

ORIGINAL ARTICLE

Distinct soil microbial diversity under long-term organic and conventional farming

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Low-input agricultural systems aim at reducing the use of synthetic fertilizers and pesticides in order to improve sustainable production and ecosystem health. Despite the integral role of the soil microbiome in agricultural production, we still have a limited understanding of the complex response of microbial diversity to organic and conventional farming. Here we report on the structural response of the soil microbiome to more than two decades of different agricultural management in a long-term field experiment using a high-throughput pyrosequencing approach of bacterial and fungal ribosomal markers. Organic farming increased richness, decreased evenness, reduced dispersion and shifted the structure of the soil microbiota when compared with conventionally managed soils under exclusively mineral fertilization. This effect was largely attributed to the use and quality of organic fertilizers, as differences became smaller when conventionally managed soils under an integrated fertilization scheme were examined. The impact of the plant protection regime, characterized by moderate and targeted application of pesticides, was of subordinate importance. Systems not receiving manure harboured a dispersed and functionally versatile community characterized by presumably oligotrophic organisms adapted to nutrient-limited environments. Systems receiving organic fertilizer were characterized by specific microbial guilds known to be involved in degradation of complex organic compounds such as manure and compost. The throughput and resolution of the sequencing approach permitted to detect specific structural shifts at the level of individual microbial taxa that harbours a novel potential for managing the soil environment by means of promoting beneficial and suppressing detrimental organisms.

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Introduction

With the advent of the green revolution, agricultural productivity has been raised by increased fertilization and pesticide application, improved irrigation, soil management regimes and crops as well as massive land conversions (Tilman *et al.*, 2002). There is increasing concern, however, that agricultural intensification leads to large-scale ecosystem degradation and loss of productivity in the long term. Negative environmental implications include soil degradation, increased greenhouse gas emissions, accumulation of pesticides and diminished availability and quality of water (Tilman *et al.*, 2001; Foley *et al.*, 2005). In fact, agricultural intensification is perceived as one of the greatest threats to global biodiversity (Convention on Biological

Diversity, 2010). Low-input systems such as organic farming, which substantially reduce the use of synthetic fertilizers, pesticides, energy and mechanic stress, aim at mitigating these negative impacts in order to improve sustainable production (Gomiero *et al.*, 2011). However, we still have an incomplete understanding of the challenges, benefits and limitations of low-input farming (Tscharntke *et al.*, 2012) and the sustainability of organic farming (Wu and Sardo, 2010).

One of the cornerstones of agricultural management is proper stewardship of soil. Soil provides fundamental ecosystem services including nutrient cycling, water regulation, transformation of organic materials and toxic compounds as well as control of pests and diseases (Doran and Zeiss, 2000). At the system level, the microbiome plays an integral role in virtually all soil processes (Barrios, 2007), such that microbial abundance, activity and composition will largely determine sustainable productivity of agricultural land (van der Heijden *et al.*, 2008). In this light, the ability to manage the soil microbiome for the presence of beneficial and absence of

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detrimental organisms could offer a promising approach to improve sustainable agricultural production. Effects of agricultural management on the soil microbiome are, however, complex and diverse (Bünemann *et al.*, 2006; Nelson and Spaner, 2010), and retrieving universally valid conclusions on organic and conventional farming systems is difficult. In general, it has been reported that low-input farming systems promote higher abundance and diversity of most organisms, and although the positive effects on the macrobiota are largely consistent across studies, the impact on the microbiota seems less clear (Hole *et al.*, 2005; Postma-Blaauw *et al.*, 2010). The enormous complexity of microbial life and the technical constraints to properly measure its components have so far limited our understanding of the relationships between low-input farming and microbial diversity. Novel high-throughput DNA sequencing technologies offer ways to explore the soil microbiota at higher resolution, coverage and throughput, and have the potential to shed more light on the community- as well as taxon-level responses to agricultural management (Taberlet *et al.*, 2012).

