# Long-term effects of organic farming on fungal and bacterial residues in relation to microbial energy metabolism

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SHORT COMMUNICATION

# Long-term effects of organic farming on fungal and bacterial residues in relation to microbial energy metabolism

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**Abstract** Samples from the bio-dynamic, bio-organic, and conventional trial, Therwil, Switzerland, were analyzed with the aim of determining the effects of organic land use management on the energy metabolism of the soil microbial biomass and on the fraction of microbial residues. The contents of adenylates, adenosine triphosphate (ATP), glucosamine, muramic acid, and galactosamine were significantly largest in the biodynamic organic farming (BYODIN) treatment and significantly lowest in the conventional farming treatment with inorganic fertilization (CONMIN). In contrast, the ergosterol-to-ATP ratio and fungal C-to-bacterial C ratios were significantly lowest in the BYODIN treatment and significantly largest in the CONMIN treatment. No clear treatment effects were observed for the ergosterol content and the adenylate energy charge (AEC), the ATP-to-microbial biomass C ratio and the ergosterol-to-fungal C ratio. Ergosterol, an indicator for saprotrophic fungal biomass, and fungal residues were significantly correlated. The microbial biomass carbon-to-nitrogen ratio showed a negative relationship with the AEC and strong positive relationships with the ratios ergosterol-to-microbial biomass C, ergosterol-to-ATP and fungal C-to-bacterial C. In conclusion, the long-term application of farmyard manure in combination with organic farming practices led to an increased accumulation of bacterial residues.

**Keywords** DOK trial · Amino sugars · Ergosterol · Adenylates · ATP

## Introduction

Organic farming aims to improve soil quality and pest control, thereby reducing environmental impacts of conventional farming by considering soil biological processes (Mäder et al. 2002). The effects of organic farming on soil organic matter and soil biological properties have been intensively investigated in the bio-dynamic, bio-organic, and conventional (DOK) trial in Therwil, Switzerland (Mäder et al. 2002; Fließbach et al. 2007; Birkhofer et al. 2008). An important reason for the positive effects of organic farming is the application of farmyard manure (Raupp and Oltmanns 2006), as demonstrated in the DOK trial and in several other long-term field experiments (Edmeades 2003; Ludwig et al. 2007; Heinze et al. 2009).

Amino sugars are the most important indicators for the presence of microbial residues in soil (Amelung et al. 2008) and contribute significant amounts of between 5% and 12% to soil organic N (Stevenson 1983; Amelung 2001). The most important amino sugars in soil are muramic acid, glucosamine, and galactosamine, although a variety of other amino sugars also exist. Little is known about the origin of galactosamine in soil, although it accounts for 30-50% of the amino sugar content (Amelung et al. 1999). The chitin of fungal cell walls is the major source of glucosamine in soil, although bacterial cell walls and the exoskeleton of soil invertebrates also make some contribution (Parsons 1981). Muramic acid is one of the most specific biomarkers, as it occurs exclusively in bacterial cell walls, especially in the murein skeleton of Gram-positive species. Due to their specificity, muramic acid and

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glucosamine give important information on the contribution of fungi and bacteria to microbial residues and thus to soil organic matter (Joergensen and Wichern 2008). However, a close relationship has also been observed between fungal glucosamine and ergosterol, an important measure for the biomass of saprotrophic fungi in arable and grassland soils (Appuhn et al. 2006). On the basis of amino sugar analysis, there is evidence that the long-term application of farmyard manure in combination with organic farming practices promotes the accumulation of bacterial residues (Scheller and Joergensen 2008). However, the previous study was based on a comparison of just two sites and not on a long-term field experiment, making generalization of results difficult.

Microbial activity indices, such as basal respiration and nitrogen mineralization analyzed in the DOK trial (Birkhofer et al. 2008), suggested an increased accessibility of soil organic matter to soil microorganisms in the conventional farming system. This increase may affect specifically the relationships between microbial biomass and adenylates (adenosine triphosphate, ATP; adenosine diphosphate, ADP; and adenosine monophosphate, AMP) as indicators of microbial energy metabolism (Dilly and Nannipieri 2001; Dilly 2006; Chander and Joergensen 2007). In the present study, identical soil samples used in a previously published comprehensive multi-method comparison (Birkhofer et al. 2008) were further analyzed with the aim of determining the effects of organic land use management on the energy metabolism of soil microorganisms and on the fraction of microbial residues. Amino sugars give information on the long-term shift in soil organic matter quality and adenylates on the actual energy metabolism. The catabolic processes might be affected by the long-term-shifts in the ratio of fungal to bacterial residues, because fungi are thought to use organic substrates more efficiently, i.e., they form more biomass from the same amount of substrate than bacteria (Sakamoto and Oba 1994; Jastrow et al. 2007).

