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Effect of biodynamic soil amendments on microbial communities in comparison with inorganic fertilization

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ABSTRACT

Cattle farmyard manure application is an important tool for maintaining soil fertility in organic agriculture, especially in biodynamic systems. The first objective was to investigate whether application of biodynamic preparations (CMBD treatment) causes positive effects additional to those of composted cattle farmyard manure fertilization (CM treatment). The second objective was to investigate the response of microbial cell-wall and cell-membrane biomarkers to the CM and CMBD treatments in comparison with inorganic fertilization plus straw return (MIN treatment). The third objective was to re-assess conversion values from the phospho-lipid fatty acid (PLFA) 16:1ω5 to arbuscular mycorrhizal fungal (AMF) biomass as well as those from ergosterol and the PLFA 18:2ω6,9 to saprotrophic fungal biomass. Application of biodynamic preparations did not cause any positive effects additional to those of composted farmyard manure fertilization. In the CM and CMBD treatments, bacterial PLFA content was 33% higher than in the MIN treatment, whereas bacterial muramic acid (MurN) content was 55% higher. The AMF indicator PLFA 16:1ω5 as well as neutral lipid fatty acid (NLFA) 16:1ω5 were both increased by roughly 80%, as the NLFA/PLFA ratio of 16:1ω5 varied only in a small range around 3.8. This indicates negligible interference from bacteria, suggesting that PLFA 16:1ω5 is a suitable marker for AMF biomass in soil. The indicators for saprotrophic fungi, the ergosterol content and the contribution of 18:2ω6,9 to total PLFA (mol%) were 40 and 60% higher, respectively, in the CM and CMBD than in the MIN treatments. In contrast, fungal GlcN was not affected by the fertilizer treatments. An increased ergosterol/fungal GlcN ratio indicates a shift in fungal community from AMF towards saprotrophic fungi in arable soils.

1. Introduction

Composted farmyard manure application is an important tool for maintaining soil fertility in organic agriculture (Mäder et al., 2002; Raupp and Oltmanns, 2006), which is mainly characterized by abandoning easily soluble inorganic fertilizers (Hartmann et al., 2015). Several long-term field experiments have demonstrated positive farmyard manure effects on soil organic C (SOC) and microbial biomass C (MBC) contents (Edmeades, 2003; Ludwig et al., 2007). Most of these experiments were carried out on silt loams (Hepperly et al., 2006; Jenkinson, 1990; Mäder et al., 2002; Marinari et al., 2006) and some on clay soils (Witter et al., 1993; Elfstrand et al., 2007). Fewer experiments with farmyard manure are available on sandy soils (Christensen, 1996; Ellmer et al., 2000), especially under biodynamic organic management (Heinze et al., 2010; Raupp and Oltmanns 2006). Biodynamic agriculture is the oldest organic farming system, with a history going back more than 90 years, based on the anthroposophical concept of Rudolf Steiner (Koepf et al., 1990, Zaller and Köpke, 2004). Biodynamic farming systems are characterized by specific preparations as compost additives and field sprays (Carpenter-Boggs et al., 2000a; Reganold et al., 1993; Turinek et al., 2009). These preparations should

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stimulate nutrient transformation processes (Reganold, 1995; Zaller and Köpke, 2004).

Biodynamic farming has been intensively investigated in the DOK trial at Therwil, Switzerland (Mäder et al., 2002; Fließbach et al., 2007; Birkhofer et al., 2008). In this trial, highest SOC and MBC contents were usually observed in the biodynamic treatment. However, in the DOK trial, farmyard manure was obtained from two different farms, so that the differences observed may not have been caused by the biodynamic preparations alone. In the long-term experiment on a sandy soil near Darmstadt, Germany (Abele, 1987; Bachinger, 1996), inorganic fertilization was compared with applying composted farmyard manure of the same origin. In this case, treatments differed only in the addition of biodynamic preparations to compost and field (Raupp, 2001).

Farmyard manure not only increases SOC and MBC, it also affects the microbial community. This is indicated by increasing the cell-wall component muramic acid (MurN) content (Joergensen et al., 2010) and lowering the cell-membrane component ergosterol content in comparison with inorganic fertilizer treatments (Heinze et al., 2010; Sradnick et al., 2014). As cell-wall components are accumulating during decomposition, MurN is a highly specific indicator for the contribution of bacteria to SOC (Amelung, 2001; Joergensen and Wichern, 2008). The ergosterol is an indicator for the contribution of saprotrophic fungi to the microbial biomass in agricultural soils (Joergensen and Wichern, 2008) as arbuscular mycorrhizal fungi (AMF) do not contain ergosterol (Olsson et al., 2003). In contrast to ergosterol, fungal glucosamine (GlcN) remained unaffected by the different fertilizer treatments (Joergensen et al., 2010; Murugan et al., 2014; Sradnick et al., 2014).

