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

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## Abstract

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

## Main Text

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

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

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

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

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

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

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

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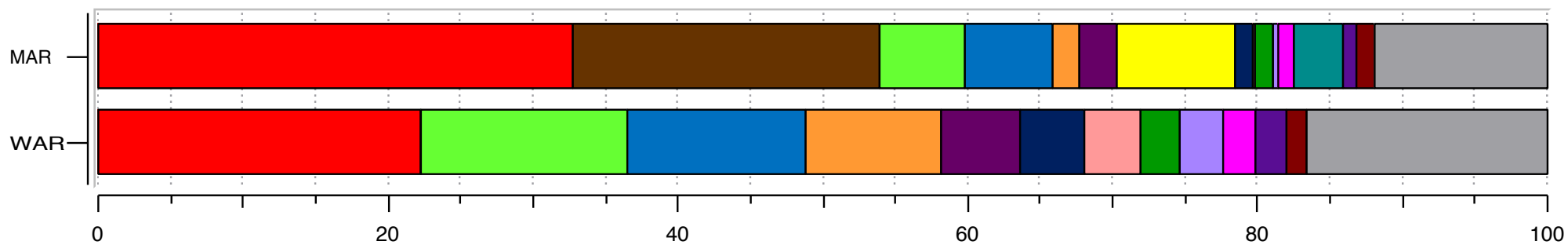
## Figure 1

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

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

## Figure 2

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



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