## Material and methods

### Study site and sampling

The DOK trial in Therwil, Switzerland is a long-term agricultural experiment established in 1978 by the Agroscope Reckenholz Tänikon research station and the Research Institute of Organic Agriculture. The soil is a haplic Luvisol on deep deposits of alluvial loess. Mean precipitation is 785 mm per year with an annual average temperature of 9.5°C (Mäder et al. 2002). More information on the soil chemical properties, soil management, and yields can be obtained from Birkhofer et al. (2008). Samples were taken from wheat plots of two organic farming systems (biodynamic organic farming, BIODYN; bioorganic, BIOORG), one conventional (CONFYM) system, receiving farmyard manure, and a second conventional system, mimicking stockless farming (CONMIN, receiving mineral fertilizers only) on 9 May 2005 (Birkhofer et al. 2008). One sample of bulk soil (1 kg) was taken at 0-5 cm depth from randomly chosen locations at the northern and southern end from all four replicated plots of each farming system, which gave eight independent samples.

### Analytical procedures

Adenine nucleotides (ATP, ADP, and AMP) were extracted with an alkaline dimethyl sulfoxide buffer as described by Joergensen and Raubuch (2005) from moist sample equivalent to 3 g oven-dry soil. The adenylate energy charge (AEC) was calculated on a molar basis as (ATP+0.5 × ADP)/(AMP+ ADP + ATP). The fungal cell membrane component ergosterol was extracted from 2 g of moist soil with 100 ml ethanol (Djajakirana et al. 1996). Then, ergosterol was determined by reversed-phase high performance liquid chromatography (HPLC) with 100% methanol as the mobile phase and detected at a wavelength of 282 nm. Glucosamine and muramic acid were determined according to Appuhn et al. (2004). Moist soil samples of 500 mg were hydrolyzed with 6 M HCl for 6 h at 105°C. After HCl removal and centrifugation, the samples were transferred to vials and stored at −18°C before the HPLC measurements. After derivatization with ortho-phthaldialdehyde, fluorometric emission of amino sugars was measured at a wavelength of 445 nm after excitation at a wavelength of 340 nm (Agilent 1100, Agilent, Palo Alto, USA).

Fungal C (μg $g^{-1}$) was estimated by multiplying the content of fungal glucosamine in μg $g^{-1}$ by 9 (Appuhn and Joergensen 2006). Fungal glucosamine was calculated by subtracting bacterial glucosamine from total glucosamine, assuming that muramic acid and glucosamine occur at a 1:2 molar ratio in bacteria (Engelking et al. 2007). Bacterial C (μg $g^{-1}$) was calculated by multiplying the content of muramic acid in μg $g^{-1}$ by 45 (Appuhn and Joergensen 2006). The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105°C). The significance of management effects was analyzed by a one-way analysis of variance using the Scheffé post hoc test, which is robust against the violation of normality and inhomogeneity of the variances (StatView Reference Manual, SAS Inst. Inc.). All statistical calculations were performed by StatView 5.0 (SAS Institute Inc.).

## Results

The contents of microbial biomass C, adenylates, ATP, glucosamine, muramic acid, and galactosamine were significantly largest in the BYODIN treatment and significantly lowest in the CONMIN treatment (Table 1). The ergosterol-to-ATP ratio and the fungal C-to-bacterial C ratio were significantly lowest in the BYODIN treatment and significantly largest in the CONMIN treatment. The ergosterol-to-microbial biomass C ratio was lowest in the BIOORG treatment and significantly largest again in the CONMIN treatment. However, the difference between BIOORG and BIODYN were not significant for this ratio. The same was true for the other microbial indices, with the exception of the adenylates. Also, the differences between the treatments BIOORG and CONFYM were not significant for the contents of adenylates, ATP, glucosamine, muramic acid, bacterial C, or galactosamine. No clear treatment effects were observed for ergosterol and fungal C, AEC, the ATP-to microbial biomass C ratio, and the ergosterol to fungal C ratio.

Adenylates were significantly correlated with microbial biomass C ($r$=0.80, $P$<0.001, $n$=32), bacterial C and galactosamine, but not with ergosterol and fungal C. These two fungal indices were significantly correlated. However, the relationships between fungal C, bacterial C and galactosamine, i.e., within the different fractions of microbial residues were stronger. The microbial biomass carbon-to-nitrogen ratio (C/N ratio) showed a negative relationship with the AEC and especially strong positive relationships with the ratios ergosterol to microbial biomass C, ergosterol to ATP. and fungal C to bacterial C (Table 2). These three ratios, expressing the fungal contribution to microbial biomass and to microbial residues in soil, were all significantly interrelated.

