2], [53], [54]. Bulk density and SOC content represent important weighted
406 indicators in soil quality assessment for their role in water infiltration, storage and supply, as
407 well as CEC, N and P contents for nutrient storage and supply [11]. Among the selected
408 biological indicators, basal respiration is considered a physiological trait of microbial
409 communities, and glucose-induced respiration is assumed to be proportional to active
410 microbial biomass [44]. In this study, we focused on the microbial component of soil
411 functioning, although we are aware that other biological indicators, such as those related to
412 meso- or macro-fauna, could have been helpful for soil quality assessment [9], [55].

413 Our aim was to aggregate information obtained using different parameters into one
414 integrative soil quality index based on several soil functions: water, C and nutrient storage
415 and cycling and sustainability of biological activities. In the present work, we initially had a
416 large number of indicators (30 in total), and several methods for SQI calculation offered to us.
417 Comparing three different SQI constructions, i.e., additive SQI, weighted additive SQI and
418 statistically modelled SQI, [56] showed that the last one was more efficient in time and cost.

419 The method for index calculation proposed by [10] allowed us to objectively select the most
420 relevant indicators to assess changes in physical, chemical and biological soil quality in the
421 studied field plots. Without this statistical approach, we could have overestimated the positive
422 effect of tree rows on soil quality because of the co-variability of the measured indicators.
423 However, this method considers as relevant for the first step of minimum dataset selection
424 only parameters that significantly changed statistically according to the treatments, which
425 could be considered a subjective choice.

426 This is the first time, to the our knowledge, that soil quality was assessed in temperate
427 alley cropping through the use of an integrative and weighted SQI. We showed a strong
428 positive effect from the 21-year-old trees and accompanying permanent grass cover in the tree
429 rows of the studied alley cropping system on soil physicochemical indicators compared with
430 the interrow and monocropping systems; this effect was restricted to the tree rows (**Table 1**).
431 The increase in SOC content in the tree rows led to an increase in those soil properties that are
432 usually related to it, such as CEC and exchangeable cation contents [10] (**Table 1**).

433 We showed that the SQI was significantly improved in an alley cropping system in
434 comparison with a monocropping system, considering the spatial heterogeneity induced by
435 these systems. This indicates here that the positive effect from the trees and herbaceous cover
436 on the weighted entire alley cropping SQI was significant beyond the tree rows. The tree
437 rows, which represent approximately 13% of the field plot surface area, yielded a significant
438 beneficial effect on SQI over approximately 20% compared with monocropping (**Fig. 3**).
439 These findings should be put in perspective, as the results depend on one site and one
440 sampling date. Additional sub-annual sampling would be necessary to reject a possible
441 seasonal effect. Even though not significant ($P = 0.08$), there was a 10% increase in the SQI
442 weighted interrow (i.e., 1-2 m + 2-4 m + 4-6.5 m) compared with the neighbouring

443 monocropping plot. Repeating these indicator measurements in time and increasing the
444 number of replicates would allow us to see if this trend could be significant or not.

445

446 *4.2. What explains the higher SOC content in the alley cropping system?*

447 In our study, the topsoil of tree rows showed SOC contents 20-30% higher than that of
448 the interrow and 50% greater than that of the monocropping plot (**Table 2**), which is in line
449 with former results at the same study site [57]. Within and close to tree rows, belowground
450 litter, as aboveground litter can also be enhanced, as tree roots have been shown to colonize
451 the topsoil at the same agroforestry site until 1.5 m in the interrow [58]. In addition, a
452 substantial portion of roots can originate from (i) herbaceous cover root systems, which can
453 colonize the topsoil close to the tree row, as shown by [59] in younger walnut tree alley
454 cropping, or ii) weed colonization with low dispersal abilities, originating from herbaceous
455 cover [60]. Perennial plants can indeed allocate up to 10-15% more C belowground than
456 crops [61], [62]. The absence of tillage in the tree rows likely favoured some accumulation of
457 both C and N [63].

458 The novelty of our study is that it provides greater insight into the pools of C found in
459 the topsoil at various locations in the alley cropping system than in the monocropping system.
460 We demonstrate that the additional C found in the topsoil comes from fractions with a higher
461 C:N ratio (> 16) (**Table A.2**), which is more similar to that of plant debris than that of smaller
462 size fractions with a C:N ratio < 10, i.e., close to the C:N ratio of soil microbial communities
463 and soil organic matter [64], [65]. These coarser fractions can be more easily mineralized by
464 microorganisms, suggesting a less stable C pool.

