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*Short Note*

# In Winter Wheat, No-Till Increases Mycorrhizal Colonization thus Reducing the Need for Nitrogen Fertilization

**Julien Verzeaux [1], David Roger [1], Jérôme Lacoux [1], Elodie Nivelle [1], Clément Adam [1], Hazzar Habbib [1], Bertrand Hirel [2,*], Frédéric Dubois [1] and Thierry Tetu [1]**

[1] Ecologie et Dynamique des Systèmes Anthropisés (EDYSAN, FRE 3498 CNRS UPJV), Laboratoire d'Agroécologie, Ecophysiologie et Biologie intégrative, Université de Picardie Jules Verne, 33 rue St Leu, Amiens Cedex 80039, France; julienverzeaux@gmail.com (J.V.); david.roger@u-picardie.fr (D.R.); jerome.lacoux@u-picardie.fr (J.L.); elodienivelle@gmail.com (E.N.); clementadam80@gmail.com (C.A.); hazzar.habbib@u-picardie.fr (H.H.); frederic.dubois@u-picardie.fr (F.D.); thierry.tetu@u-picardie.fr (T.T.)

[2] Adaptation des Plantes à leur Environnement. Unité Mixte de Recherche 1318, Institut Jean-Pierre Bourgin, Institut National de la Recherche Agronomique, Centre de Versailles-Grignon, R.D. 10, Versailles Cedex F-78026, France

* Correspondence: bertrand.hirel@versailles.inra.fr; Tel.: +33-1-30-83-30-89

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**Abstract:** Arbuscular mycorrhizal fungi (AMF) play a major role in the uptake of nutrients by agricultural plants. Nevertheless, some agricultural practices can interrupt fungal-plant signaling and thus impede the establishment of the mycorrhizal symbiosis. A field experiment performed over a 5-year period demonstrated that both the absence of tillage and of nitrogen (N) fertilization improved AMF colonization of wheat roots. Moreover, under no-till conditions, N uptake and aboveground biomass production did not vary significantly between N-fertilized and N-unfertilized plots. In contrast, both N uptake and above ground biomass were much lower when N fertilizer was not added during conventional tillage. This finding strongly suggests that for wheat, no-till farming is a sustainable agricultural system that allows a gradual reduction in N fertilizer use by promoting AMF functionality and at the same time increasing N uptake.

**Keywords:** winter wheat; tillage; nitrogen fertilization; arbuscular mycorrhizal fungi; nitrogen uptake

## 1. Introduction

Arbuscular mycorrhizal fungi (AMF) form obligate symbioses (mycorrhizas) with most cultivated plants [1] and provide many benefits to plants throughout their growth [2]. The intraradical colonization of plant roots by AMF results in the formation of some specialized structures including vesicles for nutrient storage and arbuscules for exchanging nutrients with the host plant. Such symbiotic associations significantly enhance the uptake capacity of plants for nutrients [2,3] beyond the depletion zone surrounding a root, especially for the inorganic phosphate ion and the ammonium ion [4]. In current intensive agricultural systems, soil management practices may be detrimental to an efficient fungal-plant symbiotic interaction [5], notably nitrogen (N) fertilization [6], which at the same time may reduce the diversity of fungal communities [7]. Conventional tillage (CT) causes a physical disruption of the hyphal network in the soil, thus reducing the density of propagules in the rooting zone [8]. However, knowledge of the combined effects of N fertilization and tillage on AMF colonization and N uptake in the field remains limited [2], especially for an important crop such as winter wheat. The present study has demonstrated that over a 5-year period, tillage and N

fertilization reduced AMF colonization of wheat roots and that plant N uptake capacity was reduced in the absence of N fertilization under CT conditions.

