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Short communication

# The role of soil fungi and bacteria in plant litter decomposition and macroaggregate formation determined using phospholipid fatty acids

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ABSTRACT

Although microbial-derived carbon (C) inputs to soil are increasingly acknowledged as an important source of soil organic matter (SOM), the contribution of different microbial compounds to soil C transformation and their role in aggregation remain poorly understood. This study assessed the contribution of soil fungi and bacteria to the decomposition of maize residues by means of extracted phospholipid fatty acids (PLFAs) and $^{13}$C in specific PLFAs and investigated the importance of soil fungi in the formation of macroaggregates. Sieved soil (<250 μm) was incubated for 28 days with and without addition of maize residues and fungicide. Our results show a significant relation between the amount of fungal PLFA 18:2ω6 and the amount of macroaggregates. Further, the amount of macroaggregates was higher in the treatment with the higher amount of maize-derived C in fungal PLFA, suggesting that fungal activity is important for macroaggregate formation. Based on an increased incorporation of maize-derived C into actinomycetes in fungicide treatments, we suggest that actinomycetes may take over the role of soil fungi in the decomposition of SOM. Our study underpins the important role of soil fungi in the decomposition of organic matter and structure formation in the soil, and shows that during inhibition of soil fungi other soil microorganisms are promoted and adopt their function in the soil food web.


## 1. Introduction

Soil fungi have been recognized to positively affect soil aggregation (e.g. Bossuyt et al., 2001; Denef et al., 2001), which in turn is important for maintaining high soil quality, i.e. conditions favourable for plant and microbial growth like soil porosity, aeration, infiltration of water, and stability against erosion of soils. In a previous study, Helfrich et al. (2008) investigated the effect of maize residue decomposability and fungal biomass on the dynamics of macroaggregate (>250 μm) formation and the associated partitioning of litter-derived C and N in a three months' incubation experiment. They found that the application of fungicide decreased the amount and catabolic activity of the microbial biomass (decreasing $CO_2$-emissions and decreasing proportions of maize-derived C in $CO_2$—C) and led to less macroaggregation. However, Ergosterol, which was used as a fungal biomarker was found inappropriate in periods of rapid decline of the microbial biomass, such as in the fungicide treatments (Helfrich et al., 2008). Phospholipid fatty acids (PLFAs) are regarded a good measure of living microbial biomass. Because they are major constituents of the membranes of all living cells that are not found within storage products and are degraded quickly after cell death, they have the potential to mirror even rapid changes in the microbial community (Amelung et al., 2008). Combined with substance specific $^{13}$C contents, PLFA analyses allow C source elucidation in living microbial biomass (Amelung et al., 2008; Kramer and Gleixner, 2006; Kaur et al., 2005).

The present study assessed (i) the contribution of soil fungi and bacteria to the decomposition of maize residues of different decomposability (leaves, roots) by means of specific PLFA contents and $^{13}$C in specific PLFAs and investigated (ii) their importance in the short-term formation of macroaggregates. The results from this study give the opportunity to get new insights into the initial C uptake upon degradation of plant litter by different microbial groups and its role for macroaggregation in an agricultural soil.

## 2. Materials and methods

Soil samples for PLFA analysis were derived from the first 28 days of an incubation experiment (Helfrich et al., 2008; Table 1).

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**Table 1**
Overview of the treatments. Quantification of PLFA was carried out on samples taken after 0, 14 and 28 days of incubation. Determination of specific $^{13}C$ values was done on samples taken at the start (0 days) and after 28 days of incubation.

| Treatment | Description | Days incubated |
|---|---|---|
| S0 | Control soil | 0 |
| SC0 | Control soil + Captan | 0 |
| S14 | Control soil | 14 |
| SC14 | Control soil + Captan | 14 |
| SL14 | Soil + maize leaves | 14 |
| SLC14 | Soil + maize leaves + Captan | 14 |
| SR14 | Soil + maize roots | 14 |
| SRC14 | Soil + maize roots + Captan | 14 |
| S28 | Control soil | 28 |
| SC28 | Control soil + Captan | 28 |
| SL28 | Soil + maize leaves | 28 |
| SLC28 | Soil + maize leaves + Captan | 28 |
| SR28 | Soil + maize roots | 28 |
| SRC28 | Soil + maize roots + Captan | 28 |

In brief, soil of a silty loam (stagnic Luvisol derived from loess) under wheat monoculture ($C_3$ vegetation; soil organic C 12.6 ± 0.1 g kg$^{-1}$; $\delta^{13}C$ −26.5 ± 0.1‰ V-PDB; means ± standard deviation) was incubated after destruction of all naturally occurring macroaggregates (sieving <250 μm) in the dark at 15 °C and 60% of the maximum water-holding capacity. Maize leaves (C/N 27.4; $\delta^{13}C$ −12.7 ± 0.2‰ V-PDB; means ± standard deviation) or roots (C/N 86.4; $\delta^{13}C$ −11.9 ± 0.2‰ V-PDB; means ± standard deviation) were oven-dried at 40 °C, milled <500 μm and added to the soil at a rate of 2.1 mg C g$^{-1}$ soil. Each treatment was carried out with and without addition of 0.3 g Captan per 100 g soil (50 W wettable powder, 89% active ingredient), which had been used as a fungicide in a number of studies (e.g. Bailey et al., 2002; Bossuyt et al., 2001; Denef et al., 2001) and was found to have little effect on soil bacteria (Ingham, 1985). Macroaggregates were obtained by wet-sieving >250 μm (Helfrich et al., 2008).