465 The increased GM:AX ratio in the f-LF fraction from the middle of the interrow to the
466 tree row suggests that a higher proportion of microbial-derived carbohydrates was present in
467 the tree row (**Table 3**). This may have induced higher and faster microbial turnover based on

468 more organic matter recycling through microorganisms and could indicate that the production
469 of organic compounds is easier to stabilize [66], [67]. The RDA confirmed that the increased
470 GM:AX ratio favours more fast-growing microorganisms, i.e., microbial communities with
471 higher GN bacteria proportions (**Fig. 4**).

472

473 *4.3. Spatial heterogeneity of microbial soil properties in the alley cropping system*

474 Our work demonstrated that an agroforestry system such as the one we studied here
475 may considerably impact soil microbial communities through its biomass, activity and
476 structure, resulting in the creation of within-plot spatial heterogeneity.

477 Higher microbial biomass and activities in and close to the tree row may represent a
478 higher potential capacity to decompose organic matter and improve nutrient cycling.
479 MicroResp® appeared not to be the most appropriate method to evaluate the activity of
480 microorganisms in the carbonate-rich soil; however, as carbonate content was similar
481 everywhere, it allowed us to compare positions. Our qCO₂ results indicate a similar
482 physiological activity of the microbial biomass regardless of the position within the
483 agroforestry plot [68]. This result was surprising because of the different tillage management
484 practices between the tree row and interrow [69]. However, [70] showed in a meta-analysis
485 that similar qCO₂ values were observed for tilled and no-tilled plots in long-term (10 years)
486 plots. This might indicate that qCO₂ is not sensitive to soil tillage or that microorganisms from
487 the tree row are, at the time of our sampling, as efficient in using C as those in the interrow or
488 in the monocropping system.

489 In tree rows, the MBC/SOC ratio was significantly higher than that in the interrow or
490 in the monocropping plot (i.e., 23 versus 17, data not shown). This indicates that the MBC
491 increase in tree rows was not merely due to the increase in SOC but also to some properties of

492 the soil organic matter (e.g., greater C availability) within tree rows that favour the
493 development of an abundant soil microbial biomass [71].

494 Compared with the monocropping system, the microbial biomass and substrate-
495 induced respiration were more significantly higher on the south side of the tree row than on
496 the north side in the alley cropping plot (**Fig. 1, Table 4**). This result underlines the potential
497 legacy effect of microclimate created in this alley cropping agroforestry system. Dufour et al.
498 [72] showed in the same plot that photosynthetically active radiation can be reduced by 38%
499 at 3 m on the north side when the tree canopy is well developed (end of June). Our soil
500 sampling was performed in April before walnut tree budburst began. At this stage, they
501 showed that the reduction in incident light was negligible (10%) compared with that in June
502 (50%). This suggests that the slight difference observed between the north and south sides of
503 the tree rows was due to a legacy effect of the 21-year-old practice.

504 We found that trees and the herbaceous grass community (dominated by weeds,
505 *Bromus* and *Torilis* species) in the tree rows increased PLFA diversity and biomass. These
506 two parameters were highly correlated with pH (negatively), soil moisture, DOC and total N,
507 as observed by [48] and [22]. In our case, an additional correlation with root biomass
508 highlighted its potential key role in shaping microbial community dynamics (**Table A.4**).

509 In the present study, we observed that tree rows favour a higher proportion of GN
510 bacteria than GP bacteria, leading to a lower GP:GN ratio. Environments rich in C and
511 available N are known to promote soil GN bacteria [73], which have high affinity for recent
512 plant-derived C compounds [74]. The RDA also suggested a correlation between the sugar
513 content in f-LF and the GN bacterial proportion (**Fig. 4**). In contrast, GP bacteria have been
514 shown to be less affected by low C availability [73]. Actinobacteria and other GP bacteria are
515 known to be able to feed on more complex polymers, such as older organic matter [48]. This
516 could explain their higher proportion in the middle of the interrow and in the monocropping

517 system. Variation in the GP:GN ratio suggests that microorganisms could be more
518 copiotrophic in the tree row than in the interrow of the alley cropping system as the GP:GN
519 ratio increases with decreasing C availability in soil [75] (**Fig. 2a**).

