Agriculture, Ecosystems and Environment 263 (2018) 7–17

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

# Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties

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ARTICLE INFO

*Keywords:*
Aromaticity
Dissolved organic matter
Legacy effects
MicroResp™
PLFA/NLFA
Water repellency

ABSTRACT

It is well recognized that organic soil management stimulates bacterial biomass and activity and that including cover crops in the rotation increases soil organic matter (SOM). Yet, to date the relative impact of different cover crop species and organic vs. non-organic soil management on soil bacteria and fungi and on SOM quantity and quality remains to be tested. We used a long-term (10 years) full-factorial field experiment to test the combined effects of organic vs. conventional soil management with different cover crop species (oat or rye) and the legacy effects of seven soil health treatments (SHTs: treatments with compost, chitin, marigold, grass–clover, biofumigation or anaerobic soil disinfestation (ASD), and fallow as control) on microbial community biomass, structure and catabolic activity and on SOM quantity and quality (dissolved organic carbon (DOC), aromaticity and water repellency).

Microbial community traits were assessed using PLFA/NLFA analyses and multi-substrate induced respiration. We found that organic soil management enhanced total microbial biomass by increasing bacterial, saprotrophic and arbuscular mycorrhizal fungal biomass; and increased total microbial catabolic activity, associated with maintaining high microbial efficiency (low $qCO_2$). Effects of organic management were amplified by oat as cover crop, which enhanced the abundance of saprotrophic fungi resulting in a higher fungal:bacterial ratio. Total SOM concentration was similar among treatments, however the most easily accessible fraction, i.e. DOC, was higher in organic compared to conventional soils. Also, the aromaticity of the DOC was lower in organic than in conventional systems, which was associated with lower water repellency. There was a legacy effect of SHTs on fungal:bacterial ratio in that chitin and marigold showed higher fungal:bacterial ratio compared to compost, biofumigation and ASD even 6 years after the last application.

We conclude that organic soil management enhances the abundance of all microbial groups and their total catabolic activity, associated with a higher concentration and lower aromaticity of dissolved organic matter. These effects can be enlarged by the growth of specific cover crops and the application of certain soil health treatments.

## 1. Introduction

Decades of intensive agriculture have diminished soil organic matter (SOM) content, thereby reducing fertility and biodiversity of arable lands (Moore et al., 2004; Gardi et al., 2013). Consequently, important soil ecosystem services such as nutrient cycling, water regulation, carbon (C) storage and functional biodiversity are in many cases impaired. Microbial communities are crucial to maintain soil functioning since they are the main decomposers of fresh organic matter, which drives biogeochemical nutrient cycling (Swift et al., 1979; Hättenschwiler et al., 2005). The effects of organic management on microbial communities is well documented, at least with respect to the increase of bacterial abundance and enzyme activities (Lori et al., 2017). However the combined effect of soil management with cover crops on microbial and SOM traits and the legacy effects of organic amendments are largely unknown and warrant further investigation in order to understand management impacts on SOM and soil functioning (Lori et al., 2017). This study integrates the long-term effect of agricultural practices (conventional vs. organic and two different cover crop species) and the legacy effects of seven soil health treatments

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https://doi.org/10.1016/j.agee.2018.04.018
Received 2 January 2018; Received in revised form 20 April 2018; Accepted 23 April 2018
Available online 03 May 2018

(SHT) based on the addition of organic amendments, on the composition and functionality of the soil microbial community and on SOM properties. The main aim of this study is to elucidate the effect of agricultural practices on microbial community and SOM traits that impact on C cycling in agro-ecosystems.

Usually, in organic agriculture large amounts of C are incorporated into the soil via organic fertilizers that replace mineral fertilizers, and which are known to increase SOM content (Lal, 2009). Recently it was shown that the increase of SOC stocks in organic versus conventional agriculture (Gattinger et al., 2012) is strongly dependent on (cover) crop residue decomposability traits (e.g. leaf C:N) (García-Palacios et al., in press). These traits differ among plant species, even for plant species belonging to the same plant family, but they can also be modified via soil feedbacks, for example the addition of N based fertilizers can increase leaf N concentrations and decrease leaf C:N ratio. This implies that growth of different cover crop species can alter SOM quantity, quality and accumulation rate. Besides the beneficial effects of cover cropping to enhance SOM content, cover crops can also increase microbial biomass and activity and induce changes in microbial community structure (Buyer et al., 2010). These shifts are likely dependent on cover crop chemical traits and plant-soil biotic interactions. For instance, leguminous plant species associated with N-fixing rhizobacteria produce low C:N residues, and thereby impact on microbial N mineralization activity and soil N availability (Kumar and Goh, 1999). Similarly, previous studies showed that different cover crop species can associate to different fungal communities (Benitez et al., 2016; Detheridge et al., 2016).

The net change in the soil C pool results from the balance between the C input and C losses that are mainly driven by two factors: 1- the input of C in the soil via plant material and other organic amendments and 2- the metabolic capacities of the soil biota (Six et al., 2006; De Deyn, 2013). The first factor, the chemical composition of the OM input, has a strong impact on the rate and efficiency with which the microbes break down the OM and form SOM (Bending et al., 2002), resulting in changes in the composition of the soil microbial community (De Deyn et al., 2008; Wickings et al., 2012). For instance, growth and further incorporation of plant species, such as *Tagetes patula* or *Brassica* spp. can have an impact on soil biota by exuding secondary metabolites with fungicidal and bacterial effect (Korthals et al., 2014). With respect to the second factor, the metabolic capacities of the soil biota, saprotrophic fungi are generally more efficient at decomposing complex SOM than bacteria and need less N per unit C to build their own biomass (Hodge et al., 2000). Thus, higher saprotrophic fungal biomass increases soil C and N retention relative to bacteria (de Vries et al., 2012; De Deyn, 2013) since fungal tissues accumulate more C and are more recalcitrant than bacterial tissues. Furthermore, higher functional diversity of the microbial community will enhance the breakdown of the OM, especially for complex processes such as chitin degradation that requires the functional complementarity of a diverse microbial community that involves bacteria and fungi (Beier and Bertilsson, 2013). Arbuscular mycorrhizal fungi (AMF) also contribute to increase soil C storage due to their extensive mycelium and necromass (Zhu and Michael Miller, 2003), whereas they have limited capacity to decompose OM (Hodge et al., 2001). Overall, from an agro-ecosystem long term perspective, high abundance of soil microbes with high functional diversity will enhance nutrient mineralization (De Ruiter et al., 1993), and fungi particularly (saprotrophic and AMF) will promote SOM stabilization and carbon sequestration.

Despite the impact of OM amendments on soil biotic and abiotic properties is well recognized (Six et al., 2006; Mulder et al., 2013), the legacy effects (i.e. the still measurable effects of an application after a certain timespan) of organic amendments on the development of microbial communities and soil properties are still unknown. A previous study by Lupatini et al. (2017), based on the same farmland as the present study, showed that after 3 years of application of different soil organic amendments there were still shifts in the relative abundance of some bacterial groups. However, Lupatini et al. (2017) did not include fungal communities (saprotrophic and AMF), which, as we discussed above, have key functions for soil C cycling. Besides, regarding the legacy effect of soil management on abiotic soil properties, Lewis et al. (2014) concluded that the impact of different land uses (desert, agrarian and residential lands) on soil organic C can last for several centuries. However, in continuously managed farmlands where C turnover is sped up by soil management, the legacy effect of previous soil management has not yet been studied. Information about the strength of treatments with organic amendments on both biotic and abiotic soil properties is relevant to design management strategies that efficiently promote the build-up of long-lasting soil-based ecosystem services.