This different development of ergosterol and fungal GlcN in long-term trials has been interpreted as shifts in the contribution of saprotrophic fungi and AMF to fungal biomass (Murugan et al., 2013, 2014). However, this view lacks direct evidence from AMF-specific biomarkers, e.g. the phospho-lipid fatty acid (PLFA) 16:1ω5 (Olsson et al., 1995). However, the specificity has been questioned (Rousk et al., 2010a), as this PLFA has been detected in Gram-negative bacteria (Nichols et al., 1986; Zelles, 1997). The neutral lipid fatty acid (NLFA) 16:1ω5 is not present in bacteria (Olsson, 1999) and, thus, is the most specific biomarker for AMF (Hedlund, 2002). However, this NLFA is a storage component and occurs in large concentrations in spores (Olsson and Johansen, 2000), which means that the relationship of the NLFA 16:1ω5 to AMF biomass is highly variable (Bååth, 2003). This means that the relationship of NLFA 16:1ω5 to AMF biomass is unclear (Olsson and Johnson, 2005; Drigo et al., 2010). As coverage of AMF by primers is low (Kohout et al., 2014), sequencing approaches of ribosomal markers still fail to estimate sufficiently the contribution of extra-radical AMF hyphae to the soil microbial biomass (Hartmann et al., 2015). This means that practical alternatives are limited and that the continued use of PLFA 16:1ω5 and NLFA 16:1ω5 warrants further evaluation.

The first objective was to investigate whether application of biodynamic preparations causes positive effects additional to those of composted cattle farmyard manure. On the long-term Darmstadt fertilization trial, several studies were recently published using PLFA and NLFA analysis (Ngosong et al., 2009, 2010, 2012). This offers the unique possibility to compare fertilization effects on microbial cell-wall and cell-membrane biomarkers. Consequently, the second objective of the current study was to investigate the response of bacterial biomarkers (MurN, bacterial PLFA) and fungal biomarkers (fungal GlcN, ergosterol, fungal PLFA and NLFA) to the application of composted cattle farmyard manure in comparison with inorganic fertilization plus straw return. This objective was based on the following two hypotheses: (1) Composted farmyard manure specifically promotes the bacterial contribution to MBC and SOC. (2) Composted farmyard manure increases the presence of AMF mycelium, whereas straw return specifically promotes saprotrophic fungi. The third objective was to re-assess conversion values from PLFA 16:1ω5 to AMF biomass as well as those from ergosterol and PLFA18:2ω6,9 to saprotrophic fungal biomass.

2. Material and methods

2.1. Experimental site and design

The experimental site was located near Darmstadt, Hessia, Germany (49° 50′ N, 8° 34′ E), managed by the Forschungsring. The soil is a Haplic Cambisol (FAO-WRB 2014) with 86% sand, 9% silt, and 5% clay, which has been developed from alluvial sediments of the river Neckar (Abele, 1987; Bachinger, 1996). Mean annual precipitation is 590 mm and mean annual temperature is 9.5 °C. The experimental site has been converted from an oak-pine forest to arable land between 1904 and 1920. Before the trial started, the site was managed as a biodynamic farm for at least 20 years (Abele, 1987). The experiment was started in 1980 with four fields (A, B, C, D; Abele, 1987). Only field A was chosen in the current project, because the treatments have remained unchanged since 1980 and because edge effects of a nearby forest were absent (Heinze et al., 2010). Also the other references used as a data source in the current study were solely based on field A (Ngosong et al., 2009, 2010, 2012; Sradnick et al., 2014).

Three fertilizer types and three application rates were implemented as factors in a split block design with four replicates of 5 m × 5 m each. The fertilizer types were: (MIN) inorganic fertilizer calcium ammonium nitrate, superphosphate, potassium chloride (since 1996 potassium magnesia) plus straw return, (CM) composted farmyard manure, and (CMBD) composted farmyard manure with the addition of biodynamic compost and field preparations. These fertilizer types were added at three fertilizer rates, corresponding to 60, 100, and 140 kg N ha^{-1} for cereals and 50, 100, and 150 kg N ha^{-1} for root crops (Bachinger, 1996). No fertilizers were applied when legumes were planted.

Cattle farmyard manure was composted before application for three months if added to winter rye, or for six months if added to spring wheat and root crops (Raupp and Oltmanns, 2006). The composted farmyard manure contained on average 320 mg ash, 354 mg C, 26 mg N, 5.3 mg P, and 45 mg K g^{-1} dry matter in 2004 and 2005 (Raupp, unpublished results). The biodynamic compost preparations, 0.5 g each of *Achillea millefolium*, *Chamomilla recutita*, *Taraxacum officinale*, *Valeriana officinalis, Urtica dioica*, and the bark of *Quercus robur,* were added separately to 1 t of composted farmyard manure (Koepf et al., 1990). The field preparation horn manure (BD 500), which is based on cow dung, was spread after tillage, sowing and stem elongation (200 to 300 g ha^{-1}). The field preparation, horn silica (4 g ha^{-1}), which is based on ground quartz, was spread at tillering, flowering, and corn filling (Koepf et al., 1990). The annual C input rates were roughly 10% (averaged over all fertilizer rates) higher in the CM and CMBD treatments than in the MIN treatment plus straw return (Heitkamp et al., 2009).

Except fertilization, all treatments were managed identically, i.e. cropping, mechanical weeding, and moldboard tillage down to 25 cm. The crop rotation was usually red clover (*Trifolium pratense*) or lucerne (*Medicago sativa*), spring wheat (*Triticum aestivum*), potatoes (*Solanum tuberosum*) or carrots (*Daucus carota* ssp. *sativus*), and winter rye (*Secale cereale*). The crop in 2006 was berseem clover (*Trifolium alexandrinum*), followed by oil radish (*Raphanus sativus* spp. *oleiformis*) as winter cover crop. In the CM and CMBD treatments, the last farmyard manure was applied in September 2004 and urine in April 2005. In the MIN treatments,