520 Moreover, high-recalcitrant litter inputs, such as walnut leaves, i.e., litter known to be
521 rich in tannins and lignin [76], could have favoured the fungal pathway within or close to the
522 tree row, while we did not observe this. We hypothesize that the herbaceous cover under the
523 tree row adding labile litter and rhizodeposits buffers the recalcitrant effect of walnut litter.
524 These results raise the question of the importance of considering herbaceous cover in future
525 alley cropping studies.

526

527 **5. Conclusion**

528 Through the design of our sampling strategy, we evaluated changes in soil quality
529 according to the distance from the tree rows in the interrow of an alley cropping system and
530 compared them with a neighbouring monocropping system. The use of an integrative soil
531 quality index related to water and nutrient storage and to the support of biological activity
532 simplified the comparison of the various positions within the alley cropping plot and with the
533 monocropping plot. After 21 years, soil quality was significantly improved in the tree rows
534 compared with the interrow positions and monocropping. The tree rows and its permanent
535 herbaceous cover enriched the soil in organic matter through an increased input of above- and
536 belowground litters and possibly through enhanced rhizodeposition. The SQI was
537 significantly greater under tree rows and beyond until a 2-m distance from the rows, which
538 was clear evidence of spatial heterogeneity within the cultivated interrows. For the first time,
539 we showed that by considering the SQI of the weighted entire plot of the alley cropping
540 system that soil quality was significantly improved compared with that of the monocropping
541 system.

542

543 **Acknowledgments**

544

545 This work was supported by “La Fondation de France” and the French National
546 Program EC2CO Biohefect, France. The authors want to thank Annette Bérard (INRAE) at
547 UMR EMMAH in Avignon and Josiane Abadie (INRAE) at UMR Eco&Sols in Montpellier
548 for their help during MicroResp™ analyses, Delphine Mézière and Lydie Dufour (INRAE) at
549 UMR SYSTEM in Montpellier for their knowledge of the study site and their advice on the
550 sampling location and Paula Fernandes (CIRAD) at UR HORTSYS for her help with the
551 PLFA.