The broad spectrum of agricultural practices further limits comparability among different studies (Hole *et al.*, 2005; Gomiero *et al.*, 2011). Whereas organic systems are commonly defined by management practices lacking the application of synthetic fertilizers and pesticides, the definition of conventional management is more variable. Fertilization and plant protection schemes as well as crop rotation and soil tillage strategies often vary across conventional farming systems. Commonly, conventional management practices rely on the use of synthetic fertilizers and pesticides and often avoid the use of organic fertilizers. However, as organic amendments have been shown to exert positive effects on various soil properties (Rosen and Allan, 2007), more integrated conventional fertilization strategies seek to use a combination of synthetic and organic fertilizers.

However, only a few agroecosystem experiments exist that compare organic and conventional management strategies with different fertilization and plant protection regimes over an extended period of time (Raupp *et al.*, 2006) that is ultimately required for evaluating sustainability of land-use regimes (Rasmussen *et al.*, 1998). The Swiss DOK (German abbreviation for *dynamic, organic and conventional agricultural management*) experiment represents a unique system to compare the long-term effects of organic and conventional management on ecosystem properties (Raupp *et al.*, 2006). Since 1978, 96 plots have been managed according to five different farming systems along with a 7-year crop rotation in three temporally shifted parallels (Mäder *et al.*, 2002). These farming systems differ in plant protection and fertilization regimes, whereas factors such as tillage and crop rotation are kept constant. The DOK experiment includes two conventional

approaches, an exclusively minerally fertilized system and an integrated system with a fertilization scheme combining mineral and organic fertilization, and contrasts these to three organic systems with different fertilization schemes but all lacking the use of chemicals.

Over the years, organic systems revealed an increase in microbial biomass and activity, largely driven by quantity and quality of farmyard manure (Fliessbach *et al.*, 2007; Birkhofer *et al.*, 2008). Whereas management effects on microbial bulk parameters have been well documented, the impact on soil microbial community composition was more difficult to assess. The first-generation molecular tools used to examine shifts in community structures such as genetic profiling and phospholipid fatty acid analyses demonstrated structural differences among the various organic and conventional systems (Hartmann and Widmer, 2006; Hartmann *et al.*, 2006; Widmer *et al.*, 2006; Esperschuetz *et al.*, 2007). However, diversity coverage and phylogenetic resolution strongly limited the assessment of both α - and β -diversity as well as a thorough identification of microbial groups indicative of specific management regimes.

In this context, we employed a 454-pyrosequencing approach (Margulies *et al.*, 2005) of bacterial and fungal ribosomal markers to examine the response of soil microbial diversity to >20 years of continuous organic and conventional farming in the DOK experiment. At the farming system level, we aim to identify the major agricultural factors driving differences in α - and β -diversity across management and crop regimes. Based on the initial community-level assessment, we then aim at harnessing the power of the sequencing approach to identify soil microbial taxa that have adapted to conditions characteristic of long-term agricultural intensification or low-input farming. In the long term, the capability to monitor individual microbial taxa may improve our potential to manage agricultural soils for sustainable productivity by promoting beneficial and suppressing pathogenic microorganisms.

Materials and methods

The DOK long-term experiment

The DOK experiment compares five different farming systems (three organic and two conventional) that differ in fertilization and plant protection regimes (Table 1). The biodynamic (BIODYN) and bioorganic (BIOORG) systems exclusively receive organic fertilizers (farmyard manure and slurry, FYM), whereas one conventional system (CONFYM) features an integrated fertilization scheme based on a combination of organic and mineral fertilizers. All three FYM-based systems received the same amount of 1.4 livestock units per hectare and year, but of different qualities. The system-specific manure