The composition and abundance of SOM determine to a large extent soil water regulation (Saxton and Rawls, 2006), which is important in crop production in terms of nutrient and water availability and prevention of leaching. In this study, we focus particularly on soil water repellency (SWR), which, when high, reduces soil water infiltration, and can influence plant germination, productivity and nutrient leaching (Doerr et al., 2000). Moreover, low SWR is indicative of improved soil structure and soil C sequestration (Bachmann et al., 2008). Addition of organic amendments in agricultural lands can have an effect on water repellency because OM decomposition releases hydrophobic molecules that enhance SWR (Doerr et al., 2000) and therefore water drops take longer time to penetrate into the soil. Therefore, it could be expected that addition of complex organic matter could result in enhanced SWR.

In this research, we use the “Vredepeel long-term experiment” that during 10 years had a consistent management regarding conventional/organic management and cover crop treatments to study shifts in soil properties related to C cycling (microbial and SOM traits). Furthermore, we study the legacy effect of seven soil health treatments (SHT: addition of compost, chitin, *T. patula,* grass–clover, biofumigation or anaerobic soil disinfestation, and fallow as control). As microbial traits we characterized the microbial community structure, the relative abundance of the different microbial functional groups (including bacteria, saprotrophic fungi and AMF), and its catabolic profile. As SOM traits we characterized the SOM concentration, the dissolved organic carbon (DOC) and the aromaticity of the DOC. Fig. 1 shows the relationships that we expected among the measured variables. We hypothesized that:

**H1.** Organic management compared to conventional promotes activity and abundance of bacteria, saprotrophic fungi and AMF (H1a); and different cover crop species from the same plant family (*Grammineae*) result in distinct microbial community structures due to differences in cover crop residue decomposability traits (H1b)

**H2.** Organic management compared to conventional increases SOM, DOC concentration and aromaticity of the DOC (H2a), while cover crop identity does not affect SOM traits (H2b).

**H3.** SHTs leave long-term legacy effects on microbial community structure and functionality (H3a) and on SOM quality (H3b).

**H4.** Soil management effects on SOM feed back to cover crop productivity and chemical composition (H4a), resulting in different cover crop litter traits (H4b).

**H5.** Soil management effects on SOM feed back to differences in SWR. Particularly, we expect that soils under organic management receive larger amounts of OM resulting in increased SWR.

## 2. Material and methods

### 2.1. Study site and experimental design

This study was conducted at Vredepeel long-term experimental farm (The Netherlands: N − 51° 32′ 24.958′, E − 5° 51′13826′). The soil type is a Hortic Podzol (FAO, 2015), texture is 1.1% clay, 3.7% silt and

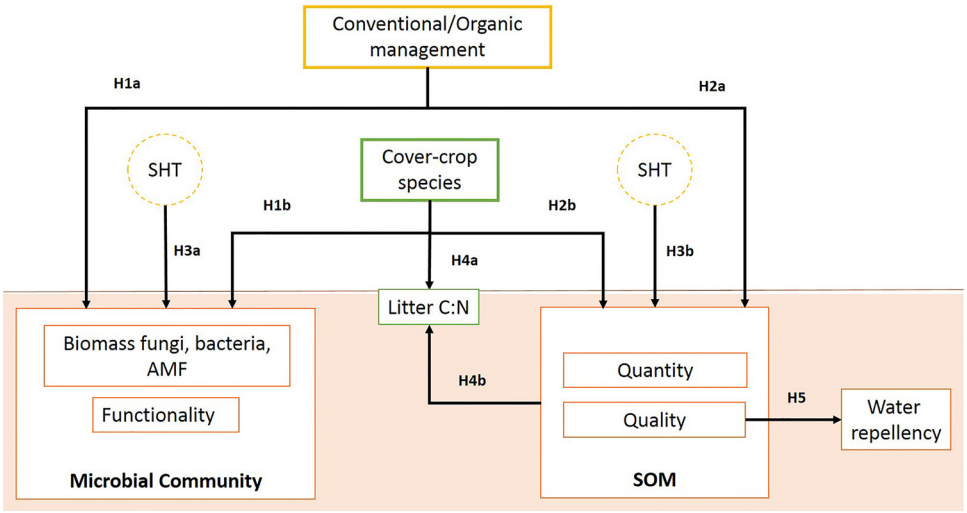


**Fig. 1.** Schematic diagram of the study system and the relations with the hypotheses. Soil microbial community properties (structure and catabolic activity) are affected by soil management (conventional or organic: H1a) and cover crop (oat or rye: H1b). Similarly, soil organic matter (SOM) properties (quantity and quality) are affected by soil management (H2a) and cover crop (H2b). There is a legacy effect of the soil health treatments (SHT) on microbial community and soil organic matter (SOM) properties. Cover crop litter traits and soil water repellency will be indirectly affected by the soil management effects on SOM (H4 and H5 respectively).

94.9% fine sand, the mean annual temperature is 10.2° C and the mean annual precipitation is 766 mm (Korthals et al., 2014). Since 2006, different agricultural practices have been applied using a split-plot randomized block design with four levels of replication (Fig. A1). In spring 2006, the farm was divided in 16 plots (60 m x 6m) that were split into 10 smaller subplots (6 m × 6 m). Plots were randomly arranged in 4 blocks to account for spatial variability. A combination of two treatments: agricultural management (organic and conventional) and cover crop type (*Avena strigosa* and *Secale cereale*) were applied to the bigger plots. Ten soil health treatments (SHT) were applied to the smaller subplots, we selected 7 SHTs to carry out this research. There were 28 treatment combinations in total, these were replicated 4 times (one per block), so the total number of studied subplots was 112.

Conventional and organic soil management differed in fertilization and weed management. While conventional plots received CAN fertilizer (calcium-ammonium-nitrate fertilizer) and pig slurry, organic plots received pig manure and pig slurry (Table A1). From the start of the long-term experiment in 2006 up to the year in which this study was carried out (2015) the organic plots had received 3 times more OM via the organic fertilizers than the conventional plots (Table A1). As a result the conventional plots received 71% of total N added to the organic plots. Weeds were controlled by glyphosate (Round-Up©) in the conventional treatment and by harrowing the first 5 cm of soil and hand-weeding in the organic treatment.

Since 2012 two cover crop species (*A. strigosa* and *S. cereale*) were grown yearly from August till February (Table A2). Main difference between the two cover crop species is that *S. cereale* (hereafter called rye) is a host for the root-lesion nematode *Pratylenchus penetrans* whereas *A. strigosa* (hereafter called oat) is not (Beers, 2010).

Soil health treatments were applied in 2006 and in 2009 in order to reduce the soil pathogenic fungus *Verticillium dahliae* and the nematode *P. penetrans* (See Table 2: Korthals et al., 2014). The SHTs consisted in the application of different types of OM in the top 20 cm soil. These SHTs are briefly explained below (more details can be found in Korthals et al. (2014).

- Grass/clover: Growth and incorporation of a mixture of four different rye-grass species and two clover species.
- Marigold: Growth and incorporation of *Tagetes patula*.
- Chitin: Addition of chitin-rich material based on shrimp debris.
- Compost: Addition of compost consisting of 65% wood, 10% leaves, and 25% grass.
- Biofumigation: Growth of *Brassica juncea* replenished with Broccoli (cv. Montop).
- Anaerobic soil disinfestation (ASD): A mixture of rye-grass incorporated in the soil, irrigated with water and covered with a virtually impermeable film of plastic during 3 months.
- Control: Fallow.

Several crops have been grown since 2006 (potatoes, lily, carrot, maize, peas and wheat: Table A2). Yield results from the previous years showed that yield is higher in conventional than in organic plots and in plots with oat growth versus rye (Table A3).