552

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Figure 1

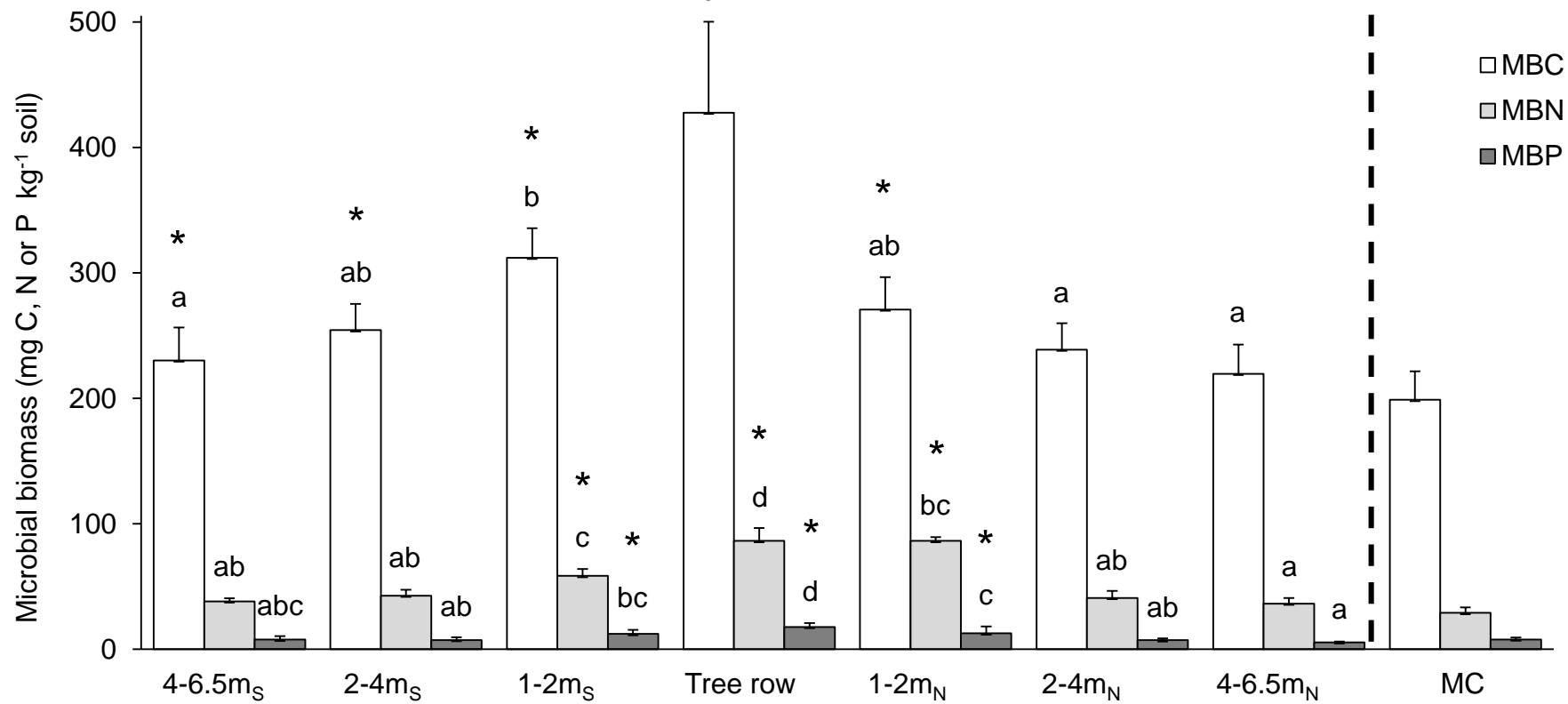


Figure 2a

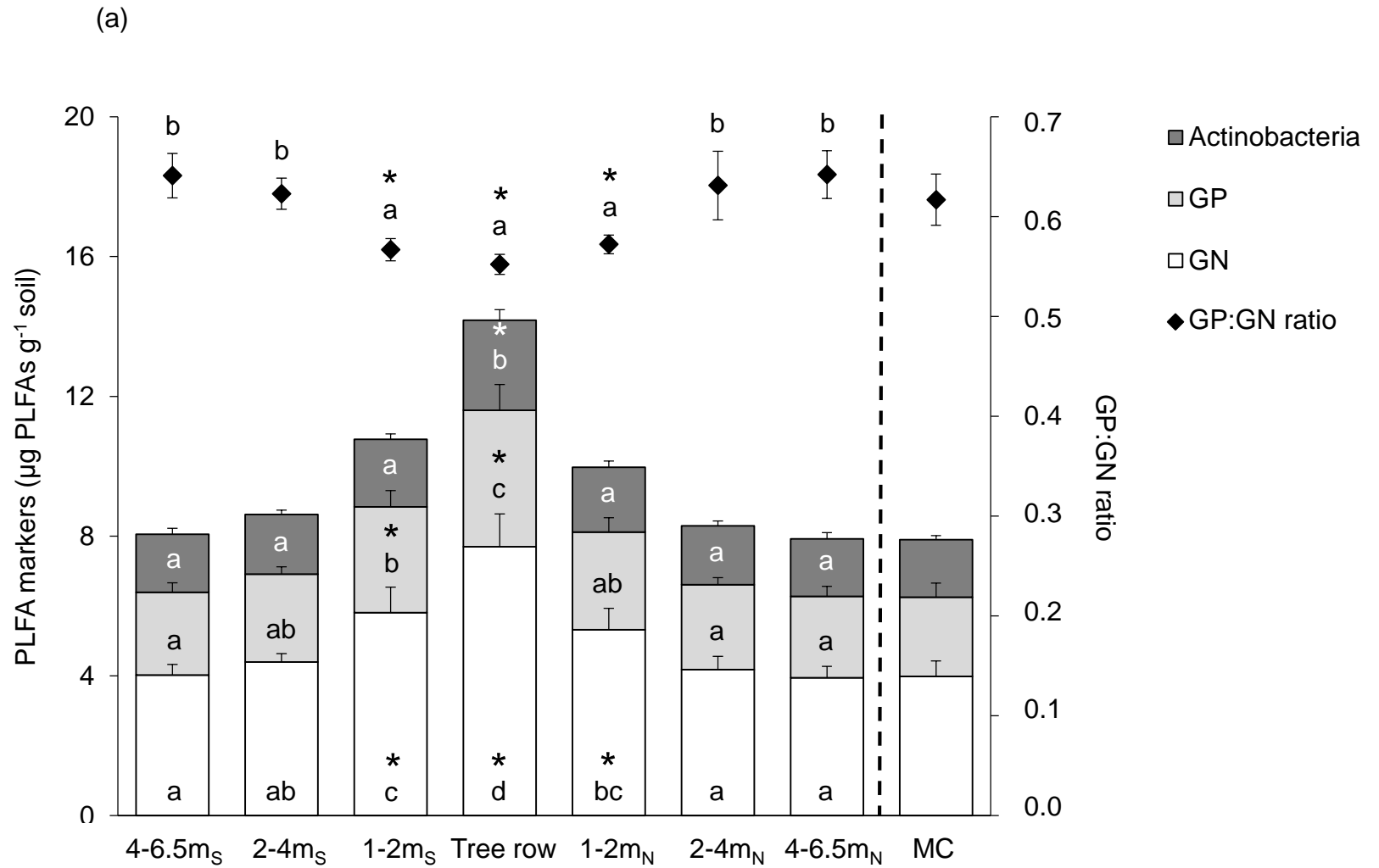


Figure 2b

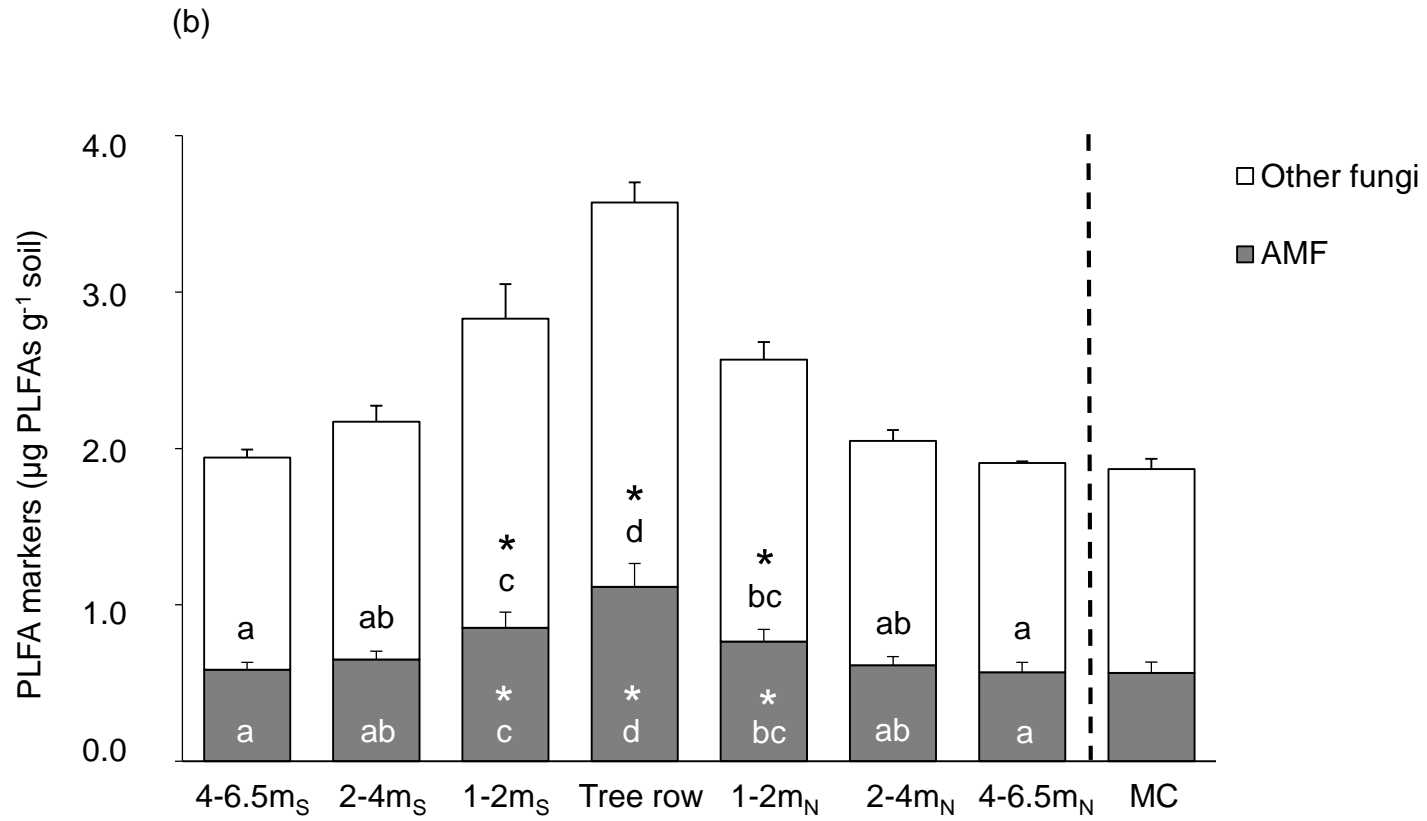


Figure 4

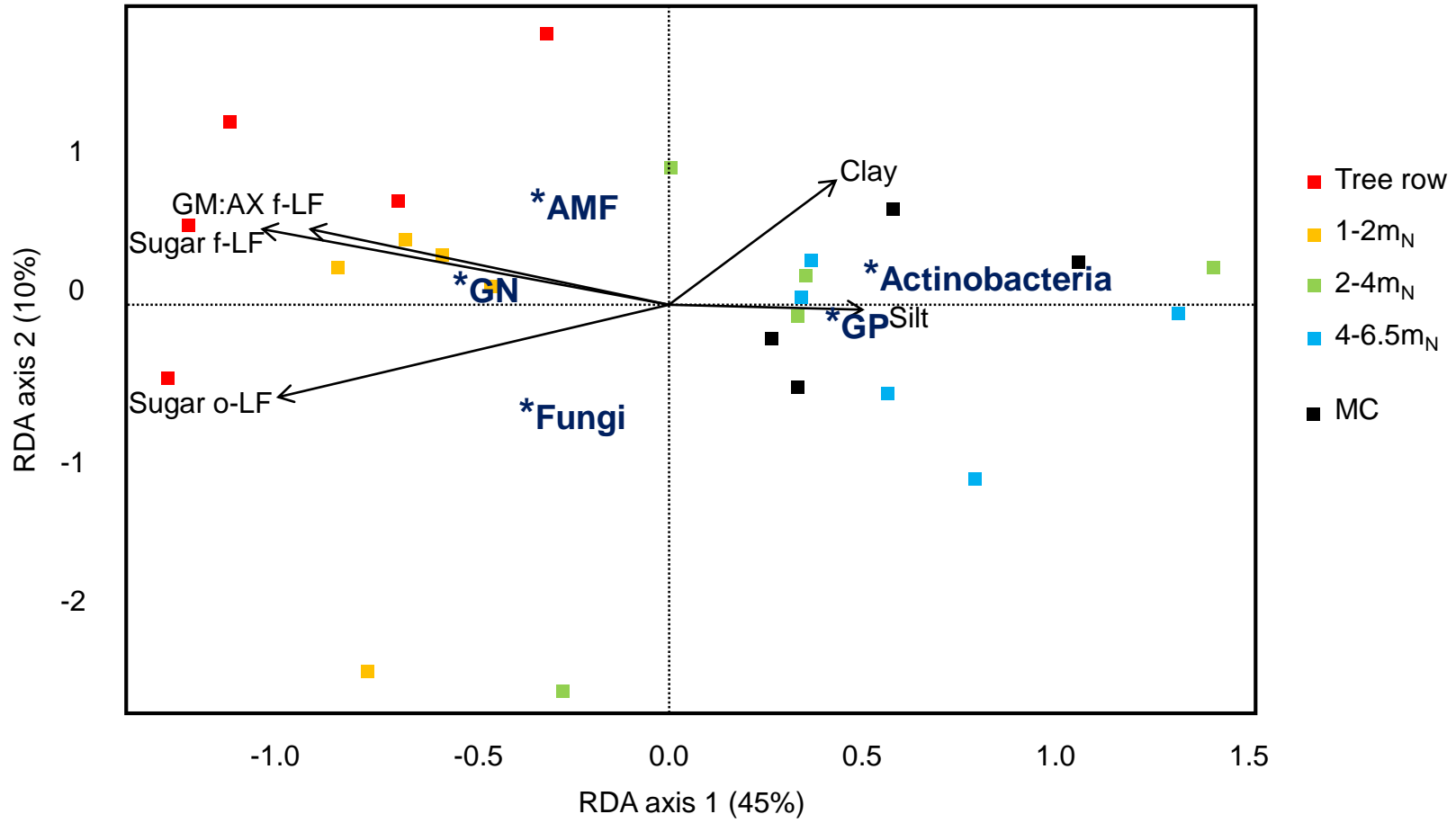


Table 1

Parameter	Tree row	1-2 m _N	2-4 m _N	4-6.5 m _N	MC
Clay (< 2 μm) (g kg ⁻¹ soil)	178 ± 9 ^a	173 ± 24 ^a	186 ± 7 ^a	184 ± 13 ^a	177 ± 10
Silt (2-50 μm) (g kg ⁻¹ soil)	157 ± 24 ^{a*}	165 ± 43 ^{a*}	157 ± 23 ^{a*}	158 ± 17 ^{a*}	208 ± 16
