

# Abstract template

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## Perennial crops for sustainable soil management – Symbiotic fungi benefit from cultivation of a perennial cereal in Europe

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### Abstract summary

*Thinopyrum intermedium* (intermediate wheatgrass) is used as a model for future perennial cereal grain production and the influence of agricultural perennialization was assessed in terms of the biomass of arbuscular mycorrhizal fungi (AMF) in soil. Cultivating a perennial cereal (*T. intermedium*) proved to promote higher biomass of AMF in comparison to conventional, but also organic, annual wheat production as well as in comparison to annual wheat and rye under no-tillage cultivation. Our results indicate improved conditions for mycorrhizal fungi in perennial cereal production stands, which may provide enhanced plant nutrient status of the crop and overall crop performance, due to larger hyphal networks and improved soil structure.

**Keywords:** Mycorrhizal fungi, soil fungi, soil ecology, perennial cereal grain crops, agriculture, soil ecosystem services

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### Introduction, scope and main objectives

Intensive agricultural practices are important drivers to the global loss of soil biodiversity and soil functions (Tsiafouli et al 2015). Thus, innovative management practices to improve soil biodiversity needs to be implemented in contemporary agriculture. *Thinopyrum intermedium* (intermediate wheatgrass) provide a perennial, multifunctional and multipurpose cereal crop (Duchene et al 2019). A shift from systems based on annual to perennial grain-crop production with permanent to semi-permanent plant cover, without the negative consequences of inverting soil tillage, is expected to benefit the delivery of soil ecosystem services (Crews et al 2016). The soil microbial community structure and biomass can be used as early indicators of the effects of changed cropping practices on the soil quality. In particular, the hyphal growth of fungal organism groups, i.e. saprotrophic (Barreiro et al 2018) and mycorrhizal fungi (Hydbom et al 2017), benefit from less disturbance, and extensive management in general (Barreiro et al 2019). This would fulfil part of the second Global Sustainable Development Goals (SDGs), calling for more resilient agricultural practices, enhancing both productivity and ecosystem quality. Increased soil biodiversity would also contribute to SDGs 12, 13 and 14 through improved efficiency in the management of natural resources, improved climate resilience and reduced nutrient loss. We determined the effect of agricultural perennialization of arable cropping systems by *T. intermedium* on the soil microbiome. We hypothesize that biomass of arbuscular mycorrhizal fungi (AMF) will be higher in the soil under a perennial cereal crop compared to under annual cereals.

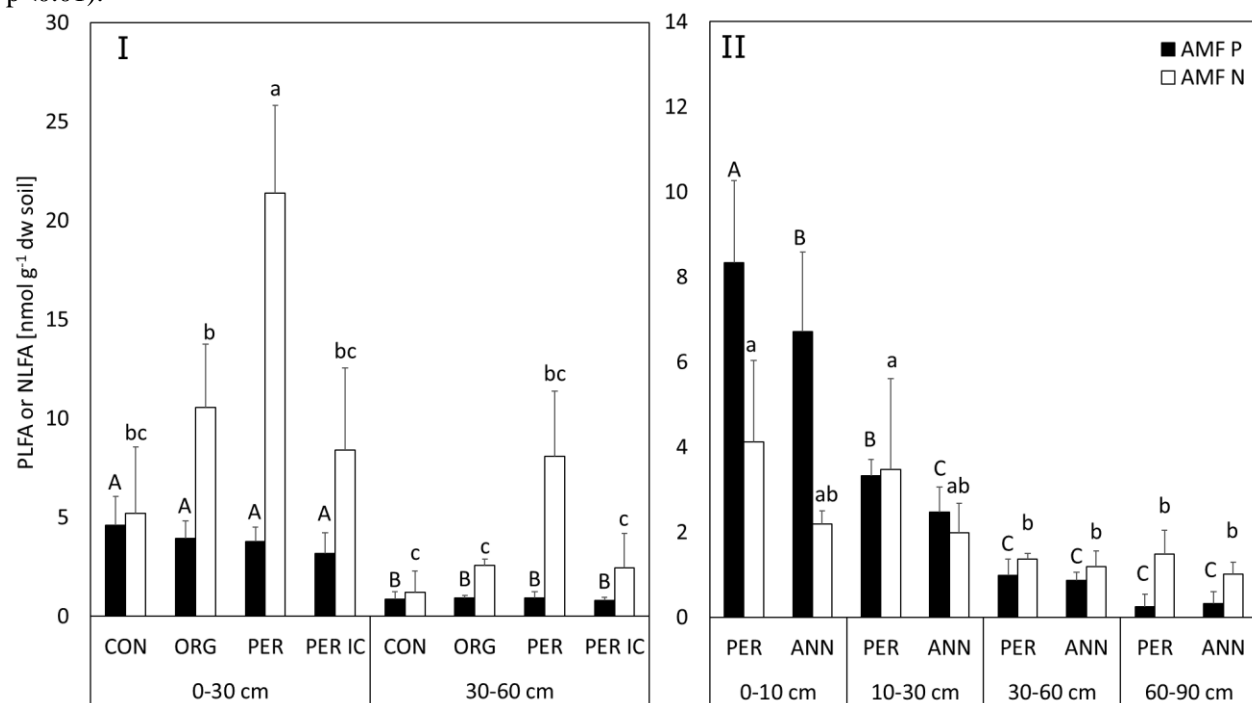
### Methodology

Mycorrhizal biomass indicators were analyzed in two field experiments. One field experiment was carried out at Alnarp (55.65°N, 13.06°E) in southernmost Sweden (established 2016), and another one in Maubec (50.37°N, 4.48°E) in southeast France (established in 2017). The Swedish experiment included sole crop stands of *T. intermedium* under organic management, intercrops of *T. intermedium* and *Medicago sativa* (lucerne) under organic management, and *Triticum aestivum* (winter wheat) under conventional and organic management. The French experiment included conventional sole crop stands of *T. intermedium* in an annual