### 2.2. Soil sampling and soil abiotic parameters

In August 2015 eight soil samples were collected randomly yet with approximate distance of 1 m between the samples from each plot using a soil auger of 20 cm depth and 2 cm diameter and samples were pooled per plot. The pooled samples were sieved over 2 mm and split in two subsamples: one subsample was freeze-dried for phospholipid and neutral lipid fatty acids (PLFA and NLFA, respectively) analysis, and the other one was kept at 4 °C to assess the chemical and physical soil properties as well the functional profile of the microbial communities using MicroResp™ (see Section 2.4).

### 2.3. PLFA and NLFA extraction

The extractions of PLFA and NLFA were performed on 3 g of freeze-dried soil from every plot according to the methods described in Frostegård and Bååth (1996) and Hedlund (2002) based on the Bligh and Dyer method (Bligh and Dyer, 1959; White et al., 1979). Total microbial biomass was quantified as the sum of all detected PLFAs biomarkers (Zelles, 1999) and the NLFA biomarker 16:1w5 (Klamer and Bååth, 2004). PLFAs i15:0, a15:0, 15:0, i16:0, 16:1w9, i17:0, a17:0, cy17:0, 18:1w7 and cy19:0 were used as bacterial biomarkers, and 18:2w6 was used as indicator of the saprotrophic fungi (Hedlund, 2002). The ratio of 18:2w6 to bacterial biomarkers was used as indicator of the relative abundance of saprotrophic fungi and bacteria (Bardgett et al., 1996). NLFA 16:1w5 was used as biomarker of arbuscular mycorrhizal fungi (Olsson, 1999).

### 2.4. Catabolic response profiling (MicroResp™)

The catabolic response profile was analysed using MicroResp™ (Campbell et al., 2003). This method quantifies the substrate induced respiration (SIR) of the microbial community to several C- and N-sources and provides information about its functionality. The method uses a 96 deep-well plate which is filled homogenously with soil and connected to a detection plate that allows measuring the amount of $CO_2$

respired by the soil microorganisms in each well separately. After six hours of incubation at 25 °C, the detection plate is read using colorimetric analysis (Campbell et al., 2003).

One week after sampling, the soil samples were adjusted to 65% water holding capacity (WHC) and added to the deep-well plate using the standard device to add around 0.6 g (300 µl volume) of soil to each well. Filled plates were incubated in dark for six days at 25 °C to re-establish the microbial activity. To avoid changes in soil moisture the deep-well plates were covered with parafilm and kept in a closed plastic box with wet paper towels and a dish with soda-lime. Ten different C- and N-sources that differed in their structural complexity were added to the wells (n = 4) to measure SIR. These sources were: amino acids (containing N-): L-arginine, L-lysine, D-alanine, glucosamine; carboxilic acids: citric acid, oxalic acid, DL-malic acid; carbohydrates: D+ glucose and saccharose; a complex organic polymer: lignin; and a fatty acid ester polymer: Tween 80. These substrates were prepared in a concentration of 30 mg $g^{-1}$ soil water, except for L-Arginine for which the concentration was 15 mg $g^{-1}$ soil and for Tween-80 which concentration was 7.5 mg $g^{-1}$ soil water. 25 µl of each substrate and deionized water were added into the deep-well plate (four replicate wells per substrate per soil sample). The 112 samples were analysed in four consecutive days, 28 soil samples each day that matched with the soil samples from each field block. Plates were connected to the detection plate with a rubber seal and incubated for 6 h at 25 °C. The absorbance of the indicator dye was measured in the detection plate at 570 nm before and after the incubation period using a microplate reader (Vmax, Molecular Devices, Sunnyvale, CA, USA). Absorbance data were normalized by subtracting the average time zero measurements for each plate from the measured colour development per well after the incubation time (Campbell et al., 2003). The data were converted to $CO_2$ concentration using a calibration curve; $\%CO_2 = 0.02 \cdot A_{570}{}^{-3.11}$ ($R^2 = 0.93$) where% $CO_2$ is the concentration in the headspace after incubation and $A_{570}$ is the normalized absorbance (Brolsma et al., 2015). For each substrate the median $CO_2$ concentration change (n = 4) over time was converted to respiration rate (µg $CO_2$–C.$g^{-1}$ dry soil $h^{-1}$) using the formula provided in MicroResp™ procedure, and corrected for median respiration rate of the controls (deionized water) (Brolsma et al., 2015).

### 2.5. Soil chemical and physical analysis

SOM was calculated using weight loss on ignition (Hoogsteen et al., 2015). First, soil moisture was calculated for each plot by measuring the weight loss of 10 g of fresh soil after drying it at 105 °C until constant weight. SOM was calculated by heating the dried soil sample at 550 °C during 4 h and measuring the weight loss once the oven temperature had dropped to 150 °C.

A soil subsample was air dried at 60 °C during two days to measure pH, total N and C, dissolved organic carbon (DOC) and aromaticity of the DOC. Soil pH was measured in a 1:5 soil to deionized water solution, that had been previously shaken for 1 h. Total N and total C were calculated by soil combustion and gas chromatography using a CN Element Analyzer (LECO TRUSPEC CN, CEBAS-CSIC, Spain). DOC was obtained via 1:10 soil to $CaCl_2$ (0.01 M) extraction (Houba et al., 2000). The extractions were equilibrated by horizontally shacking for 1 h and centrifuging at 3500 g during 10 min. The supernatant was filtered through a 0.20 µm cellulose acetate membrane filter and a subsample of the extract was used to measure total DOC concentration in a TOC-5050A analyser (Shimadzu Corporation, Kyoto, Japan). Aromaticity was quantified by measuring the specific ultraviolet absorbance (SUVA) of the DOC extract using a spectrophotometer (Genesys 10S UV–VIS, Thermo Fisher Scientific Inc., Waltham MA, USA). Specific UV (SUVA, l $g^{-1}$ $cm^{-1}$) absorbance at 254 nm was calculated using the following equation (Amery et al., 2008):

$$SUVA = A_{254} * 1000/b * [DOC]$$

where $A_{254}$ is absorbance at 254 nm, b is the length path (cm) and DOC the dissolved organic carbon concentration (mg $l^{-1}$) of the solution.

Soil water repellency was assessed by measuring the potential water drop penetration time (WDPT) (Dekker et al., 1998). In brief this method measures the average time that 3 deionized-water drops take to penetrate in the soil using a soil subsample of each plot that was dried at 60 °C during 3 days and stored at 19 °C to equilibrate with the ambient air temperature. Each soil subsample was put in a circular tray with a diameter of 5 cm using a spoon and taking care of creating a horizontal soil surface for each soil sample by gently flattening the soil surface with the spoon.

### 2.6. Cover crop properties

A greenhouse experiment was carried out to measure cover crop productivity and chemical properties of the cover crops growing in soil from the SHT-control plots. Soil was collected in March 2016 from the SHT-control plots under conventional and organic agricultural management and the cover crop plots (oat and rye; N = 16). Soil was kept at 4 °C until sieved over 2 mm. Then, soil moisture was calculated and adjusted to 60% of the soil water holding capacity by adding deionized water (resulting moisture was 13.83% of soil moisture). Each soil replicate was used to fill 1 pot (5 l, 21 cm diameter) and the amount of soil added to each pot ranged from 3230 g to 4145 g. Oat and rye were grown in soil from the respective cover crop treatments. Twelve seeds of oat and 10 seeds of rye were grown in each type of soil simulating real field seed density of 80 kg $ha^{-1}$ and 100 kg $ha^{-1}$, respectively. Plants were grown during 47 days, regularly watered to maintain the same soil moisture and fertilized with a Hoagland nutrient solution (Hoagland and Arnon, 1950).

Shoots were cut at the soil surface and shoot biomass was measured. Leaf dry matter content (LDMC) was measured following (Pérez-Harguindeguy et al., 2013). Afterwards, leaves were dried in the oven at 60 °C for 2 days, grinded and sent out for chemical analyses. Leaf litter N and C concentrations were measured using a CN Element Analyzer (Analyzer (Flash 1112 EA, Thermo-153 Finnigan, Bremen, Germany).