Sand (50-2000 μm) (g kg ⁻¹ soil)	109 ± 18 ^{a*}	101 ± 22 ^a	105 ± 25 ^a	103 ± 22 ^a	76 ± 20
Bulk density (g cm ⁻³)	1.24 ± 0.1 ^{a*}	1.41 ± 0.1 ^a	1.23 ± 0.1 ^{a*}	1.30 ± 0.1 ^a	1.43 ± 0.1
CaCO ₃ (g kg ⁻¹ soil)	539 ± 11 ^a	545 ± 22 ^a	540 ± 26 ^a	539 ± 18 ^a	527 ± 20
Moisture	14.3 ± 1.8 ^{c*}	13.3 ± 1.1 ^{bc*}	11.8 ± 0.7 ^a	11.9 ± 0.9 ^b	11.4 ± 0.6
pH _{water}	8.40 ± 0.05 ^{a*}	8.52 ± 0.04 ^{b*}	8.55 ± 0.03 ^b	8.57 ± 0.05 ^b	8.58 ± 0.03
SOC (g kg ⁻¹ soil)	19.0 ± 3.5 ^{b*}	16.4 ± 4.5 ^{ab}	14.6 ± 3.0 ^a	13.8 ± 2.6 ^a	11.8 ± 1.0
DOC (mg kg ⁻¹ soil)	34.5 ± 5.6 ^{b*}	23.4 ± 2.8 ^a	17.4 ± 4.9 ^a	18.1 ± 3.2 ^a	20.3 ± 2.6
N _{tot} (g kg ⁻¹ soil)	1.8 ± 0.2 ^{c*}	1.4 ± 0.1 ^b	1.3 ± 0.1 ^{ab}	1.2 ± 0.1 ^a	1.1 ± 0.1
C:N	10.9 ± 1.6 ^a	11.4 ± 2.0 ^a	11.2 ± 2.3 ^a	11.5 ± 3.9 ^a	10.2 ± 3.1
Mineral N (mg kg ⁻¹ soil)	31 ± 5 ^{b*}	16 ± 4 ^{a*}	11 ± 2 ^a	11 ± 2 ^a	11 ± 2
Olsen P (mg kg ⁻¹ soil)	8.3 ± 2.1 ^b	5.8 ± 1.2 ^a	5.2 ± 1.6 ^a	5.6 ± 2.3 ^a	5.8 ± 1.3
CEC (cmol ⁺ kg ⁻¹ soil)	14.7 ± 1.9 ^{b*}	13.0 ± 1.6 ^a	12.5 ± 1.4 ^a	12.4 ± 1.3 ^a	11.8 ± 0.4
Exch. Ca (cmol ⁺ kg ⁻¹ soil)	14.7 ± 1.6 ^{b*}	13.3 ± 1.5 ^a	13.1 ± 1.1 ^a	12.8 ± 1.1 ^a	12.5 ± 0.5
Exch. Mg (cmol ⁺ kg ⁻¹ soil)	0.73 ± 0.12 ^{b*}	0.47 ± 0.10 ^{a*}	0.39 ± 0.09 ^{a*}	0.38 ± 0.07 ^{a*}	0.29 ± 0.02
Exch. K (cmol ⁺ kg ⁻¹ soil)	0.51 ± 0.08 ^{b*}	0.42 ± 0.08 ^{ab*}	0.36 ± 0.08 ^a	0.35 ± 0.07 ^a	0.31 ± 0.02