Phospholipid fatty acids were extracted following the method of Bligh and Dyer (1959) and Zelles and Bai (1993). Soil lipids were extracted using a mixture of chloroform, methanol and phosphate buffer (1:2:0.8 vol). Lipid extracts were separated into neutral, glycol- and phospholipids on silicic acid columns. Phospholipids were methylated and the obtained fatty acid methyl ester (FA-ME) were further separated into saturated, polyunsaturated and monounsaturated fatty acids and quantified by gas chromatography (GC: HP 6890 Series, AED: G 2350 A, Agilent Technologies, United States) using a HP ultra 2 column (50 m × 0.32 mm I.D., 0.25 μm film thickness) in the split mode. Compound-specific determination of $\delta^{13}C$ values of individual PLFA-ME was performed with GC/MS-C-IRMS in triplicate (GCQ, Thermoquest, Germany); (Delta + XL, Finnigan MAT, Germany). To obtain $\delta^{13}C$ values of the PLFAs, the $\delta^{13}C$ values of PLFA-ME were corrected for the methyl-C which was added during methylation. The amount of maize-derived C used as microbial C source in percent was calculated by dividing the isotopic shift between PLFAs from soil with and without addition of maize litter through the isotopic shift between the soil used for the incubation and the maize plant material (Balesdent and Mariotti, 1996). The obtained PLFAs were grouped into grampositive bacteria (Gram$^+$) excluding actinomycetes