### 2.7. Statistical analysis

Microbial community structure and catabolic response profile were analysed by Permutational MANOVA (PerMANOVA) with the “adonis” function from the “vegan” R package (Oksanen et al., 2013). Bray-Curtis distance matrix of the PLFA and AMF- NLFA abundance data was tested against the paired interactions of agricultural management, cover crop type and SHT. Permutations were constrained within blocks. When factors interactions were significant, pairwise comparisons followed by Bonferroni correction were used to identify the differences among treatments.

Effects of shifts in soil microorganism biomass (bacteria, saprotrophic fungi, AMF and total), the catabolic response to each applied C source, basal respiration ($CO_2$ respiration under water addition), microbial metabolic quotient ($qCO_2$ = basal respiration/glucose-SIR; respiration per unit of biomass) and soil chemical and physical properties were assessed using linear mixed-effects models with the “lmer” function from the “lme” R package (Bates et al., 2015). Agricultural management system, cover crop type and SHT were included as fixed factors while Block and the SHT nested within the interaction term of agriculture and cover crop were added as random effects. The dependent variables were transformed when the residuals of the model were not normally distributed. Significance of pairwise comparisons was tested using least significant difference Fisher test.

Cover crop litter properties growing in soil from the SHT-control plots were assessed by calculating litter biomass, LDMC, and litter C:N ratio. The effects of the agricultural management and the cover crops on litter properties were tested using lineal mixed-effects models with

**Table 1**
Effects of agricultural management system (Agr; conventional or organic), cover crop species (Ccrop; oat or rye), soil health treatment (SHT: for explanation of SHT, see text) and their interactions on the microbial community composition (PLFA and NLFA) and catabolic profile (MicroResp™). Bold *P* values indicate significant effects.

| PerMANOVA | PLFA and NLFA | | | | MicroResp™ | | | |
|---|---|---|---|---|---|---|---|---|
| | Df | F | $R^2$ | *P* | Df | F | $R^2$ | *P* |
| Agr. management system | 1 | 14.08 | 0.12 | **0.001** | 1 | 2.62 | 0.023 | **0.04** |
| Cover crop species | 1 | 3.70 | 0.03 | **0.008** | 1 | 1.251 | 0.01 | 0.19 |
| SHT | 6 | 0.52 | 0.03 | 0.93 | 6 | 1.331 | 0.07 | 0.06 |
| Agr x Ccrop | 1 | 1.38 | 0.01 | 0.22 | 1 | 2.595 | 0.02 | **0.04** |
| Agr x SHT | 6 | 0.44 | 0.02 | 0.98 | 6 | 0.925 | 0.05 | 0.30 |
| Ccrop x SHT | 6 | 0.54 | 0.02 | 0.92 | 6 | 0.465 | 0.02 | 0.87 |
| Residuals | 90 | | 0.76 | | 90 | | 0.80 | |
| Total | 111 | | 1 | | 111 | | 1 | |

Block as a random factor.

Partial redundancy analysis (pRDA) was used to represent the variation of microbial community composition (PLFA and AMF-NLFA abundance matrix) explained by the soil parameters and the agriculture and cover crop treatment, using the "vegan" R package (Oksanen et al., 2013). Possible block effects were controlled by adding the "condition" term within the "rda" function. Significance of the pRDA, canonical axes and explanatory variables were tested with the "anova.cca" function. Adjusted $R^2$ was calculated with the function "RsquareAdj".

All graphs were designed using R software (R Development Core Team, 2013) and Adobe Illustrator CC (2015).

## 3. Results

### 3.1. Microbial community composition and catabolic profile (H1)

Agricultural management (organic or conventional) and cover crop (oat or rye) induced shifts in the abundance and community structure of the soil microbes (Table 1). Organic management increased the bacterial and fungal biomass (both saprotrophic fungi and AMF), hence increasing total soil microbial biomass (Fig. 2a, Table 2). Additionally, the cover crop oat resulted in higher abundance of saprotrophic fungi compared to rye (Fig. 2a; Table 2). Consequently, organically managed plots showed higher fungal:bacterial ratio under oat than under rye (Fig. 2b, Table 2).

The catabolic profile of the soil microbial community was affected by the interaction between agricultural management system and cover crop identity (PerMANOVA; Table 1). Pairwise comparison of agricultural management and cover crop treatments indicated that the biggest difference in the ordination structure of the catabolic profile was between organic management and rye as cover crop and conventional management and rye as cover crop ($F = 5.69$, *P* adjusted = 0.084). Likewise, the metabolic quotient (i.e., an indicator of the efficiency of the microbial community at mineralizing C sources; high qCO2 indicates low efficiency), showed an interactive effect between cover crop identity and agricultural management (Table 2), resulting in higher $qCO_2$ for the conventional rye plots (Fig. 3a).

Basal respiration was higher in organically managed plots and in plots with rye (Table 2; Fig. 3b,c). When the responses of the microbial community to the different substrates were analysed separately, we observed that microbial communities from organic and rye plots were better able to metabolise some complex amino acids and carboxylic acids as indicated by their higher respiration rates under these conditions. However, there were no differences under the addition of easily degradable C sources such as glucose or saccharose (Table 2; Fig. 3b,c).

### 3.2. SOM properties (H2)

Agricultural management did not affect SOM quantity (mean and standard error: 3.85% ± 0.06%), whereas the quality of the SOM differed between agricultural managements: DOC concentration was higher and DOC aromaticity was lower under organic management (Fig. 4a,b; Table 2). Oat versus rye as cover crop did not influence the measured SOM properties (Table 2).

### 3.3. Legacy effects of the SHTs (H3)

There were legacy effects of SHT on microbial community traits. Particularly, plots where chitin and marigold had been added showed higher fungal:bacterial ratios compared to soils subjected to biofumigation, ASD or compost addition (Fig. 2c, Table 2). Moreover, there was also a legacy effect of SHT on microbial community basal respiration and metabolic capacity to degrade lignin and Tween80 (Table 2). Soils in which grass/clover was grown as SHT showed higher basal respiration and higher respiration rate under the addition of lignin and Tween80 compared to chitin and marigold (Fig. 3d). Legacy effects on the measured SOM properties were not detected (Table 2).

### 3.4. Feedback effects to cover crop traits (H4)

Overall, when grown in the greenhouse under controlled conditions and equal water regime, oat produced more dry mass ($F_{(1,12)} = 19.71$, $P < 0.001$) and had higher leaf dry matter content (LDMC) than rye ($F_{(1,12)} = 17.3$, $P < 0.01$ Fig. 5a,b). Organic management also increased cover crop biomass and N content of both oat and rye ($F_{(1,12)} = 28.46$, $P < 0.001$; $F_{(1,12)} = 22.85$, $P < 0.001$; Fig. 5a, c). Moreover, the C:N ratio of the cover crops grown in the greenhouse was mainly determined by the agricultural management of the soil in the field, with lower C:N when the cover crops were growing in soil from organically managed plots ($F_{(1,12)} = 31.06$, $P < 0.001$; Fig. 5d).

### 3.5. Effects on soil water repellency (SWR) (H5)

Soil under organic management showed lower SWR compared to soil under conventional management (Fig. 4c; Table 2). The pH of organically managed soils was also slightly, but very significantly, higher than under conventional management (Fig. 4d; Table 2). Cover crop and SHT did not significantly affect water repellency of the soil (Table 2).