## 2. Results and Discussion

In the field experiment, both CT and N fertilization strongly decreased wheat root colonization by AMF ($p < 0.001$). The highest AMF root colonization was obtained with no-till without N fertilization (NTN0, 35% of root length colonized by AMF) and the lowest with conventional tillage with N fertilization (CTNX, 2%) (Figure 1A). It is known that soil disturbance and N enrichment resulting from plowing [8,9] and N fertilization [6] reduce the ability of plants and AMF to form mycorrhizas. Moreover, such a reduction seems to be greater when both plowing and N fertilization are used. In line with this observation, Mbuthia *et al.* [10] reported that no-till (NT) and low N fertilizer application were associated with the presence of more AMF in the soil. This could explain why in the present study there were larger amounts of AMF in the roots following NT without any N fertilization (N0). It is also well known that mycorrhizal functioning is strongly influenced both by plant nutrient status and by soil nutrient availability [6]. When nutrient availability is high, root exudate composition can be qualitatively modified, thus decreasing fungal colonization. Moreover, it has been shown that root exudates can stimulate hyphal growth and branching [11], through an attractive effect [12]. Recently, it has been reported that in sorghum, N fertilization negatively influences the production and exudation of strigolactones that are essential host recognition signaling molecules involved in AMF colonization [13]. It is therefore likely that low N availability induces changes both in the root system and in the composition of the root exudate, thus inducing the different processes controlling AMF colonization.

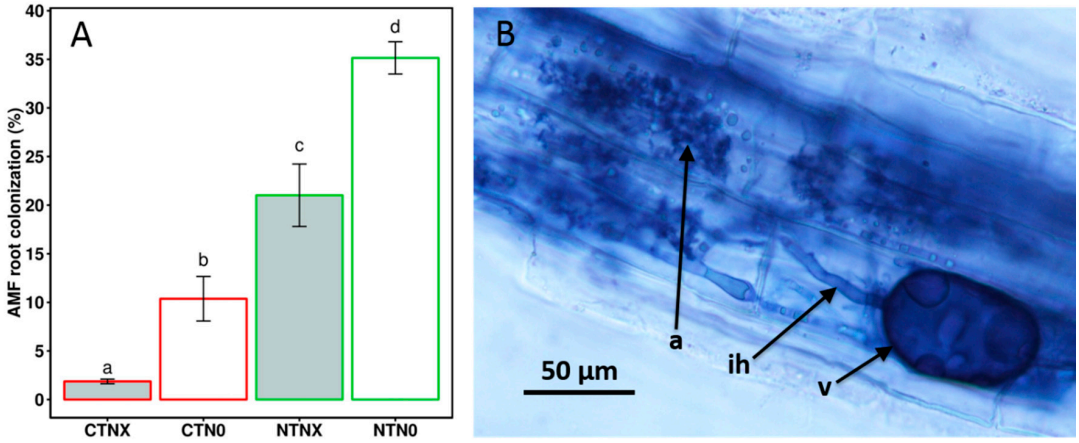


**Figure 1.** (**A**) Effect of tillage and nitrogen (N) fertilization on the percentage of wheat root length colonized by arbuscular mycorrhizal fungi (AMF). Values are means ± standard error. Letters, a, b, c and d indicate differences among treatments according to the Conover post-hoc test ($p < 0.05$), following a significant Kruskal-Wallis test ($p < 0.001$). CT: conventional tillage; NT: no-till; NX: chemical N fertilization; N0: without N fertilization; (**B**) Wheat root colonized by arbuscular mycorrhizal fungi. a: arbuscule, v: vesicle, ih: intraradical hypha.

The wheat aboveground (AG) biomass at anthesis was the highest under CTNX and NTN0, and was the lowest under conventional tillage without N fertilization (CTN0, $p < 0.05$; Table 1). In addition, there was a much lower AG biomass production in the absence of N fertilizer under CTN0, compared to CTNX. In contrast, total AG plant biomass remained similar between NX and N0 under NT conditions. It was therefore not surprising to observe the same differences for plant N uptake between the different soil treatments and N fertilization conditions ($p < 0.05$; Table 1). When the intraradical colonization of plant roots by AMF is established, the formation of specialized structures for the exchange of nutrients such as arbuscules, enhances the absorbing capacity of the root for water and nutrients [14] and,

