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Importance of fungi in a 63 years old long-term field experiment with 20 years of maize growth

Muhammad Shahbaz [a,c,*], Barry Thornton [b], Gunnar Börjesson [a]

[a] Department of Soil and Environment, P.O. Box 7014, Swedish University of Agricultural Sciences, SE-750 07, Uppsala, Sweden
[b] The James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK
[c] Centre for Environmental and Climate Research, Lund University, 223 62, Lund, Sweden

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ABSTRACT

Microbial diversity and their activity in the rhizosphere and bulk soil areas were measured in a long-term field trial (started in 1956), where maize has been grown for the last 20 years with and without N fertilisation. Various microbial groups and their substrate feeding strategies (i.e. demonstrating activities) were identified through phospholipid fatty acids (PLFAs) analysis and their $\delta^{13}C$ values. Microbial abundance (esp. fungi) remained unaffected by long-term N fertilisation. However, fungi dominated over bacteria with 2–3 times higher biomass in the rhizosphere than bulk soil. The $\delta^{13}C$ of PLFAs showed that fungi had the highest values, particularly in fertilised rhizosphere areas, indicating that this was the most active group (than any other microbial group) for assimilation of maize rhizodeposits.

The rhizosphere contains a 2–20 times higher fraction of active microorganisms compared to bulk soil, which makes it a hotspot for microbial processes essential to plant growth [1]. The ability of the rhizosphere to stimulate microbial activity has been long known. Despite that, studies have seldom addressed the differences in the responses of the microbial groups to fertilisation (e.g. N) in bulk and rhizosphere soils. This calls for a better understanding of microbial processes in the rhizosphere and non-rhizosphere for critical evaluation of the plant-soil interactions and fertilisation effects on soil organic matter (SOM) turnover and nutrients release.

Soil microorganisms and their activities are extremely diverse, determined by several factors such as pH, soil moisture, fertilisation and availability of organic C or resources from plants (e.g. rhizodeposition). Microbial activity and their dependence on resource availability as affected by plant rhizodeposits or fertilisation can be estimated e.g. through C source elucidation in living microbial biomass [2,3]. In this regards, a shift from C3 to C4 plants introduces a distinct natural C isotope labelling that helps in estimating the SOM turnover and microbial substrate feeding strategies i.e. their activities [4–6]. Our previous investigations, in a long-term maize field, show that patterns of microbial succession to be contrasting between years, with fungi very active in the mid-season in 2012, while being more dominant towards the end of the growing season in 2017 [2,7]. However, it was unclear how succession of different microbial groups and their activities varies between rhizosphere and bulk soil. To that end, we hypothesized that microbial groups (esp. fungi and bacteria) and their activities depends on long-term N fertilisation and should be different in the rhizosphere compare to bulk soil.

The experiment at Ultuna (59′82° N, 17′65° E), Sweden was started in 1956 with a transition of C3 vegetation to C4 (silage maize) in 2000, which has resulted in a substantial shift in the SOM isotopic composition [2,7]. The soil is a clay loam with 36.5% clay that had 1.5% organic C at start [8]. The experiment is a block design with 4 replicates of each treatment and each plot has 2 × 2 m dimension. The treatments used for the investigation of this study were bare fallow, cropped unfertilised and cropped fertilised with calcium nitrate at a dose of 80 kg (N) ha^{-1} yr^{-1}. All treatments (including bar fallow) are fertilised with equal amounts of P and K (20 and 38 kg ha^{-1} yr^{-1}). The maize (variety 'Yukon') was sown on June 17, 2019 in three rows in each plot with 0.5 m distance in between. The harvest was completed on September 18, 2019. This included removal of all plant material except stubbles and roots. The following day, soil samples were taken between 9 and 10 a.m. from the 4 replicates in all treatments. Bulk soil samples were taken from all plots down to 10 cm depth between maize crop rows. For rhizosphere samples, five stubbles were lifted up from each plot and the soil adjacent to roots within 1 cm distance was saved for analysis. All samples were

* Corresponding author. Department of Soil and Environment. P.O. Box 7014, Swedish University of Agricultural Sciences, SE-750 07, Uppsala, Sweden.
E-mail addresses: muhammad.shahbaz@cec.lu.se, shahbazmu@yahoo.com (M. Shahbaz).

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Table 1
Phospholipid fatty acids (PLFAs; nmol g soil^{-1}) in different microbial groups. Levels in rows not connected by same letter are significantly different.