Table 2

Fraction	Tree row	1-2 m _N	2-4 m _N	4-6.5 m _N	MC
f-LF (g C kg ⁻¹ soil)	3.6 ± 1.3 ^{b*}	2.1 ± 0.9 ^a	1.5 ± 0.5 ^a	1.3 ± 0.5 ^a	1.2 ± 0.3
o-LF (g C kg ⁻¹ soil)	2.2 ± 2.2 ^{b*}	1.7 ± 1.7 ^{ab*}	1.4 ± 1.4 ^a	1.2 ± 1.2 ^a	1.0 ± 1.0
50-2000 μm (g C kg ⁻¹ soil)	3.6 ± 0.9 ^b	2.3 ± 0.3 ^{ab}	2.0 ± 0.2 ^a	2.0 ± 0.1 ^a	1.5 ± 0.1
<50μm (g C kg ⁻¹ soil)	12.0 ± 2.1 ^{b*}	9.0 ± 1.8 ^a	8.6 ± 1.9 ^a	7.9 ± 1.8 ^a	7.8 ± 1.0

Table 3

Fraction	Tree row	1-2 m _N	2-4 m _N	4-6.5 m _N	MC
Total (mg sugar C g ⁻¹ soil)	4.4 ± 0.2 ^b	4.1 ± 0.1 ^a	4.0 ± 0.2 ^a	4.0 ± 0.2 ^a	4.0 ± 0.3
f-LF (mg sugar C g ⁻¹ soil)	0.57 ± 0.19 ^{b*}	0.35 ± 0.11 ^a	0.32 ± 0.07 ^a	0.28 ± 0.04 ^a	0.29 ± 0.08
o-LF (mg sugar C g ⁻¹ soil)	0.38 ± 0.05 ^{a*}	0.31 ± 0.07 ^{a*}	0.29 ± 0.20 ^a	0.22 ± 0.09 ^a	0.16 ± 0.06
< 50µm (mg sugar C g ⁻¹ soil)	2.26 ± 0.4 ^a	2.15 ± 0.2 ^a	1.87 ± 0.7 ^a	2.10 ± 0.2 ^a	2.36 ± 0.1
GM:AX Total	0.95 ± 0.06 ^a	0.96 ± 0.04 ^a	1.00 ± 0.04 ^a	0.99 ± 0.04 ^a	0.97 ± 0.05
GM:AX f-LF	0.85 ± 0.15 ^{b*}	0.73 ± 0.09 ^{ab}	0.71 ± 0.09 ^{ab}	0.67 ± 0.09 ^a	0.62 ± 0.08
GM:AX o-LF	0.95 ± 0.03 ^a	0.91 ± 0.07 ^a	0.97 ± 0.04 ^{a*}	0.95 ± 0.04 ^a	0.90 ± 0.03
GM:AX < 50µm	1.05 ± 0.01 ^a	1.03 ± 0.02 ^a	1.04 ± 0.02 ^a	1.02 ± 0.02 ^a	1.03 ± 0.01

Table 4

Substrate	Tree row	1-2m _N	1-2m _S	2-4m _N	2-4m _S	4-6.5m _N	4-6.5m _S	MC
Water	0.57 ± 0.09 ^{ab}	0.59 ± 0.20 ^{ab}	0.72 ± 0.25 ^b	0.45 ± 0.12 ^{ab}	0.38 ± 0.11 ^a	0.36 ± 0.10 ^a	0.35 ± 0.06 ^a	0.43 ± 0.16
Glucose	2.65 ± 0.47 ^{b*}	2.12 ± 0.50 ^{ab*}	2.49 ± 0.50 ^{b*}	1.97 ± 0.41 ^{ab*}	1.98 ± 0.31 ^{ab*}	1.72 ± 0.41 ^a	1.81 ± 0.30 ^{ab*}	1.30 ± 0.21
Trehalose	1.99 ± 0.57 ^{a*}	1.80 ± 0.61 ^{a*}	1.98 ± 0.51 ^{a*}	1.39 ± 0.35 ^a	1.44 ± 0.22 ^{a*}	1.17 ± 0.52 ^a	1.11 ± 0.14 ^a	1.05 ± 0.19
Alanine	1.50 ± 0.56 ^{c*}	1.03 ± 0.61 ^{abc*}	1.44 ± 0.50 ^{bc*}	0.95 ± 0.20 ^{abc}	0.99 ± 0.34 ^{abc}	0.62 ± 0.16 ^a	0.72 ± 0.17 ^{ab}	0.73 ± 0.12
qCO ₂	0.22 ± 0.02 ^a	0.28 ± 0.05 ^a	0.29 ± 0.08 ^a	0.24 ± 0.09 ^a	0.19 ± 0.05 ^a	0.21 ± 0.03 ^a	0.20 ± 0.03 ^a	0.33 ± 0.11