Table 1 Detailed management characteristics of the DOK long-term field experiment (Therwil, Switzerland)

| System | Organic (low-input) farming systems | | | Conventional (high-input) farming systems | |
|---|--------------------------------------|--------------------------------------|-------------------------------|---|--|
| | Unfertilized (NOFERT) | Biodynamic (BIODYN) | Bioorganic (BIOORG) | Conventional (CONFYM) | Mineral (CONMIN) |
| <i>Fertilization scheme</i> | | | | | |
| Farmyard manure and slurry (FYM) ^a | — | Composted FYM | Rotted FYM | Stacked FYM | — |
| Mineral | — | — | Rock powder, magnesia | Synthetic (NPK) | Synthetic (NPK) |
| <i>Inputs (kg ha⁻¹ y⁻¹)^b</i> | | | | | |
| Dry matter | 0 | 3177 ± 410 | 3303 ± 494 | 3404 ± 525 | 0 |
| Organic matter | 0 | 1818 ± 243 | 2176 ± 326 | 2514 ± 419 | 0 |
| N _{tot} | 0 | 93 ± 10 | 102 ± 13 | 181 ± 16 | 133 ± 10 |
| N _{min} | 0 | 27 ± 3 | 34 ± 5 | 120 ± 10 | 133 ± 10 |
| P | 0 | 18 ± 3 | 24 ± 4 | 36 ± 3 | 36 ± 2 |
| K | 0 | 220 ± 22 | 189 ± 22 | 269 ± 22 | 262 ± 19 |
| Ca | 0 | 150 ± 25 | 125 ± 20 | 159 ± 38 | 238 ± 70 |
| Mg | 0 | 27 ± 4 | 25 ± 4 | 34 ± 5 | 34 ± 6 |
| <i>Plant protection scheme</i> | | | | | |
| Weed control | Mechanical | Mechanical | Mechanical | Mechanical and herbicides ^c | Mechanical and herbicides ^c |
| Disease control | Indirect methods ^d | Indirect methods ^d | Indirect methods ^d | Chemical (thresholds) ^c | Chemical (thresholds) ^c |
| Insect control | Plant extracts, biocontrol | Plant extracts, biocontrol | Plant extracts, biocontrol | Chemical (thresholds) ^c | Chemical (thresholds) ^c |
| Special treatments | Biodynamic preparations ^e | Biodynamic preparations ^e | CuSO ₄ in potatoes | Plant growth regulators ^c | Plant growth regulators ^c |

Abbreviations: BIODYN, manured biodynamic; BIOORG, manured bioorganic; Ca, calcium; CONFYM, manured conventional; CONMIN, minerally fertilized conventional; DOK, German abbreviation for *dynamic, organic and conventional agricultural management*; FYM, farmyard manure and slurry; K, potassium; Mg, magnesium; NOFERT, unfertilized biodynamic; N_{min}, mineral nitrogen; N_{tot}, total nitrogen; P, phosphorus. ^aFYM was applied at 1.4 livestock units per hectare and year. FYM processing differed for the three stocked farming systems, that is, BIODYN (composted for 8–12 months), BIOORG (rotted for 3 months) and CONFYM (stacked for 4–8 months).

^bAverage (mean ± s.e.) annual nutrient amendments between 1992 and 2007 (nutrient input through plant residues are not included). N_{tot} in FYM was measured according to Kjeldahl and refers to the sum of organic and ammonium N. N_{min} in FYM refers to ammonium N only.

^cHerbicides (1–2 treatments per year) and fungicides (2–3 treatments per year) were applied according to threshold values. Pest control was performed in potatoes on a regular basis and in winter wheat on a rare basis. Plant growth regulators (Cycocel, OHP Inc., Mainland, PA, USA) were routinely applied to winter wheat.

^d*Bacillus thuringiensis* subsp. *tenebrionis* (Novodor FC, Valent BioScience Corporation, Libertyville, IL, USA) was applied in all organic farming systems as biocontrol agent against potato beetle. No other microbial inoculants (for example, biocontrol, effective microorganisms) were used.

^eBiodynamic preparations (Reganold, 1995) P500 (cow manure fermented in a cow horn) and P501 (silica incubated in a cow horn) were amended at rates of 250 g and 4 g hectare and year, respectively. Composting additives were P502 (*Achillea millefolium*, L.), P503 (*Matricaria recutita*, L.), P504 (*Urticaria dioica*, L.), P505 (*Quercus robur*, L.), P506 (*Taraxacum officinale*, Wiggers) and P507 (*Valeriana officinalis*, L.). A decoct of shave-grass (*Equisetum arvense*, L.) has been applied once during vegetational growth to wheat and potatoes as a protective agent against plant diseases at rates of 1.5 kg ha⁻¹.