as such, speeds up plant growth [15,16]. Moreover, the mycorrhizosphere, as a spatial extension of the rhizosphere associated with the hyphosphere [17], is known to increase the soil volume in which the N-cycling processes can occur. This could partly explain why N uptake decreased only when there was no N fertilization and when root colonization by AMF was reduced under CT condition (CTN0). In contrast, under NT conditions, in the absence of N fertilization, N uptake was maintained by the plant-fungus symbiosis. The extraradical hyphae of AMF are able to take up and assimilate inorganic N [18,19], originating from N fertilizer or released following the decomposition of patches of organic matter. Furthermore, Hodge *et al.* [4] provided evidence that AMF were also able to acquire N directly from the soil organic material, which is able to stimulate hyphae growth. In this study, the higher root colonization of wheat by AMF under NT could be explained by the fact that these soils are characterized by higher contents of fresh organic matter in the upper layer because there is no soil inversion. In the absence of N fertilization, the hyphosphere may prospect for organic nutrients, thus maintaining whole plant N uptake capacity. However, we observed that soil N and C contents were not higher in NT compared to CT (Table 1). Such a finding can be explained by the fact that fresh organic matter was eliminated by sieving before our analysis and by the absence of cover crops during winter periods from the beginning of the experiment. Nevertheless, the soil C:N ratio was significantly higher in NT plots ($p < 0.001$), suggesting a higher availability of C originating from the crop residues present in the upper soil layer. Taken together, our results suggest that the ecological impact of N transfer from AMF to wheat might be higher when the physical environment enhances spore germination and hyphae growth (*i.e.*, lack of physical disturbance in NT) and when mineral N availability is low (*i.e.*, lack of intensive N fertilization in N0), thus maintaining the emission of chemical signals by roots. It can be concluded that direct-seeding mulch-based cropping systems, by stabilizing soil structure and by promoting organic nutrient utilization instead of using inorganic fertilizers, appears to be suitable for sustainable wheat production. In the future, long-term studies will be required to fully assess the impact of cropping systems and the mode of N fertilization on plant-AMF interactions, including the recognition of signaling molecules between the fungi and its host.

**Table 1.** Impact of nitrogen fertilization and tillage on agronomic traits and soil parameters of wheat plants.

| | H (*p*) | CTNX | CTN0 | NTNX | NTN0 |
|---|---|---|---|---|---|
| AG biomass (g· plant$^{-1}$) | 10.61 (0.014) | 7.02 ± 0.43 $^{b}$ | 4.88 ± 0.27 $^{a}$ | 6.07 ± 0.30 $^{ab}$ | 7.13 ± 0.65 $^{b}$ |
| Plant N concentration (mg· g$^{-1}$) | NS | 10.35 ± 0.45 | 9.56 ± 0.40 | 10.89 ± 0.66 | 9.68 ± 0.55 |
| Plant N uptake (mg· plant$^{-1}$) | 9.25 (0.03) | 72.37 ± 4.95 $^{b}$ | 46.98 ± 4.06 $^{a}$ | 66.83 ± 7.03 $^{ab}$ | 68.02 ± 5.37 $^{ab}$ |
| Soil N (g· kg$^{-1}$) | 12.49 (0.006) | 1.46 ± 0.01 $^{b}$ | 1.45 ± 0.01 $^{b}$ | 1.40 ± 0.03 $^{b}$ | 1.31 ± 0.02 $^{a}$ |
| Soil C (g· kg$^{-1}$) | NS | 13.15 ± 0.17 | 12.93 ± 0.10 | 14.10 ± 0.33 | 13.17 ± 0.35 |
| Soil C:N ratio | 17.64 (0.0005) | 9.01 ± 0.08 $^{a}$ | 8.93 ± 0.10 $^{a}$ | 10.08 ± 0.14 $^{b}$ | 10.04 ± 0.21 $^{b}$ |
| Soil compaction (MPa) | NS | 0.13 ± 0.01 | 0.11 ± 0.03 | 0.23 ± 0.05 | 0.20 ± 0.03 |

In H: Values of the Kruskal-Wallis test with its probability in brackets. Letters, a, ab and b indicate differences among treatments according to the Conover post-hoc test ($p < 0.05$), following a significant Kruskal-Wallis test ($p < 0.001$). CT: conventional tillage; NT: no-till; NX: with chemical N fertilization; N0: without N fertilization. NS: not significant. AG biomass: aboveground biomass. Values correspond to the mean ± standard error of plant and soil parameters among the four treatments.