Sample type	Bare fallow	Cropped				Prob > F
		unfertilised		fertilised		
	bulk soil	bulk soil	rhizosphere	bulk soil	rhizosphere	
Total PLFAs	33.8 c	56.8 b	64.3 ab	63.4 b	77.3 a	0.0003 **
Gram positive	5.0 c	8.6 b	9.4 b	9.7 ab	11.7 a	0.0002 **
Gram negative	6.4 b	12.1 a	12.0 a	12.1 a	14.1 a	0.0008 **
Fungi	0.6 b	2.1 ab	4.7 a	1.7 ab	4.5 a	0.1355
Actinobacteria	0.8 c	1.2 b	1.2 b	1.5 a	1.6 a	<0.001 ***
Stress indication	0.6 a	0.5 a	0.5 a	0.5 a	0.5 a	0.4136

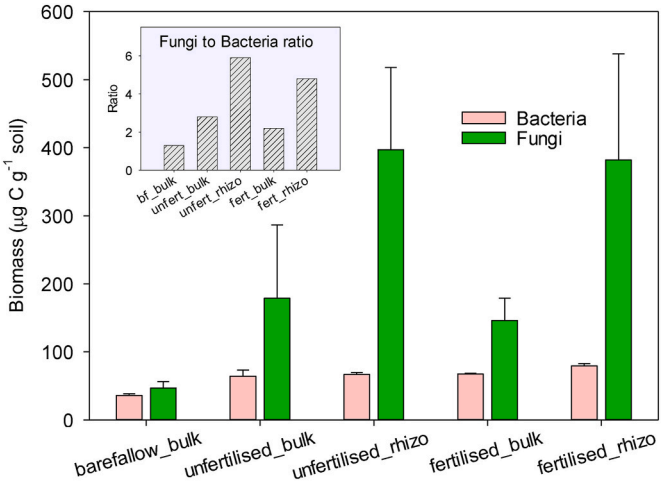

Fig. 1. Biomass (µg C g soil^{-1}) of fungi and bacteria (gram-positive and gram-negative) under different long-term management practices. Inset shows fungi to bacteria ratio (abbrevation: bf = barefallow, unfert/fert = unfertilised/fertilised) . Error bars are S.E (n = 4).

immediately frozen (within 2 h), except for samples analysed for water content and SOM measured by loss on incineration. Soil sampling that is made as a routine in this experiment were performed a few days later for total C, total N and pH.

Freeze-dried soil samples were analysed for $\delta^{13}C$ content in phospholipid fatty acids (PLFAs) according to methods described by *Shahbaz* et al. [7]. 27 different PLFAs biomarkers were determined. PLFAs including a15:0, i15:0, i16:0, and i17:0 represented gram-positive bacteria and cy17:0 and cy19:0 represented gram negative bacteria. Fungal biomass was represented by PLFA 18:2ω6,9, while the PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω7, 16:1u9, i17:0, a17:0, cy17:0, 17:0, 18:1ω7 and cy19:0 were used to represent total bacterial biomass. PLFAs nmol concentrations were converted to biomass C using the following factors: bacterial PLFAs: 363.6 nmol = 1 mg C; and fungal PLFA (18:2): 11.8 nmol = 1 mg C [9,10].

Statistics were done with a linear mixed model, including blocks as random effect.

Due to a very dry period in June the maize plants had problems to establish, and yields were lower than normal (Table S1) when compared with mean yields over the period 2000–2019; i.e. 2563 and 6043 kg ha^{-1}, for unfertilised and fertilised treatments, respectively. The development of SOM contents depended on long-term treatments, and the effect of bare fallow and the fertilisation strategy is substantial, which is also manifest in soil water holding capacity (Tables S1 and S2).

Total PLFAs (Table 1) were correlated to SOM contents, with lowest values in the bare fallow. Gram-positive bacteria and actinobacteria followed the same pattern, including higher abundance in rhizosphere compare to bulk soil samples. However, Gram-negative bacteria were similar in all samples from the cropped plots (Table 1). The ratios