types ranged from stacked (CONFYM) to rotted (BIOORG) to composted (BIODYN) manure, with increasing aeration resulting in carbon loss (Table 1) and decreasing carbon-to-nitrogen ratios ranging from 20 to 18 and 13, respectively (Fließbach et al., 2007). Between 1992 and 2007, the organic system received ~28% (BIODYN) and 13% (BIOORG) less organic matter and ~49% (BIODYN) and 44% (BIOORG) less total nitrogen when compared with the integrated conventional system (CONFYM) (Table 1). The second conventional system (CONMIN) exclusively received mineral fertilizer. The third organic system (NOFERT) received no fertilizer at all. Overall, the FYM-based organic systems (BIODYN and BIOORG) received 58–77% of the nutrients (nitrogen, phosphorus, potassium, calcium and magnesium) that were applied to the conventional systems (CONFYM and CONMIN) (Table 1).

Plant protection in the conventional systems was performed with respect to thresholds using chemical weed (herbicides), disease (fungicides) and insect

(insecticides) control as well as synthetic plant growth regulators according to Swiss standards (Federal Department of Economic Affairs Education and Research, 2008a), similar to those in the European Union. Organic plant protection was performed according to the respective guidelines (Lampkin, 1990). BIOORG was managed using mechanical and biological but no chemical plant protection strategies according to the Swiss organic standards (Federal Department of Economic Affairs Education and Research, 2008b). Both BIODYN and NOFERT were managed according to biodynamic regulations (Demeter Schweiz, 2012), using mechanical and biological but no chemical plant protection strategies as well as special biodynamic preparations (Reganold, 1995). It is important to understand that the DOK experiment compares holistic farming systems rather than controlling the variation of each individual component. Therefore, the main factors differentiating the systems, that is, plant protection measures and differences in fertilization, cannot be completely isolated.

Collection of soil samples (each representing a pool of 8 to 14 cores per plot, 2.5 cm diameter, 0–20 cm depth) and extraction of total nucleic acids have been described previously (Widmer *et al.*, 2006; Schneider *et al.*, 2010). The DOK field (1.4 ha) contains 96 plots (5 × 20 m each) arranged as a randomized split–split block with four replicates of each treatment and crop. For this study, 40 plots representing all five farming systems (NOFERT, BIODYN, BIOORG, CONMIN and CONFYM) and two different stages in the crop rotation (winter wheat and grass-clover) were selected. Samples were collected in March 2000 (after 21 years of continuous management) and a complete crop rotation later in March 2007. A total of 80 samples (5 systems × 2 crops × 2 time points × 4 replicates) were analysed.

Fertilizer and soil chemical properties

Chemical properties of fertilizers and soils have been determined according to Swiss standard protocols (Agroscope, 2011). Fertilizer contents have been measured annually since 1992. In FYM, total nitrogen (N_{tot}) as the sum of organic and ammonium N was measured using the Kjeldahl method on a AutoKjeldahl-System K-370 (Büchi Labortechnik AG, Flawil, Switzerland). FYM organic matter was determined by combustion at 600 °C. Elemental analyses of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were performed from dried and ground material calcinated at 450 °C. After HCl extraction of ashed filtrates, properties were measured by inductively coupled plasma-optical emission spectroscopy. Mineral fertilizers (NPK) in CONMIN and CONFYM consisted of calcium ammonium nitrate, triple superphosphate and potassium magnesium sulphate.

Soil chemical properties have been measured biannually. The pH of 60° dried soil samples was determined in a soil suspension with deionized water (1:10, w/v). Soil organic carbon was analysed by titration after wet oxidation in concentrated H_2SO_4 and 2 M $K_2Cr_2O_7$. Total soil nitrogen was measured using the Kjeldahl method. Plant available soil P and K were determined photometrically and by flame atomic emission spectroscopy, respectively, in extracts from CO_2 saturated water. Mg was determined by flame atomic absorption spectroscopy in $CaCl_2$ extracts.