## Discussion

The present results of adenylates and ATP as indices for microbial biomass (Dyckmans et al. 2003) mirrored the general positive effects of organic farming on soil biological properties observed repeatedly in the DOK trial (Mäder et al. 2002; Birkhofer et al. 2008). In contrast to these general characteristics, the content of saprotrophic fungi, as indicated by the ergosterol content (Joergensen and Wichern 2008), remained constant, and was not improved by the application of farmyard manure and other organic farming practices, suggesting a shift in the microbial community structure. However, this was not supported either by direct bacterial counts or by bacterial and fungal PLFA (Birkhofer et al. 2008).

An increased microbial biomass in combination with constant saprotrophic fungi means a shift in the microbial community structure towards bacteria and towards biotro-

**Table 1** Effects of land use management on different microbial properties in the DOK trial at Frick, Switzerland

| Microbial properties | BIODYN | BIOORG | CONFYM | CONMIN | CV (±%) |
|---|---|---|---|---|---|
| Soil pH (0.1 M KCl)[a] | 5.8 | 5.4 | 5.3 | 4.5 | 4 |
| Soil organic C (mg g$^{-1}$ soil)[a] | 16.0 | 14.4 | 13.5 | 12.3 | 10 |
| Microbial biomass C (µg g$^{-1}$ soil)[a] | 470 a | 430 a | 310 b | 230 c | 13 |
| Adenylates (nmol g$^{-1}$ soil) | 4.0 a | 3.2 b | 3.1 b | 2.1 c | 16 |
| ATP (nmol g$^{-1}$ soil) | 2.8 a | 2.5 a,b | 2.1 b | 1.3 c | 18 |
| Ergosterol (µg g$^{-1}$ soil) | 0.35 a | 0.31 a | 0.33 a | 0.32 a | 12 |
| Glucosamine (µg g$^{-1}$ soil) | 780 a | 740 a,b | 710 a,b | 660 b | 12 |
| Muramic acid (µg g$^{-1}$ soil) | 68 a | 60 a,b | 55 b,c | 45 c | 14 |
| Galactosamine (µg g$^{-1}$ soil) | 300 a | 260 a,b | 230 b,c | 210 c | 12 |
| Bacterial C (mg g$^{-1}$ soil) | 3.0 a | 2.7 a,b | 2.5 b,c | 2.0 c | 14 |
| Fungal C (mg g$^{-1}$ soil) | 6.2 a | 5.9 a | 5.7 a | 5.4 a | 12 |
| Microbial biomass C/N[a] | 5.7 b | 5.8 b | 7.7 a | 7.9 a | 12 |
| AEC | 0.78 a,b | 0.84 a | 0.79 a,b | 0.75 b | 5 |
| ATP/microbial biomass C (µmol g$^{-1}$) | 6.0 a | 5.6 a | 7.0 a | 5.9 a | 15 |
| Ergosterol/microbial biomass C (‰) | 0.76 c | 0.72 c | 1.11 b | 1.44 a | 19 |
| Ergosterol/ATP (g mol$^{-1}$) | 130 b | 130 b | 160 b | 250 a | 24 |
| Ergosterol/fungal C (mg g$^{-1}$) | 5.7 a | 5.3 a | 5.9 a | 6.0 a | 13 |
| Fungal C/bacterial C | 2.0 b | 2.2 b | 2.3 b | 2.7 a | 9 |

Different letters within a row indicate a significant difference ($P$<0.05, Scheffé test)

*CV* pooled coefficient of variation between replicate samples ($n$=4×2); *BIODYN* biodynamic, with composted farmyard manure and biodynamic preparations; *BIOORG* bioorganic, with rotted farmyard manure; *CONFYM* conventional, with stacked farmyard manure; *CONMIN* conventional, with inorganic fertilizer application

[a] Data taken from Birkhofer et al. (2008)