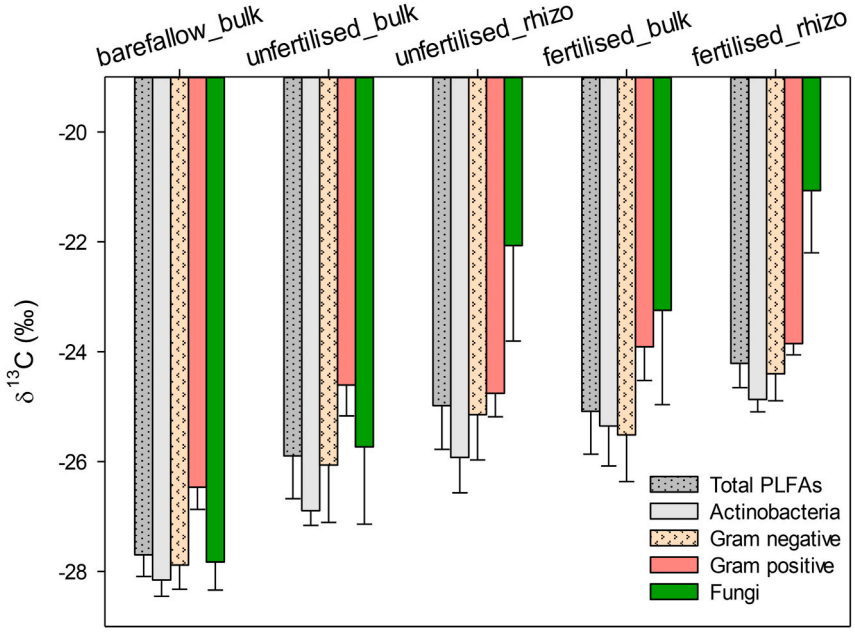

Fig. 2. $\delta^{13}C$ values (‰) in different microbial groups. Gram positive bacteria are mean values for phospholipid fatty acids (PLFAs) i15:0, a15:0, br17:0 and a17:0; weighted based on ratios, and Gram negative bacteria are mean values for PLFAs 16:1ω7c, 16:1ω5c and 18:1ω7; weighted based on ratios. Error bars are S.E. (n = 4).

between fungi and bacteria appeared to be 1.3 for the bare fallow, 2.8 and 2.2 for the cropped unfertilised and fertilised bulk soils respectively (Fig. 1). Similarly, fungi to bacteria ratio in the rhizosphere of unfertilised and fertilised treatments was 5.9 and 4.8, respectively, indicating fungi dominance. The variation was rather high, but not exceptional, with a coefficient of variation of 8.0% for total PLFAs and 4.9% for fungi. *Malik* et al. [11] analysed PLFAs and determined fungi to bacteria ratios to be between 2.8 and 4.2 in a grassland diversity experiment in Germany. The ratios of fungi to bacteria we found in rhizosphere samples must therefore be regarded as elevated. If we regard fungi as the active part, they are also within the range of 2–20 reported by *Kuzyakov* and *Blagodatskaya* [1] for ratios between rhizosphere and bulk soil. The higher abundance of fungi (ca. six times) than bacteria in the rhizosphere (Fig. 1) is consistent with earlier studies where higher rates of root litter derived C in PLFAs of fungi compared to bacteria were observed [12]. It has been reported that with N fertilisation fungal abundance decreases as plant become less reliant on the symbiosis [3, 13]. However, in contrast to our hypothesis, this was not the case as fungi were comparable both in the rhizosphere and bulk soils either under fertilised or unfertilised conditions (Fig. 1).

Similar to biomass data, $\delta^{13}C$ values in the PLFAs (Fig. 2) followed the same pattern, with the lowest, most negative, values in the bare fallow, which can be explained by maize never having been grown on these plots. Remarkably, along with 2–3 times higher abundance in the rhizosphere than bulk soil (Fig. 1), the highest $\delta^{13}C$ values were found in the fungal PLFAs (Fig. 2) that indicate strong assimilation of maize material. Fungi is considered to have a higher ability to degrade complex root litter due to fast colonisation of hyphae combined with effective enzymes release [14]. We may see the rhizosphere and the bulk soil as two different stages in a succession, where the rhizosphere is the first step in assimilation of plant debris and the bulk soil is a later development [15]. The investigation made at this site in 2017 showed that fungi were increasing throughout the growing season, while $\delta^{13}C$ values in fungi seemed to drop down a few weeks after harvest in the fertilised treatment [7]. In this study, presence of N fertilisation effect on $\delta^{13}C$ but absence on fungal biomass in the rhizosphere, might indicate the existence different types of fungi in these two different treatments [16]. Nevertheless, these findings suggested that fungi abundance and activity is intimately connected more with rhizodeposits than any other microbial group, which indicate their activity and dominent potential role in SOM decomposition processes.

In conclusion, the long-term N fertilisation (for 63 years) did not show strong effect on fungi or total microbial biomass (as indicated by PLFAs) both in the rhizosphere and bulk soil. Fungi dominated over all other microbial groups with 2–3 times higher abundance in the rhizosphere than bulk soils. The $\delta^{13}C$ contents in PLFAs showed fungi to be the most active group in assimilating maize rhizodeposits, particularly in the fertilised treatment. Overall, the results shows the necessity to include plants and its rhizosphere in measurements of microbial SOM turnover in soil.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2020.103264.

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