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Carbon partitioning in a walnut-maize agroforestry system through arbuscular mycorrhizal fungi

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1 **Abstract**

2 In tree-based intercropping systems, roots of trees and crops are interacting and could
3 influence ecosystem services provided by soil microorganisms. Here, the analysis of
4 diversity of arbuscular mycorrhiza fungi (AMF) associated with roots of walnut and
5 maize reveals differences. Of interest, *Funneliformis* genus is mainly associated with
6 maize roots, and one OTU related to an uncultured *Glomus*, might form a common
7 mycorrhizal network linking roots of both plants. In addition, the analysis of ¹³C of
8 mycelium living in the surrounding environment of roots, suggest that part of the
9 carbon derived from walnut trees could be transferred to maize plants. Our results
10 suggest that in temperate agroforestry ecosystems, AMF could participate in the
11 redistribution of nutrients between connected plants.

12

13 **Main Text**

14 Agroforestry combine, in the same plot of land, silvicultural **and/or** forestry
15 productions with agricultural production (crops or livestock), and is synonymous with
16 agro-sylvo-pastoralism (www.worldagroforestry.org). In one system of agroforestry, the
17 tree-based intercropping systems, crops are located in alleys between rows of trees (Jose
18 *et al.*, 2000; Bainard *et al.*, 2011). Productivity of these systems depends on the net
19 difference between benefits and costs among uses and biophysical components of the
20 agro-environment (Thevathasan and Gordon, 2004). **In tree-based intercropping**
21 **systems, tree benefits are both economical and ecological by (i) producing wood, (ii)**
22 **maintaining and improving soil structure and biodiversity conservation (Price and**
23 **Gordon, 1999; Stamps and Linit, 1998), (iii) reducing wind speed and soil evaporation**
24 **(Jose *et al.*, 2004), (iv) increasing carbon (C) storage both in wood and soil, (v) recycling**
25 **leached nutrient through deep tree roots (Jose *et al.*, 2004) and (vi) allowing a better**
26 **resilience to climate change (Moorhead and Dickens, 2012; Nehrlich *et al.*, 2012). In**
27 **contrast, trees could have negative impacts on growth and yield of crops as they could**
28 **reduce light access by shading crops, with C4 plants more vulnerable than C3 (Reynolds**
29 ***et al.*, 2007), and as they are better competitors for soil nutrients. In these system,**

30 reducing the application of inorganic fertilizers (Thevathasan and Gordon, 2004) lead to
31 lowering yield.

32 Roots of trees and crops are intermingled, and their interaction could influence soil
33 microorganisms, playing a central role in nutrient cycling and in the production of tree-
34 based intercropping systems by ecosystem services they provide. These include
35 arbuscular mycorrhizal fungi (AMF), living in a mutualistic symbiosis with the majority
36 of plant roots. AMF belong to the phylum Glomeromycota (Tedersoo *et al.*, 2018), with
37 more than 200 species described based on spore morphology (Schüssler and Walker,
38 2010). AMF receive carbon from their host plant (Bryla and Eissenstat, 2005). In return,
39 the extra-radical mycelium (ERM) forage the soil and provide the host plant with
40 mineral nutrients (Smith and Read, 2008). AMF display a little host specificity and their
41 ERM can colonize simultaneously several plants from the same or different species
42 (Barto *et al.*, 2012), forming a Common Mycorrhizal Network (CMN), allowing to
43 transfer and exchange nutrients and signals among plants (Wipf *et al.*, 2019). In
44 temperate agroecosystems, the abundance and community composition of AMF as well
45 as the functioning of CMN are strongly and negatively influenced by conventional
46 agricultural practices and crop management techniques as tillage and inorganic
47 fertilization (Helgason *et al.*, 1998; Oehl *et al.*, 2003; Jumponen *et al.*, 2005; Brito *et al.*,
48 2012; Brigido *et al.*, 2017). Sustainable management practices may lead to positively
49 influence the composition, the richness and the abundance of AMF communities when
50 comparing agricultural fields with grasslands (Burrows and Pflieger, 2002; Jansa *et al.*,
51 2003; Sturmer and Siqueira, 2011). In addition, tree-based intercropping systems may
52 lead to the maintenance of the CMN regarding low soil disturbance or tillage
53 (Hailemariam *et al.*, 2013) and to the faster establishment of root colonization by AMF
54 when switching from one crop to another (Mason and Wilson, 1994). However, the
55 influence of tree-based intercropping systems on diversity of AMF communities, both
56 on tree and crop roots, and on the functioning of the CMN is poorly understood in
57 temperate regions (Chiffot *et al.*, 2009; Lacombe *et al.*, 2009) compared to tropical
58 regions (*i.e.* Snoeck *et al.*, 2010; Jalonen *et al.*, 2013; Dobo *et al.*, 2018). CMN have been