### 3.6. Combined treatment effects

Multivariate partial redundancy analysis (pRDA) of the microbial community composition showed that agricultural management system, cover crop identity and soil properties together explained 37.79% of the variation in the microbial community composition ($AdjR^2 = 0.33\%$); 5.71% was explained by the block effect. Overall, RDA axis 1 explained 33.4% ($F_{(1,100)} = 57.12$, $P < 0.001$) and RDA axis 2 explained 3% of the variation in the microbial community composition ($F_{(1,100)} = 5.07$, $P < 0.001$) (Fig. 6). Agricultural management system strongly influenced the soil microbial community composition ($F_{(8,100)} = 8.70$, $P < 0.001$) and ordination showed clear distinction among organically and conventionally managed plots. The position of PLFA and NLFA markers in the ordination indicate that the maximum abundance of most of the biomarkers coincides with organically managed plots (Fig. 6) which is in agreement with the previously presented results (Fig. 2a). Moreover, the positioning of the AMF and saprotrophic fungal markers (16:1w5 and 18:2w6, respectively) relative to the centroid of the organic plots indicates that organic management may favour fungal growth (Fig. 6). Also the cover crop species significantly influenced the microbial community composition, albeit to a lesser extent ($F_{(1,100)} = 4.42$, $P < 0.01$) with abundance of most PLFA markers

**Table 2**
Impact of agricultural management system (Agr), cover crop species (Ccrop), soil health treatment (SHT) and their interactions on: the biomass of the total microbial community, the biomass of different microbial groups (bacteria, saprotrophic fungi and AMF), the fungal:bacterial ratio, the multi-SIR (substrate induced respiration; MicroResp™), as well as on soil organic matter (SOM), dissolved organic carbon (DOC), aromaticity of the dissolved organic carbon (SUVA), total C and N, C:N ratio, soil water repellency (SWR) and pH. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, $0.05 < P < 0.10$ = marginally significant (mg), d.f. indicates degrees of freedom. Bold *P* values indicate significant effects.

| | Response variables | Agricultural management system(d.f. = 1) | Cover crop species (d.f. = 1) | SHT (d.f. = 6) | Agr x Ccrop (d.f. = 1) | Agr x SHT (d.f. = 6) | Ccrop x SHT (d.f. = 6) |
|---|---|---|---|---|---|---|---|
| **PLFA & NLFA** | | | | | | | |
| | Total | **30.24**$^{***}$ | 2.00 | 0.64 | 0.43 | 0.36 | 0.32 |
| | Bacteria | **47.25**$^{***}$ | **3.44**$^{mg}$ | 0.73 | 0.04 | 0.10 | 0.69 |
| | Saprotrophic fungi | **30.92**$^{***}$ | **4.03**$^{*}$ | 1.15 | 1.90 | 1.21 | 0.62 |
| | Fungi:bacteria | **4.39**$^{*}$ | 1.49 | **2.59**$^{*}$ | **3.74**$^{mg}$ | 1.60 | 0.49 |
| | AMF | **5.11**$^{*}$ | 0.16 | 0.75 | 0.02 | 0.63 | 0.58 |
| **MicroResp™** | | | | | | | |
| | Basal respiration | **3.81**$^{mg}$ | **4.01**$^{*}$ | **2.03**$^{mg}$ | 1.22 | 1.03 | 0.78 |
| | $qCO_2$ | 0.82 | 1.79 | 0.70 | **6.57**$^{*}$ | 0.76 | 0.33 |
| Carbohydrates | Glucose | 3.64 | 0.30 | 1.15 | 3.74 | 0.63 | 0.14 |
| | Saccharose | 2.25 | 0.38 | 1.00 | 1.83 | 0.62 | 0.34 |
| Amino acids | Arginine | 2.11 | **3.78**$^{mg}$ | 1.73 | 2.35 | 0.93 | 0.21 |
| | Alanine | **5.61**$^{*}$ | **2.84**$^{mg}$ | 1.54 | 2.54 | 1.06 | 0.49 |
| | Lysine | **4.86**$^{*}$ | **3.44**$^{mg}$ | 1.52 | 1.56 | 1.37 | 0.71 |
| Amino sugar | Glucosamine | **3.41**$^{mg}$ | 2.43 | 1.74 | 0.42 | 0.96 | 0.68 |
| Carboxilic acids | Oxalic acid | 2.40 | **4.84**$^{*}$ | 1.37 | 1.29 | 1.13 | 0.43 |
| | Citric acid | **3.97**$^{*}$ | 1.73 | 1.56 | 0.90 | 1.13 | 0.52 |
| Organic Polymers | Malic acid | **3.09**$^{mg}$ | 1.57 | 1.77 | 2.58 | 1.20 | 0.75 |
| | Lignin | **3.46**$^{mg}$ | **3.27**$^{mg}$ | **1.98**$^{mg}$ | 2.56 | 1.13 | 0.46 |
| Polysorbate | Tween 80 | **3.05**$^{mg}$ | 2.40 | **1.86**$^{mg}$ | 2.06 | 0.77 | 0.81 |
| **Soil Properties** | | | | | | | |
| | SOM | 1.65 | 0.21 | 0.085 | 0.30 | 0.20 | 0.18 |
| | DOC | **9.10**$^{**}$ | 2.49 | 0.178 | 1.55 | 0.61 | 0.58 |
| | SUVA | **22.35**$^{***}$ | 2.20 | 0.616 | 1.74 | 0.64 | 1.44 |
| | C | 4.15 | 0.15 | 0.314 | 0.50 | 0.82 | 0.11 |
| | N | 0.27 | 0.12 | 0.312 | 0.61 | 0.29 | 0.43 |
| | C:N | 0.35 | 0.02 | 0.883 | 1.71 | 0.29 | 0.68 |
| | WDPTp | **4.15**$^{*}$ | 0.15 | 0.314 | 0.50 | 0.82 | 0.11 |
| | pH | **18.13**$^{***}$ | 0.42 | 1.017 | 0.34 | 0.19 | 0.19 |

associated with oat as compared to rye. The ordination also suggests that oat resulted in higher SOM concentration and C:N ratio of the soil as compared to rye. The abundance of the actinomycetes (10Me18:0 and 10Me17:0; Heijboer et al., 2016) increased along with increasing SOM concentration, which also resulted in a significant role in the RDA ordination ($F_{1,100} = 6.42$, $P < 0.001$). Likewise, various PLFA markers from which some are identified as bacterial markers (i:16, a:15, 15:00, 16:1w9) were positively related to an increase in DOC. Coefficients of the RDA axis and the significance of the explanatory variables can be found in Table A4.

## 4. Discussion

Our study shows that organic soil management enhances the abundance of all microbial groups (bacteria, saprotrophic fungi and AMF) and their catabolic activity, resulting in a more active C cycling. Moreover, our data show that the effect of cover crop on microbial traits (composition and catabolic activity) depends not only on the cover crop species but also on the legacy that consecutive years of soil management have on SOM traits, and in turn on cover crop traits such as productivity and N concentration. Furthermore, with this study we were able to show that the addition of distinct organic amendments to soil can have a long-term legacy effect on microbial community structure (fungal:bacterial ratio) and microbial catabolic activity.