Pyrosequencing of bacterial and fungal ribosomal markers

Amplicon generation (bacterial 16S_{V1-V3} and fungal ITS2 of the ribosomal RNA operon) was performed as previously described (Hartmann *et al.*, 2014). Amplicons were unidirectionally sequenced using the GS-FLX Titanium technology (Roche 454 Life Sciences, Branford, CT, USA) at the Génome Québec Innovation Center Montréal, Canada. Sequence data

were denoised according to Hartmann *et al.* (2014), including elimination of sequencing errors (Quince *et al.*, 2009), PCR substitution errors (Quince *et al.*, 2011) and chimeras (Edgar *et al.*, 2011) as implemented in MOTHR (Schloss *et al.*, 2009), as well as target verification and extraction (Hartmann *et al.*, 2010; Nilsson *et al.*, 2010). Denoised sequences were clustered into operational taxonomic units (OTUs) using CROP (Hao *et al.*, 2011) at 97% sequence identity. CROP centre sequences were queried against GREENGENES (DeSantis *et al.*, 2006; McDonald *et al.*, 2011) and UNITE (Abarenkov *et al.*, 2010) using the naive Bayesian classifier (Wang *et al.*, 2007) implemented in MOTHR and a minimum bootstrap of 60%.

Statistics

All statistical tests performed in this study were considered significant at $P < 0.05$ unless indicated otherwise; however, we provide the precise P -values wherever possible. Multivariate analysis of microbial diversity was performed according to Anderson and Willis (2003) and included (1) a robust unconstrained ordination to determine the major variance components, (2) a compatible constrained analysis with reference to the hypothesis, (3) a rigorous statistical test of the hypothesis and (4) a characterization of the taxa responsible for the multivariate patterns. For this purpose, we used (1) principal coordinate analysis (PCO; Gower, 1966); (2) canonical analysis of principal coordinates (CAP; Anderson and Willis, 2003); (3) permutational analysis of variance (PERMANOVA; Anderson, 2001), permutational analysis of multivariate dispersions (PERMDISP; Anderson, 2006) and analysis of similarity (ANOSIM; Clarke, 1993); and (4) correlation-based indicator species analysis (De Cáceres and Legendre, 2009). Each method comes with its own advantages and limitations such that the combined use of these methods provides a robust assessment of the hypothesis. Differences in β -diversity were measured using Bray–Curtis similarities calculated based on normalized and square root transformed OTU abundances (Hartmann *et al.*, 2012). PCO, CAP, PERMANOVA, PERMDISP and ANOSIM were performed using the homonymous routines in PRIMER6+ (Clarke and Gorley, 2006). Significance levels calculated in CAP, PERMANOVA, PERMDISP and ANOSIM were determined with 10^5 permutations. Adjustments for multiple testing were performed using the Benjamini–Hochberg correction (Benjamini and Hochberg, 1995) in the R package MULTTEST (Pollard *et al.*, 2013). Correlations between resemblance matrices were determined using a non-parametric Mantel-type test implemented as the RELATE routine in PRIMER6+.

Estimates of α -diversity were based on evenly rarefied OTU abundance matrices and included observed richness S_{obs} and Smith–Wilson evenness E_{var} (Smith and Wilson, 1996) as calculated in MOTHR. Sampling effort was estimated using Good's

coverage (Good, 1953). Rarefaction curves of the observed richness were calculated in MOTHUR using 1000-fold resampling without replacement. Biplot correlations between PCO scores and α -diversity metrics were calculated using the 'corr.axes' function in MOTHUR. In order to maximize comparability with analysis of β -diversity, management effects on α -diversity were examined using univariate PERMANOVA, ANOSIM and PERMDISP based on Euclidean distances.

Overall management effects on soil chemistry were examined using PCO combined with multi- and univariate PERMANOVA and ANOSIM of Euclidean distances based on z -transformed data. The relationship between β -diversity and soil chemistry was analysed using nonparametric multivariate regression between the soil chemical parameters and the OTU-based resemblance matrices implemented as distance-based linear modelling (McArdle and Anderson, 2001) in PRIMER6+ and run with 10^5 permutations. Models were built using a step-wise selection procedure and the adjusted R^2 selection criterion.