59 studied in many systems, both in field (Babikova *et al.*, 2014) and controlled conditions
60 (Walder *et al.*, 2015; Rezacova *et al.*, 2018a, 2018b), and were focused on how the network
61 benefits the plant hosts. Diversity of AMF forming the CMN and associated with roots
62 as well as the C contribution of plants in the building of CMN, was never assessed in the
63 field. Here, we used walnut trees (*Juglans nigra* L., a C3-plant) and maize (*Zea mays* L., a
64 C4-plant), which display distinctly different ratios of $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$), to trace the delivery
65 of plant C to AMF. In 2017, an old walnut tree orchard was replanted in several row
66 distanced of 8 m with Fernette walnut variety after a light vibra-shank cultivator
67 treatment. On May 30 2018, 8 rows of maize were planted, at a density of 81000
68 seeds/ha, in the area between two walnut rows. Young roots, from the walnut trees and
69 from the maize plants of the second row (5m-away), were sampled for AMF diversity,
70 and traps, buried at 15 cm for 4-months, were used to collect fungal mycelium for $\delta^{13}\text{C}$
71 measurements. Hyphal traps were 0.45L bags (15 x 15 x 2 cm), made with 21 μm -nylon
72 meshes and filled with a mixture of zeolithe (Symbion, Czech Republic) and sand (1 : 1
73 v/v).

74 The AMF diversity was assessed by Illumina sequencing targeting the AMF large
75 ribosomal sub-unit. All the detected OTUs belonged to the Glomerales order (Figure 1)
76 with one OTU, (*Glomus* sp Cluster 1) showing a homology of 98.8% with an uncultured
77 *Glomus* (HQ243144), grouped 33% and 22% of the sequences isolated, respectively from
78 the maize or the walnut root system. OTUs belonging to the *Rhizophagus* and
79 *Septoglomus* genus grouped respectively 29% and 13% of the total sequences. They
80 grouped respectively 38% and 21% of the sequences in walnut roots, and only 21% and
81 4% in maize roots. *Claroideoglomus* was also 10 times more present in walnut roots than
82 in maize roots. On the opposite, sequences belonging to the *Funneliformis* genus were
83 nearly exclusively detected in maize roots (33%) compared to walnut roots (0.01%), with
84 29% of the sequences detected in maize corresponded to the *F. mosseae* species. These
85 observations are in agreement with other reports showing that *Funneliformis* are often
86 reported as tolerant to soil disturbance (Jansa *et al.*, 2002, 2003; Borriello *et al.*, 2012; Avio
87 *et al.*, 2013; Wetzal *et al.*, 2014; Peyret-Guzzon *et al.*, 2016).

88 In order to determine the plant source of the carbon found in the fungal mycelium,
89 values of $\delta^{13}\text{C}$ were determined as previously described (Courty *et al.*, 2011) in the
90 walnut or maize plant leaves as well in the mycelium. The $\delta^{13}\text{C}$ of walnut leaves (-32 ‰)
91 was significantly different from the $\delta^{13}\text{C}$ of maize leaves (-14‰) (Figure 2). **Mycelium**
92 **collected from the traps close to walnut and maize roots displayed significant $\delta^{13}\text{C}$**
93 **values** of ca. -26‰ and ca. -21‰, respectively, suggesting a strong influence of
94 neighboring plants to support AMF C needs. In addition, differences in the $\delta^{13}\text{C}$ values
95 between **the common mycelium** collected under maize plants and walnut trees, could
96 reflect a mycelium extending from the walnut trees to the maize plants. In fact, we may
97 consider that the AMF mycelium close to maize roots was receiving C, not only from
98 maize, but also from walnut trees. We do recognize that the collected mycelium could
99 have different origins (*i.e.* AMF, saprobes) and thereby impact the $\delta^{13}\text{C}$ values. However,
100 the fact that one of the most predominant OTU was detected in the roots systems of both
101 plants allows to hypothesize the presence of a CMN between these plants.