### 4.1. Effects of organic management and cover crops on the microbial community traits

In agreement with our hypothesis H1a, the addition of OM via organic fertilizers resulted in different microbial communities with enhanced abundance of all microbial functional groups (bacteria, saprotrophic fungi and AMF; Figs. 2 and 6). Consequently, the catabolic

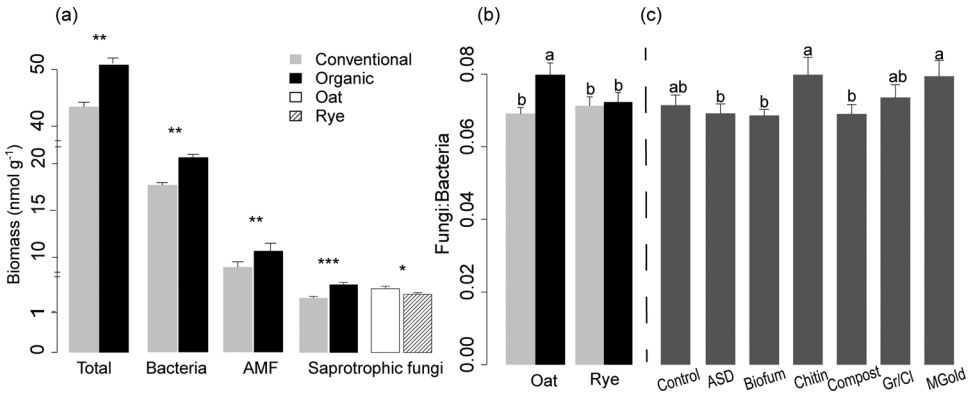


**Fig. 2.** a) Total soil microbial biomass and biomass of the different soil microbial groups (bacteria, AMF and saprotrophic fungi) under organic and conventional management. Y axis units scale is broken from 1.7 to 8.7 nmol $g^{-1}$ and from 22 to 38.5 nmol $g^{-1}$. b) Fungal:bacterial ratio in soils under organic and conventional management with oat or rye as cover crops, and c) under the different SHTs. Bars are means + 1 SE; asterisks indicate significant differences (**: $P < 0.01$; ***: $P < 0.001$) between conventional and organic management or oat and rye. Different letters indicate significant differences ($P < 0.05$).

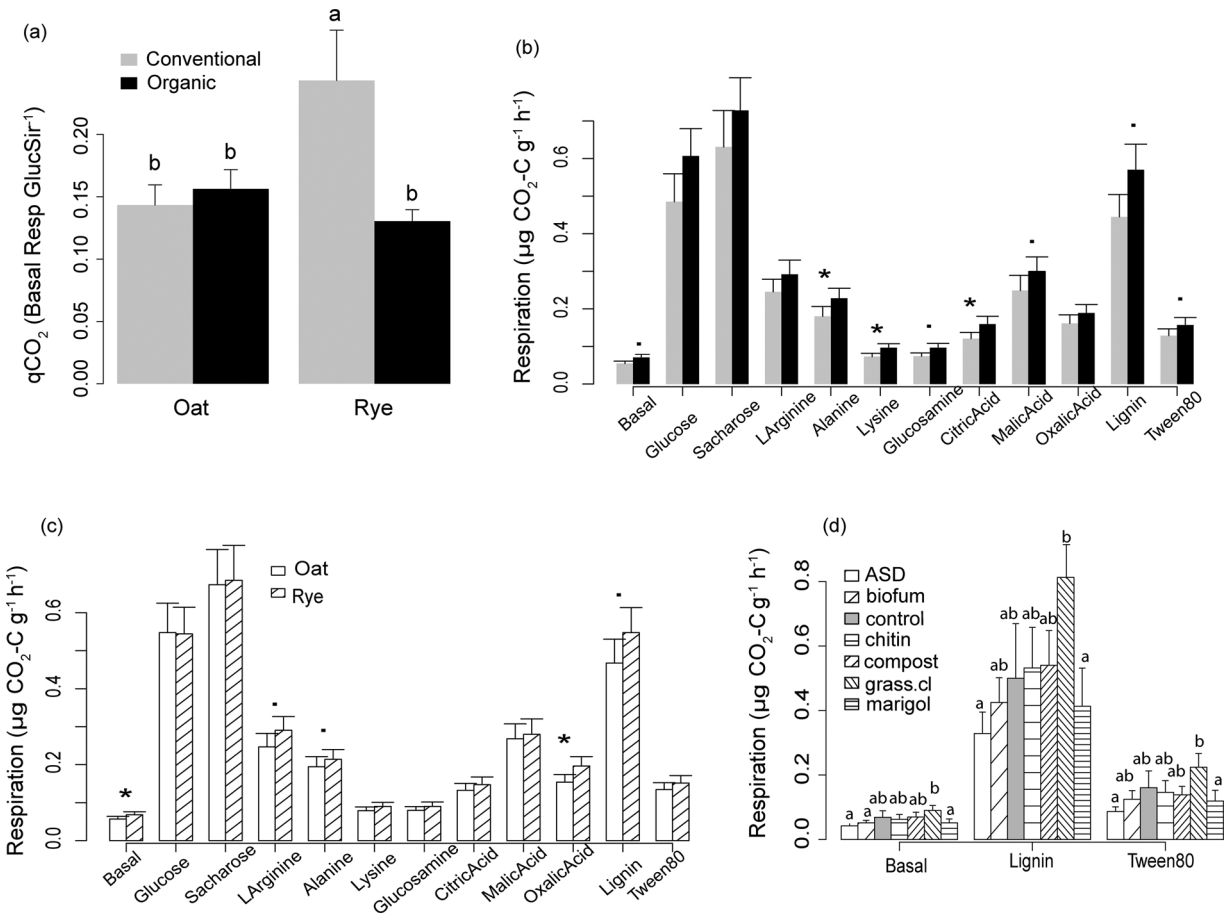


**Fig. 3.** a) Metabolic quotient ($qCO_2$) in soil from conventional or organic management systems under oat or rye growth. b) Catabolic response in soil from conventional or organic management systems. c) Catabolic response in soil from under oat or rye. d) Catabolic response in soil previously subjected to different SHTs. Bars are means + 1 SE; stars and different letters indicate significant differences between treatments ($P < 0.05$); black squares indicate marginally significant differences between treatments ($0.05 < P < 0.10$).

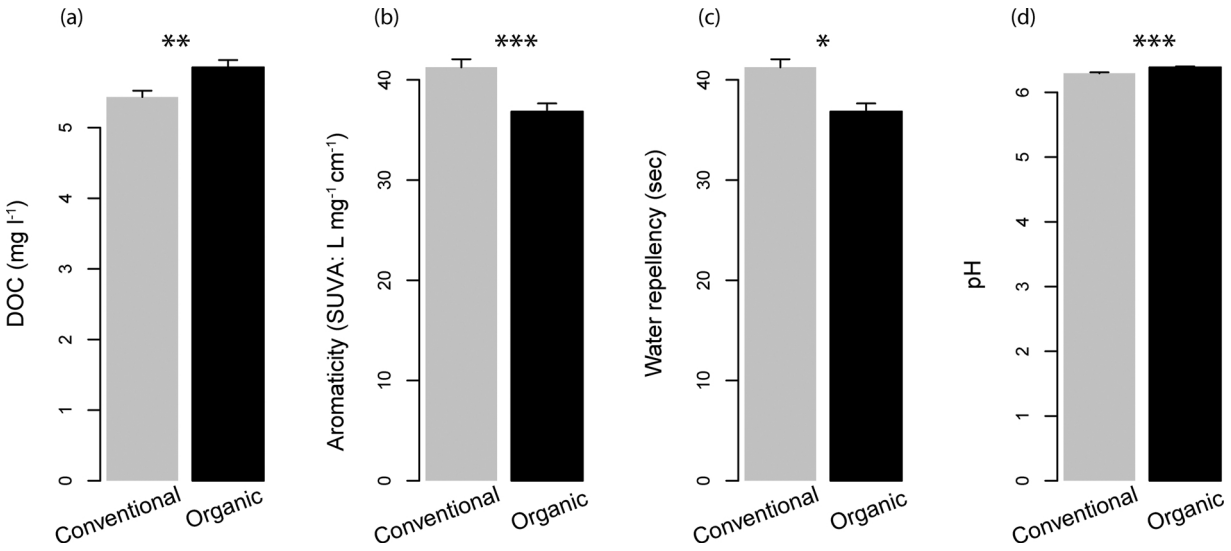


**Fig. 4.** a) Soil dissolved organic carbon (DOC), b) aromaticity of the DOC (SUVA), c) soil water repellency and d) soil pH in conventionally and organically managed plots. Bars are means + 1 SE; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

activity of the microbial community was higher in organic than in conventional plots (Fig. 3b), which implies higher mineralization rate and increased release of nutrients available for plant under organic management. Our results complement the results of a recent meta-analysis by Lori et al. (2017) which showed an increase in bacterial abundance and activity under organic farming systems compared to conventional systems, and add that the larger microbial community size in organically managed soil is caused by a simultaneous enhancement of all microbial groups.