grain crops rotation with *T. aestivum* (2017-2018), with direct sowing applied since 2007. Both experiments were replicated in four blocks for each treatment/crop. Soil sampling took place at the end of the growing season in 2018 using soil cores were four sub-samples made up one composite sample for each sampling depth and treatment plot. Phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) 16:1 $\omega$ 5 were used as indicators of AMF biomass (Olsson 1999; Frostegård and Bååth 1996; Olsson et al 1995). PLFA 16:1 $\omega$ 5 indicate both arbuscular mycorrhizal fungi (AMF) and Gram negative (G-) bacteria (Olsson 1999; Frostegård and Bååth 1996; Olsson et al 1995), thus, the NLFA 16:1 $\omega$ 5 is used as an accompanying indicator of AMF due to its high correlation with AMF spore counts in soil (Sharma and Buyer 2015). PLFAs and NLFAs were extracted using the protocol presented by Frostegård et al (1993) using CHCl<sub>3</sub>:MeOH:citrate buffer (1:2:0.8 v/v/v). The lipids were separated using pre-packed silica columns subsequently rinsed with CHCl<sub>3</sub> (neutral lipids) and MeOH (phospholipids). The neutral and phospholipids were subjected to methanolysis after adding FAME 19:0 as internal standard, and the resulting fatty acid methyl esters quantified by gas chromatography with flame ionization detector (GC-17A, Shimadzu).

## Results

In the Swedish experiment, the abundance of the one of the biomass indicators of AMF was higher in the perennial sole crop than in the three other crops at the 0-30 cm soil level ( $F_{AMF\_N}=13.48$  (df=3)  $p<0.001$ ) (Fig. 1:I). The biomass indicators of AMF ( $F_{AMF\_P}=112.6$  (df=1)  $p<0.001$ ;  $F_{AMF\_N}=38.54$  (df=1)  $p<0.001$ ) decreased from 0-30 cm to 30-60 cm depth. In the French experiment, the abundance of PLFA biomass indicator of AMF was higher in the perennial sole crop than in the annual crops at the level of 0-10 and 10-30 cm soil depth ( $F_{AMF\_P}=4.441$  (df=1)  $p<0.05$ ) (Fig. 1:II). The biomass indicators of AMF was generally higher in the upper soil level than further down the soil profile ( $F_{AMF\_P}=121.1$  (df=3)  $p<0.001$ ;  $F_{AMF\_N}=6.516$  (df=3)  $p<0.01$ ).



**Figure 1:** I) The abundance of neutral lipid and phospholipid fatty acid indicators (nmol g<sup>-1</sup> dw soil) of arbuscular mycorrhizal fungi (AMF P and AMF N) in the conventional annual wheat (CON), organic annual wheat (ORG), organic perennial wheatgrass (PER), organic perennial wheatgrass and lucerne intercrop (PER IC) in the SITES AGROECOLOGICAL FIELD EXPERIMENT (SAFE) at Lönstorp, SLU, Sweden. II) The abundance of AMF P and AMF N (nmol g<sup>-1</sup> dw soil) in perennial wheatgrass (PER) and annual wheat (ANN) at Maubec, France. Data are presented as means from each sampling depth (cm) and crop treatment with standard deviations. Different letters indicate differences at the significance level of  $p<0.05$  (ANOVA with Tukey's post hoc test using RStudio). Upper case letters are used for AMF P and lower case letters for AMF N.

## Discussion

Our hypothesis was partly confirmed in the Swedish experiment through the higher abundance of the NLFA marker for AMF biomass. The high abundance, and clear response, of the NLFA marker reflect the larger abundance of AMF spore counts (Sharma and Buyer 2015) in soil under the perennial crop. The lack of influence on the PLFA marker for AMF biomass may be due to the rather young age of the perennial stand. It has been shown that AMF colonization increases with increasing age of co-occurring plant (Grigera et al. 2007), motivating further studies to evaluate the longer-term development of the soil-crop interactions. The French experiment confirms the expectation to increase abundance of AMF biomass in soil under the perennial crop. The rather low response of the markers for AMF biomass may result from the overall application of no-tillage practices in both the annual and perennial crop, combined with the recent introduction of the perennial (as in the Swedish experiment). Further questions to explore is the potential to increase the plant's ability to acquire nutrients (Tran et al 2019) and the potential of AMF to improve soil structure and related soil functions. Intercropping with legumes, to exploit biological N<sub>2</sub> fixation, is an important measure to contribute to sustainable development (Voisin et al, 2014) through increased yield stability (Raseduzzaman & Jensen 2017) and the potential higher biomass N concentration and N accumulation in the intercropped partner (Bedoussac et al 2015). The occurrence of N<sub>2</sub> fixing bacteria and the higher levels of accessible soil N usually benefit the abundance of AMF. Our results indicate that the expected higher abundance of AMF under perennial crops is not realized when intercropped with lucerne, which could be explained the competitive interactions resulting in less *T. intermedium* roots for the AMF to colonize. Furthermore, the otherwise expected AMF colonization of lucerne roots may not have occurred and the interactions needs to be further investigated.

## Conclusions

Agricultural perennialization will lead to healthier soil microbiomes, in this case proven by higher abundance of the obligate symbiotic soil mycorrhizal fungi. The outcome is in line with the global sustainability goals meaning that the implementation of improved, less interruptive agricultural practices should be promoted.

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