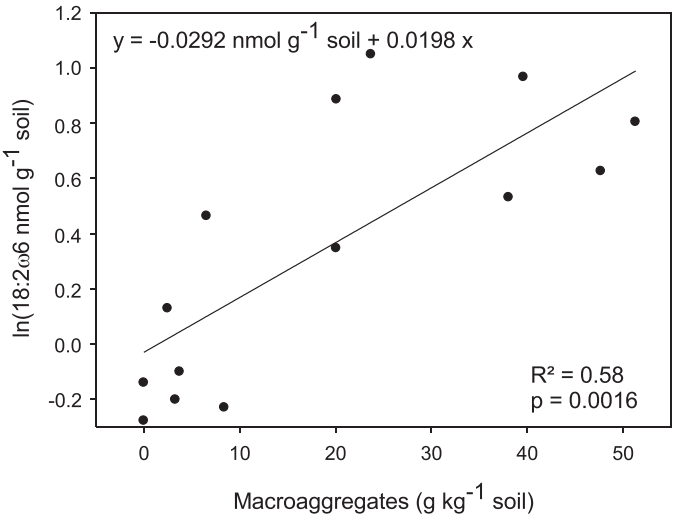


**Fig. 1.** Linear regression between the amount of lnPLFA 18:2ω6 and water-stable macroaggregates.

**Table 2**
Amounts of major and of biomarker PLFA for the investigated treatments.

| | | Amount of selected PLFAs (nmol g$^{-1}$ soil) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S0 | SC0 | S14 | SC14 | S28 | SC28 | SL14 | SLC14 | SL28 | SLC28 | SR14 | SRC14 | SR28 | SRC28 |
| i14:0 | Gram$^+$ | 0.60 | 0.65 | 0.79 | 0.79 | 0.51 | 0.66 | 0.88 | 1.05 | 1.39 | 1.10 | 0.89 | 0.95 | 1.18 | 0.95 |
| i15:0 | Gram$^+$ | 4.87 | 5.27 | 6.92 | 6.23 | 5.33 | 5.79 | 7.49 | 6.50 | 11.39 | 7.82 | 9.27 | 7.05 | 10.57 | 6.97 |
| a15:0 | Gram$^+$ | 4.13 | 4.80 | 5.60 | 5.89 | 4.35 | 5.65 | 7.31 | 9.41 | 10.68 | 10.79 | 8.46 | 9.38 | 9.23 | 9.10 |
| br16:0 | Gram$^+$ | 0.40 | 0.45 | 0.56 | 0.53 | 0.44 | 0.49 | 0.51 | 0.52 | 0.79 | 0.60 | 0.66 | 0.60 | 0.72 | 0.57 |
| i16:0 | Gram$^+$ | 1.87 | 1.86 | 2.38 | 2.32 | 1.81 | 2.19 | 3.13 | 2.82 | 4.34 | 3.19 | 3.75 | 3.02 | 3.86 | 2.82 |
| br17:0 | Gram$^+$ | 0.65 | 0.79 | 0.87 | 0.86 | 0.78 | 0.87 | 0.87 | 0.89 | 1.35 | 0.94 | 1.04 | 0.94 | 1.02 | 0.83 |
| i17:0 | Gram$^+$ | 1.18 | 1.19 | 1.52 | 1.37 | 1.20 | 1.27 | 1.58 | 1.29 | 2.45 | 1.51 | 1.97 | 1.50 | 2.17 | 1.41 |
| a17:0 | Gram$^+$ | 1.21 | 1.30 | 1.63 | 1.57 | 1.15 | 1.49 | 1.94 | 1.80 | 2.80 | 2.13 | 2.29 | 2.00 | 2.38 | 1.85 |
| br18:0r | Gram$^+$ | 0.91 | 0.99 | 1.25 | 1.25 | 1.03 | 1.34 | 1.08 | 1.17 | 1.69 | 1.31 | 1.53 | 1.33 | 1.49 | 1.13 |
| br19:0 | Gram$^+$ | 0.33 | 0.38 | 0.55 | 0.46 | 0.38 | 0.43 | 0.41 | 0.43 | 0.66 | 0.54 | 0.61 | 0.48 | 0.58 | 0.46 |
| 18:2ω6c | Fungal marker | 0.87 | 0.76 | 1.14 | 0.90 | 0.82 | 0.79 | 1.87 | 1.70 | 2.63 | 2.23 | 2.42 | 1.59 | 2.85 | 1.41 |
| n14:0 | Universal | 1.03 | 1.04 | 1.19 | 1.22 | 0.95 | 1.06 | 1.43 | 1.59 | 2.27 | 1.86 | 1.43 | 1.35 | 1.61 | 1.38 |
| n15:0 | Universal | 0.51 | 0.81 | 0.55 | 0.54 | 0.47 | 0.52 | 0.63 | 0.68 | 0.97 | 0.80 | 0.73 | 0.70 | 0.82 | 0.68 |
| n16:0 | Universal | 6.40 | 6.78 | 8.75 | 8.17 | 6.35 | 7.87 | 12.38 | 14.71 | 18.69 | 16.46 | 12.79 | 11.20 | 14.09 | 10.99 |
| n17:0 | Universal | 0.34 | 0.32 | 0.34 | 0.35 | 0.29 | 0.34 | 0.46 | 0.48 | 0.69 | 0.53 | 0.52 | 0.47 | 0.57 | 0.42 |
| n18:0 | Universal | 1.24 | 1.39 | 1.85 | 1.43 | 1.24 | 1.35 | 2.00 | 2.32 | 2.91 | 2.56 | 2.23 | 2.06 | 2.33 | 1.88 |
| 10Me17:0 | Actinomycetes | 2.84 | 3.17 | 3.66 | 3.61 | 3.41 | 3.32 | 3.43 | 3.44 | 5.08 | 3.95 | 4.43 | 3.92 | 5.63 | 3.83 |
| 10Me18:0 | Actinomycetes | 0.58 | 0.62 | 0.81 | 0.74 | 0.62 | 0.65 | 0.79 | 0.76 | 1.16 | 0.87 | 1.04 | 0.87 | 1.08 | 0.81 |
| 10Me19:0 | Actinomycetes | 1.57 | 1.72 | 2.08 | 2.06 | 1.70 | 1.90 | 2.13 | 2.06 | 3.21 | 2.46 | 2.79 | 2.32 | 2.95 | 2.23 |
| cy19:0 | Gram$^-$ | 3.40 | 4.07 | 4.14 | 3.99 | 3.35 | 3.95 | 3.73 | 4.22 | 5.62 | 4.67 | 4.78 | 4.54 | 5.16 | 4.67 |
| 16:1ω7c | Gram$^-$ | 5.02 | 4.27 | 6.08 | 6.11 | 4.70 | 4.87 | 8.90 | 6.24 | 16.09 | 8.69 | 11.05 | 6.61 | 11.38 | 6.57 |
| 18:1ω7c | Gram$^-$ | 7.83 | 7.29 | 9.84 | 9.42 | 7.54 | 8.02 | 11.76 | 9.03 | 21.90 | 12.53 | 15.19 | 9.69 | 17.71 | 10.23 |
| 18:1ω9c | Gram$^-$ | 5.61 | 5.00 | 8.52 | 6.64 | 4.66 | 5.46 | 7.30 | 6.25 | 12.95 | 8.58 | 10.35 | 6.85 | 10.26 | 6.92 |
| % of total PLFA | | 77.6 | 78.6 | 79.3 | 78.1 | 79.7 | 80.7 | 80.2 | 81.1 | 77.9 | 78.8 | 78.3 | 79.7 | 79.6 | 81.2 |