One might expect that larger addition of complex OM molecules via

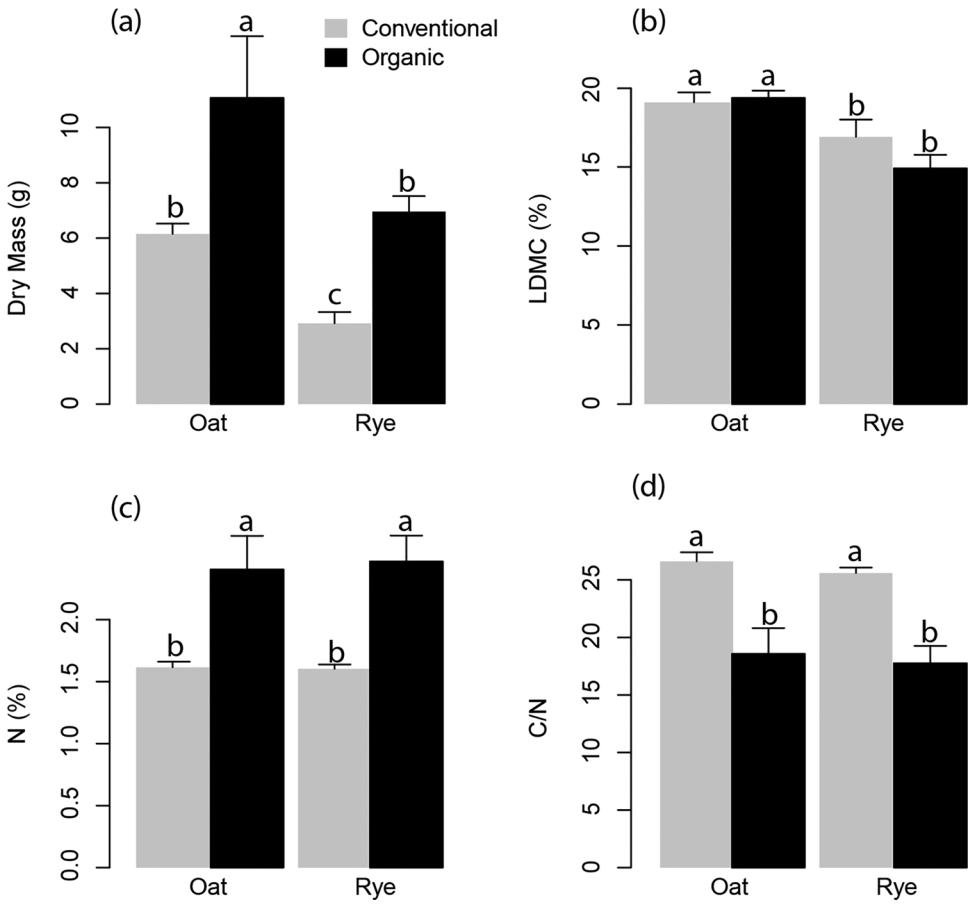


**Fig. 5.** a) Dry mass, b) leaf dry matter content (LDMC), c) nitrogen (N) percentage, and d) leaf C:N ratio of cover crops (oat or rye) growing in the greenhouse in soil collected from the control SHT plots, subjected to the combinations of conventional and organic management and oat and rye cover crops. Bars are means + 1 SE. Different letters indicate significant differences between treatments ($P < 0.05$).

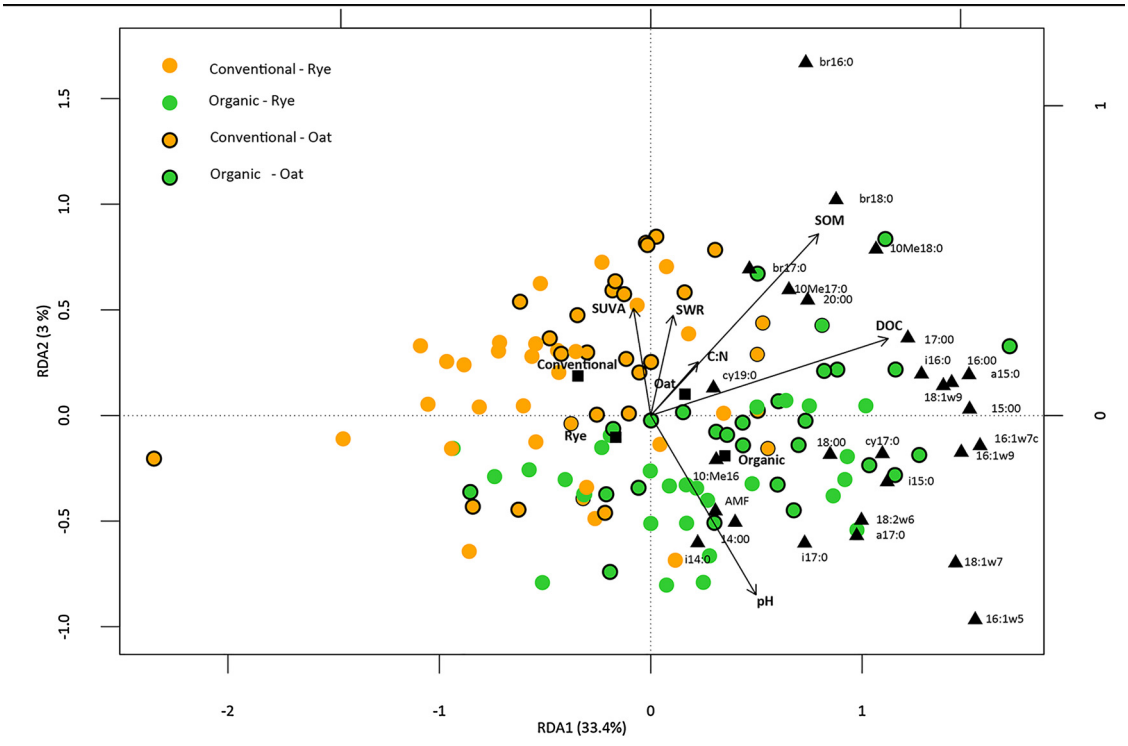


**Fig. 6.** pRDA triplot showing the relationships among plots (circles), PLFA and NLFA biomarkers (triangles), soil properties (arrows) and soil management (squares). Colours (orange and green) represent conventional and organic plots, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fertilization with manure in the organically managed plots would lead to a dominance of saprotrophic fungi versus bacteria (higher fungal:bacterial ratio) since fungi are more efficient at breaking down complex organic molecules (Hodge et al., 2000). However, changes in the fungal:bacterial ratio were dependent on the interaction between agricultural management system and cover crop. A higher fungal:bacterial ratio occurred in organic plots with oat but not in organic plots with rye (Fig. 2b), indicating that the growth of different cover crop species determined changes in microbial community structure, which is in agreement with hypothesis H1b. Oat may have stimulated a higher fungal:bacterial ratio when growing in organic plots compared to rye by the SOM feedback to cover crop traits (as we will discuss further in the Section 4.4 *SOM feedback to cover crop traits*). The amount of oat residues incorporated into organic plots is estimated to double the amount of residues added to the other treatments (Fig. 5a). Therefore, organic oat plots received an extra addition of OM, that may have contributed to the shift of microbial community structure towards saprotrophic fungal dominance.