**Table 2** Correlation coefficients between adenylates, ergosterol, fungal C, bacterial C, and galactosamine as well as between the adenylate energy charge (AEC), and the ratios ergosterol/microbial biomass C, ergosterol/ATP, and fungal C/bacterial C in the DOK trial at Frick, Switzerland ($n$=32)

| | Ergosterol | Fungal C | Bacterial C | Galactosamine | AEC | Ergosterol/ microbial biomass C | Ergosterol/ ATP | Fungal C/ bacterial C |
|---|---|---|---|---|---|---|---|---|
| Adenylates | 0.04 | 0.24 | 0.57** | 0.53** | | | | |
| Ergosterol | | 0.42* | 0.30 | 0.36 | | | | |
| Fungal C | | | 0.77*** | 0.77*** | | | | |
| Bacterial C | | | | 0.95*** | | | | |
| Microbial biomass C/N | | | | | −0.41* | 0.73*** | 0.59** | 0.58** |
| AEC | | | | | | −0.54** | −0.56** | −0.40 |
| Ergosterol/microbial biomass C | | | | | | | 0.90*** | 0.62** |
| Ergosterol/ATP | | | | | | | | 0.64** |

*$P$<0.05, **$P$<0.01, ***$P$<0.001

phic arbuscular mycorrhizal fungi (AMF), which do not contain ergosterol (Olsson et al. 2003). However, the biomarker for AMF, the neutral lipid fatty acid (NLFA) 16:1ω5, did not differ significantly between farming systems (Birkhofer et al. 2008), suggesting a decrease in the contribution of their mycelium to the microbial biomass in the organic farming treatments. The NLFA 16:1ω5 is a good indicator for AMF storage lipids (Gavito and Olsson 2008). It may also accumulate in dead fungal tissue to a certain extent and consequently does not always reflect the actual AMF biomass. A strong indication of a long-term shift towards bacterial biomass due to farmyard manure application is the absolute increase in the contents of the bacterial cell wall component muramic acid and the resulting decrease in the fungal C-to-bacterial C ratio, suggesting a marked change in the soil organic matter quality between the different treatments after an experimental period of 27 years.

In the conventional treatments, especially without farmyard manure, an increased microbial biomass C/N ratio in combination with an increased fungal C-to-bacterial C ratio indicates that the shift in the microbial community structure towards fungi had a depressive effect on the N storage of total biomass. This has not always been found (Heinze et al. 2009). Soil type- and soil management-specific interactions seem to affect the relationship of the microbial biomass C/N ratio and the ratio of fungal to bacterial tissue. This may explain the absence of any relationship between the microbial biomass C/N ratio and the ATP-to-microbial biomass C ratio repeatedly observed (Chander and Joergensen 2007). It may also explain the negative relationship between the ergosterol-to-microbial biomass C ratio and AEC never observed before suggesting a lower metabolic activity of saprotrophic soil fungi.

The shift in the ratio of fungal C to bacterial C from 2.7 in the CONMIN to 2.0 in the BIODYN treatment means that the bacterial residues as a percentage of the total microbial residues increased significantly from 27% to 33%, but that the fungal residues still dominate all treatments as always observed in soil (Joergensen and Wichern 2008). The percentages of bacterial residues are still considerably higher than those relating direct counts of bacteria to the total microbial biomass by fumigation extraction (Birkhofer et al. 2008). On average, the fraction of amino sugars consisted of 70% glucosamine, 24% galactosamine, and 6% muramic acid. These percentages are generally similar to those obtained in other soils (Amelung et al. 1999). The close correlation between galactosamine and bacterial C in the present experiment suggests that galactosamine is mainly of bacterial origin, as repeatedly stated in earlier publications (Kögel and Bochter 1985; Amelung 2001). Between 0% and 50% galactosamine has been found in laboratory cultures of bacteria and fungi (Engelking et al. 2007). Galactosamine contributed on average 4% to the total amino sugar content in cultured bacteria and 15% in cultured fungi, suggesting that fungi should contribute larger percentages of galactosamine to the amino sugar pool than bacteria. However, the function of galactosamine within microbial cells or as metabolites is still unknown.

In conclusion, ergosterol and amino sugar analysis, i.e., muramic acid and glucosamine, revealed that long-term application of farmyard manure in combination with organic farming practices led to a shift in the microbial residues towards bacterial tissue. Galactosamine is an important N component in soil closely related to bacterial residues in the present experiment, but still of unknown origin.