## 3. Materials and Methods

### *3.1. Site Description and Experimental Design*

The field experiment was conducted at the experimental site of *La Woestyne*, in Northern France (50°44′ N, 2°22′ E, 40 m above sea level). The average annual air temperature and total rainfall were 10.5 °C and 675 mm respectively, with amounts of rainfall relatively homogeneous across seasons. The soil particle size composition was as follows: silt 66.8%, clay 21.2%, and sand 12%.

Prior to the establishment of the field experiment in 2009, the field was managed with chisel plowing and a rotary power system. In order to study the effect of tillage and N fertilization on wheat colonization by AMF, the experimental field was split into four replicate plots for each of the four treatments: conventional tillage with (CTNX) or without (CTN0) N fertilization; no-till with (NTNX) or without (NTN0) N fertilization. CTN0 and NTN0 plots measured 7 m × 8 m while CTNX and NTNX

plots measured 14 m × 8 m. The crop rotation before the sampling date consisted of green peas (*Pisum sativum* L.) in 2010, maize (*Zea mays* L.) in 2011, winter wheat (*Triticum aestivum* L.) in 2011–2012, flax (*Linum usitatissimum* L.) in 2013, sugar beet (*Beta vulgaris* L.) in 2014 and winter wheat in 2014–2015. As maize was grown for silage and flax for fiber, all the aboveground structures were removed from the field. Pea haulms, wheat straw and beet leaves were returned to the soil. In all the NX plots, maize received 108 kg·N·ha$^{-1}$, wheat 160 kg·N·ha$^{-1}$, flax 80 kg·N·ha$^{-1}$ and sugar beet 160 kg·N·ha$^{-1}$ (50% urea, 25% ammonium, 25% nitrate). Green peas did not receive any N fertilization in NX plots according to European policies. The N0 plots have not been fertilized for the 5 years of experiment. In October 2014, the winter wheat used for crop sampling was sown at 12.5 cm of row spacing and 250 seeds m$^{-2}$ using an AS 400 drill (Alpego, Italia) and was fertilized in two times with 80 kg·N·ha$^{-1}$ in March and May 2015.

### *3.2. Sample Collection and Analyzes*

In June 2015, at the anthesis stage of winter wheat, six plants were randomly sampled in each of the four replicate plots for each treatment (CTNX, CTN0, NTNX and NTN0). The root system was collected by extracting 15 cm depth and 5 cm diameter soil cores directly over the cut stems of the six selected plants. Six 15 cm deep soil cores were also collected for soil N and C analysis using a 2 cm diameter auger.

The aboveground structures of plant samples were dried at 65 °C for 3 days and subsequently weighed (± 0.1 g accuracy) to determine total aboveground biomass. Each sample was then ground into a fine powder for plant total N and C analysis. Soil samples were sieved using a 2 mm mesh, dried at 35 °C for 48 h and ball-milled using a grinder MM 400 (Retsch, Haan, Germany). Soil total plant and soil N and C contents were determined using an elemental analyzer (Flash EA 1112 series, Thermo Fisher Scientific, Waltham, USA). Since the soil was free of carbonate, the soil organic C was assumed to be equal to the total soil C content.

AMF colonization of wheat was monitored in 30 root subsamples of 1 cm length by plant. Subsamples were stained with trypan blue according to Koske and Gemma [20]. Mycorrhizal infection was quantified using the method of McGonigle *et al.* [21], with 150 intersections counted for each sample.

Near the plant and soil sampling areas, penetration resistance was measured by using a penetrologger (Eijkelkamp, Giesbeek, The Netherlands) fitted with a 60 deg and 1 cm$^2$ base area cone.

### *3.3. Statistical Analysis*

All statistical analyzes were performed using the *R* software (v. 3.1.2, R Development Core Team). Mean values are given with their standard error. Plant and soil parameters, as well as AMF colonization, were compared among treatments by using a non-parametric Kruskal-Wallis one-way analysis of variance followed by a Conover post-hoc test whenever significant (*PMCMR* package, [22]).

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**Conflicts of Interest:** The authors declare no conflict of interest.

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