The growth of rye versus oat slightly enhanced the activity of the microbial community as shown by the higher basal respiration and higher $CO_2$ production after the addition of arginine, alanine, oxalic acid and lignin (Fig. 3c, Table 2). However, the efficiency of the microbial community at mineralizing C was lower in rye-conventional plots (higher $qCO_2$: Fig. 3a), meaning a higher amount of C loss via microbial respiration per unit of microbial biomass. Spohn (2015) showed that addition of litter that is harder to decompose is related to lower microbial efficiency. In our experiment, we observe that despite this could be the rule for conventional-rye plots that receive higher C:N litter residues (Fig. 5d), it is not for conventional-oat plots that also received higher C:N ratio litter but maintained the same $qCO_2$ (Fig. 3a). Therefore, there are other factors besides C:N litter ratio controlling the microbial $qCO_2$. We propose that the higher abundance of saprotrophic fungi in oat plots (Fig. 2a) improved the catabolic efficiency of the microbial community and therefore, the $qCO_2$ remained lower for both conventional and organic plots (Fig. 3a).

### 4.2. Effects of organic management and cover crops on SOM traits

Contrary to our expectations (H2a), the concentration of SOM was similar among treatments (3.85% ± 0.06%) despite the three times larger addition of OM into organic plots compared to that in conventionally managed plots. In contrast to SOM quantity, SOM quality differed between organically and conventionally managed soils, which was in accordance with our hypothesis H2a. As expected, there was also more DOC, which was of lower aromaticity in organically managed plots compared to conventionally managed plots, implying that there is more accessible C of less biochemical complexity in the soil solution in organically compared to conventionally managed soils (Fig. 4a,b).

A recent study hypothesized that labile OM, which is more efficiently utilized by microbes than complex compounds, is the dominant source of microbial products, which are the main precursor of stable SOM (microbial efficiency matrix stabilization theory (MEMS; Cotrufo et al., 2013)). These authors also demonstrate that labile C is rapidly incorporated into the microbial biomass via the dissolved organic matter microbial path and results in efficient SOM formation (Cotrufo et al., 2015). Therefore, both, OM decomposability traits and microbial community traits are determinants of SOM stabilization. Our experiment shows that organic management enhances both the decomposability of the SOM, and the abundance and the activity of the microbial community. In fact, the pRDA shows that certain PLFA bacterial markers increase in the same direction as DOC (Fig. 6). These markers likely stand for bacteria that can quickly make use of the DOC which is considered a precursor of stable SOM.

### 4.3. Legacy effects of the soil health treatments

A legacy effect of the SHTs was observed on the fungal:bacterial ratio even after 6 years from the last SHT application (H3, Table 2). Although none of the SHTs differed from the control, some significantly differed from one another (Fig. 2c): the plots with chitin amendment and marigold (*T. patula*) resulted in higher fungal:bacterial ratios compared to plots with compost, biofumigation and ASD (Fig. 2c). Although the PLFA analysis does not give information on the abundance of specific fungal and bacterial groups, previous studies showed that the addition of chitin enhanced the abundance of chitinolytic microbes (Cretoiu et al., 2013; Sharp, 2013; Lupatini et al., 2017), which in soil are mainly actinomycetes (Swiontek Brzezinska et al., 2014), and some fungi from the *Mucorales* and the *Ascomycetes* families (Gooday, 1990) capable of decomposing, lignin, chitin, pectin and creatine (Swiontek Brzezinska et al., 2014). Similarly, marigold residues, that are rich in secondary metabolites (thiophenes) are known to decrease root-lesion nematode populations (Evenhuis et al., 2004), but they are also complex molecules, difficult to degrade (Chomel et al., 2016) which may impact on microbial community structure and catabolic activity. Our results are in accordance with Topp et al. (1998), who showed that marigold residues do not alter bacterial communities, whereas they may cause a shift in microbial community structure towards fungal dominance (Fig. 2c). In contrast, compost, biofumigation and ASD may have stimulated bacterial growth resulting in lower fungal:bacterial ratios (Fig. 2c). A recent study on the bacterial communities in the Vredepeel soil (Lupatini et al., 2017) showed higher abundance of bacterial habitat specialists (i.e. taxa that are distinctive for a specific site) in the ASD and compost treatments than in the chitin and marigold treatments. Specifically, ASD favoured the presence of bacterial taxa within the *Bacillales* and *Clostridiales* (*Firmicutes*) and compost favoured the taxa within the *Proteobacteria*. Still, further research is necessary to explain the mechanisms by which these treatments lead to relative more bacteria.

We also observed a legacy effect of the SHTs on the microbial catabolic profile that indicates that grass-clover had the highest basal respiration rate, and the highest activity under the addition of lignin and Tween80 (Table 2, Fig. 3d). These results suggest that the grass-clover may have enhanced the abundance of active microorganisms that are able to degrade lignin and complex C sources such as Tween80.

### 4.4. SOM feedback to cover crop traits

As we predicted in our hypothesis H4, cover crop traits (productivity and leaf C:N) were indirectly affected by the effect of soil management on SOM traits. In our experiment, organic soils received more N than conventional soil (Table A1) which can explain why the cover crop residues had a lower C:N ratio and higher productivity when they were grown in organic as compared to in conventional soil (Fig. 5a,d). Hence, the C-input via the cover crop residues was larger and more decomposable in organic compared to conventional, which may have consequences on both microbial community structure and SOM stabilization. To maximize the benefits of cover crops growth to soil properties is therefore necessary to consider the response of cover crop traits to soil management's legacy in soil fertility.

### 4.5. SOM effects on water repellency and pH

Organic management conferred also other than the aforementioned benefits to the soil properties, namely a decrease in water repellency and a slight increase in pH (Fig. 4c,d). Sandy soils can be highly water repellent, impairing the infiltration of water in the soil and causing uneven soil water distribution. The addition of only organic fertilizers decreased water repellency as compared to plots that also received mineral fertilizers, likely by changing the structural composition and arrangement of organic compounds (Doerr et al., 2005). Indeed, in the

pRDA aromaticity and water repellency are correlated. Besides, mineral fertilizers usually cause soil acidification and consequently, pH was lower in conventional plots. There is strong evidence that microbial biomass positively correlates with soil pH (Bååth and Anderson, 2003; Aciego Pietri and Brookes, 2008). Consequently organic plots with higher pH likely support a higher activity of microbes, eventually enhancing the sustained activity of the soil food.

### 4.6. Conclusions

With our experiment we show that soil management and cover crops both influence microbial community structure and activity, and SOM quality. Organic management increases microbial biomass and catabolic activity which may directly enhance nutrient mineralization and SOM stabilization. Additionally, the effect that soil management has on soil fertility feeds back on cover crop decomposability traits which in turn affect microbial catabolic efficiency. Hence, this study highlights the importance of integrating different agricultural practices, such as organic fertilization and including cover crops to strengthen the soil properties that foster more sustainable and resilient agro-ecosystems, such as fungal:bacterial ratio and $qCO_2$. Furthermore, we showed long lasting effects of applying certain organic amendments (chitin and marigold in our experiment) in enhancing a beneficial soil property (i.e. fungal:bacterial ratio).

## Acknowledgements

We thank Irene Garcia, Sya Hoeke, Gwénaëlle Flageul and Giulia Mainardi for their help with lab analysis. We also acknowledge Johnny Visser, Harry Verstegen and colleagues of Vredepeel experimental farm. This research was funded by the Netherlands Organization for Scientific Research through the 2013–2014 BiodivERsA/FACCE-JPI joint call for research proposals, with national funders ANR, NWO, FCT (BiodivERsA/001/2014), MINECO, FORMAS, and SNSF. G.B.D.D. acknowledges NWO-ALW for financial support (VIDI grant nr 864.11.003).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.agee.2018.04.018.

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