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## References


Amelung W (2001) Methods using amino sugars as markers for microbial residues in soil. In: Lal JM, Follett RF, Stewart BA (eds) Assessment methods for soil carbon. Lewis Publishers, Boca Raton, pp 233–272

Amelung W, Zhang X, Flach KW, Zech W (1999) Amino sugars in native grassland soils along a climosequence in North America. Soil Sci Soc Am J 63:86–92

Amelung W, Brodowski S, Sandhage-Hofmann A, Bol R (2008) Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Adv Agron 100:155–250

Appuhn A, Joergensen RG (2006) Microbial colonisation of roots as a function of plant species. Soil Biol Biochem 38:1040–1051

Appuhn A, Joergensen RG, Raubuch M, Scheller E, Wilke B (2004) The automated determination of glucosamine, galactosamine, muramic acid and mannosamine in soil and root hydrolysates by HPLC. J Plant Nutr Soil Sci 167:17–21

Appuhn A, Scheller E, Joergensen RG (2006) Relationship between microbial indices in roots and silt loam soils forming a gradient in soil organic matter. Soil Biol Biochem 38:2557–2564

Birkhofer K, Bezemer TM, Bloem J, Bonkowski M, Christensen S, Dubois D, Ekelund F, Fließbach A, Gunst L, Hedlund K, Mäder P, Mikola J, Robin C, Setälä H, Tatin-Froux F, Van der Putten WH, Scheu S (2008) Long-term organic farming fosters below and aboveground biota: implications for soil quality, biological control and productivity. Soil Biol Biochem 40:2297–2308

Chander K, Joergensen RG (2007) Microbial biomass and activity indices after organic substrate addition to a selenium contaminated soil. Biol Fertil Soils 44:241–244

Dilly O (2006) Ratios of microbial biomass estimates to evaluate microbial physiology in soil. Biol Fertil Soils 42:241–246

Dilly O, Nannipieri P (2001) Response of ATP content, respiration rate and enzyme activities in an arable and a forest soil to nutrient additions. Biol Fertil Soils 34:64–72

Djajakirana G, Joergensen RG, Meyer B (1996) Ergosterol and microbial biomass relationship in soil. Biol Fertil Soils 22:299–304

Dyckmans J, Chander K, Joergensen RG, Priess J, Raubuch M, Sehy U (2003) Adenylates as an estimate of microbial biomass C in different soil groups. Soil Biol Biochem 35:1485–1491

Edmeades DC (2003) The long-term effect of manures and fertilizers on soil productivity and quality: a review. Nutr Cycl Agroecosyst 66:165–180

Engelking B, Flessa H, Joergensen RG (2007) Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biol Biochem 39:2111–2118

Fließbach A, Oberholzer HR, Gunst L, Mäder P (2007) Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric Ecosyst Environ 118:273–284

Gavito ME, Olsson PA (2008) Foraging strategies of the external mycelium of the arbuscular mycorrhizal fungi *Glomus intraradices* and *Scutellospora calospora*. Appl Soil Ecol 39:282–290

Heinze S, Raupp J, Joergensen RG (2009) Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. Plant Soil (in press). doi:10.1007/s11104-009-0102-2

Jastrow JD, Amonette JE, Bailey VL (2007) Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Clim Change 80:5–23

Joergensen RG, Raubuch M (2005) Determination of adenylates and adenylate energy charge. In: Margesin R, Schinner F (eds) Manual of soil analysis—monitoring and assessing soil bioremediation. Soil Biology, Volume 5. Springer, Berlin, pp 299–304

Joergensen RG, Wichern F (2008) Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol Biochem 40:2977–2991

Kögel I, Bochter R (1985) Amino sugar determination in organic soils by capillary gas chromatography using a nitrogen-selective detector. Z Pflanzenernähr Bodenk 148:260–267

Ludwig B, Schulz E, Merbach I, Rethemeyer J, Flessa H (2007) Predictive modelling of the C dynamics for eight variants of the long-term static fertilization experiment in Bad Lauchstädt using the Rothamsted carbon model. Eur J Soil Sci 58:1155–1163

Mäder P, Fließbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil fertility and biodiversity in organic farming. Science 296:1694–1697

Olsson PA, Larsson L, Bago B, Wallander H, van Aarle IM (2003) Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. New Phytol 159:1–10

Parsons JW (1981) Chemistry and distribution of amino sugars in soils and soil organisms. In: Paul EA, Ladd JN (eds) Soil biochemistry. Volume 5. Marcel Dekker, New York, pp 197–227

Raupp J, Oltmanns M (2006) Farmyard manure, plant based organic fertilisers, inorganic fertiliser—which sustains soil organic matter best? Asp Appl Biol 79:273–276

Sakamoto K, Oba Y (1994) Effect of fungal to bacterial biomass ratio on the relationship between $CO_2$ evolution and total soil microbial biomass. Biol Fertil Soils 17:39–44

Scheller E, Joergensen RG (2008) Decomposition of wheat straw differing in nitrogen content in soils under conventional and organic farming management. J Plant Nutr Soil Sci 171:886–892

Stevenson FJ (1983) Isolation and identification of amino sugars in soil. Soil Sci Soc Am J 47:61–65