102 In summary, $\delta^{13}\text{C}$ analysis and OTUs suggest that walnut of walnut-based intercropping
103 systems could have an impact on the C distribution. This experiment gives some
104 evidence of a physiological connection through the ERM between a perennial and a crop
105 plant. Further experiments should be carried out to investigate of the seasonal dynamics
106 of this exchange as well as the possible seasonal modifications of the AMF community.

107

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112

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250

251 **Figure 1**

252 **Stacked bar of the taxonomic assignation of the OTU obtained from the maize (MAR) or**
253 **walnut (WAR) associated roots representing 90% of the total sequences. The DNA was**
254 **extracted from five independent walnut or maize root samples and the AMF large**
255 **ribosomal sub-unit amplified as previously described (Brigido *et al.*, 2017), with the**
256 **exception that the FLR3-FLR4 primers used for the second PCR were linked to Illumina**

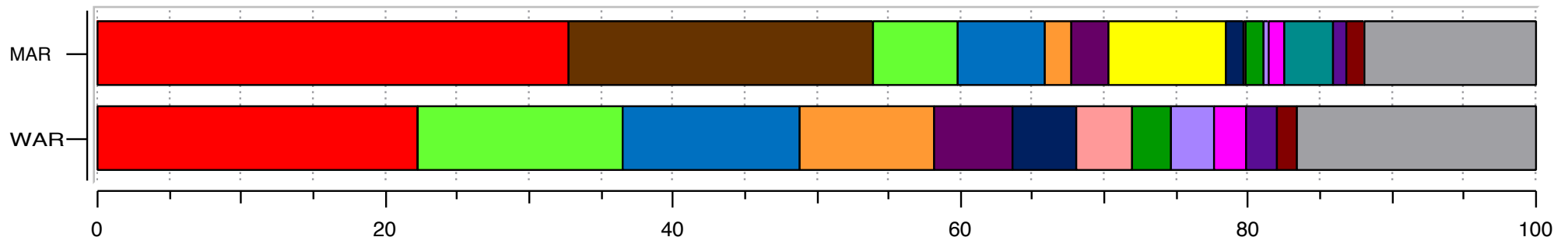
257 Miseq adaptors. Samples were pair-end sequenced on an 2x250 Illumina platform. The
258 sequences obtained were clustered using the FROGS pipeline (Escudié *et al.*, 2017)
259 implemented on the Galaxy platform (Drain *et al.*, 2019) and taxonomically assigned
260 against the MaarjAM database (Öpik *et al.*, 2010). Using as filter parameters the
261 abundance of < 0.0005, 17880 OTU representing 11% of the sequence abundance were
262 removed. The final 93 identifyied OTU belonged all to the Glomeronycota. The relative
263 abundance data were obtained after rarefaction of all samples to the lowest number of
264 reads in a sample (12524).

265

266 **Figure 2**

267 $\delta^{13}\text{C}$ values of walnut and maize roots, as well as mycelium collected from trapped
268 (n=5). Different lowercase letters indicate significant differences according to pairwise *t*-
269 tests (Bonferroni corrected, $P < 0.01$)

270



■ *Glomus* sp Cluster_1

■ *F. mosseae* Cluster_9

■ *R. irregularis* Cluster_16

■ *R. irregularis* Cluster_10

■ *S. viscosum* Cluster_23

■ *R. irregularis* Cluster_32

■ *F. mosseae* Cluster_20

■ *Glomus* sp Cluster_8

■ *S. viscosum* Cluster_77

■ *R. irregularis* Cluster_27

■ *Claroideoglomus* sp Cluster_13

■ *R. irregularis* Cluster_68

■ *F. constrictum* Cluster_4

■ *S. viscosum* Cluster_45

■ *Rhizophagus* sp Cluster_201

■ Other