The association strength (that is, the point biserial correlation coefficient R) of each OTU with a particular farming system or farming system combination was determined using correlation-based indicator species analysis (De Cáceres and Legendre, 2009) with all possible combinations (De Cáceres *et al.*, 2010) and correction for unequal sample sizes where necessary (Tichy *et al.*, 2006). Based on the rationale that an OTU can occupy a certain niche provided by multiple farming systems, considering all possible combinations is important to detect these associations (De Cáceres *et al.*, 2010). The analysis was performed in GINKGO (Bouxin, 2005) with 10^5 permutations. P -value adjustments for multiple comparisons were performed using the false discovery rate correction according to Storey (2002). Q -values were determined using QUALITY (Käll *et al.*, 2009) and associations were considered significant at $q < 0.05$. Singletons and doubletons, that is, OTUs that were represented by only one or two sequences across the whole data set, hold little indicator potential and were not included in the analysis.

Various network approaches were used to analyse the data sets. Directed networks visualizing the OTU distribution across the taxonomic tree were generated using the *prefuse layout* algorithm in CYTOSCAPE 3.0 (Shannon *et al.*, 2003). Bipartite networks were generated using the systems as source nodes and the OTUs as target nodes, with edges (that is, lines connecting nodes) corresponding to positive associations of particular OTUs with specific systems or system combinations. Bipartite networks were generated using the *edge-weighted spring-embedded layout* algorithm in CYTOSCAPE with edges weighted according to the association strength. OTU co-correlations between all pairs of significantly ($q < 0.05$) associated OTUs were

calculated using Spearman's rank correlation coefficient. P -values were adjusted using QUALITY and co-correlations were considered significant at $q < 0.01$. Based on this information, co-correlation networks were constructed using the *edge-weighted spring-embedded layout* algorithm in CYTOSCAPE with edges weighted according to the correlation coefficient.

Results

Management effects on bacterial and fungal diversity
A total of 594 340 (7429 ± 2481 per sample) bacterial 16S_{V1-V2} and 523 928 (6549 ± 1463 per sample) fungal ITS2 high-quality sequences with an average read length of 258 ± 14 and 266 ± 42 bp, respectively, were obtained for the 80 soil samples. Sequence clustering yielded 3877 (795 ± 122 per sample) bacterial and 2554 (357 ± 40 per sample) fungal OTUs, representing an average Good's coverage of $95.7 \pm 1.1\%$ and $98.4 \pm 0.4\%$, respectively. All high-quality sequences as well as the CROP-derived centre sequences that are representative of each OTU are provided in Supplementary Data 1.

The farming systems and crop types were significant drivers of bacterial and fungal β -diversity (Figure 1 and Table 2). The bacterial and fungal communities in the different farming systems were on average 10% and 13% dissimilar. The communities under different crop types were 4% and 11% dissimilar, respectively, revealing a stronger crop effect on fungi than on bacteria. In fact, the crop effect on the bacterial community was statistically only supported by PERMANOVA, but not by ANOSIM (Supplementary Table 1). These management effects were accompanied by pronounced spatial and temporal variabilities that contributed with 10–11% (plot) and 8–15% (year) to the explained variance. The temporal component showed significant interactions with the factors management and crop (Table 2); however, the management and crop effects were also significant when each year was examined separately (data not shown). Overall, bacterial and fungal community structures were significantly correlated (RELATE $R = 0.64$, $P < 0.001$).

Unconstrained PCO ordinations mainly separated FYM-based and non-FYM-based systems on the first axis, whereas the strong influence of the temporal component became evident on the second axis (Figure 1a). Despite substantial spatiotemporal variability, PERMANOVA and ANOSIM suggested the presence of distinct microbial communities in all farming systems with the exception of no differences between NOFERT and CONMIN for bacteria (Table 2). These differences became evident in the constrained CAP ordination that largely eliminated variation introduced by crop type and spatiotemporal variability (Figure 1b). All five farming systems formed distinct clusters in the ordination space, clusters that were quantitatively supported by high CAP reclassification rates in the range of 75%

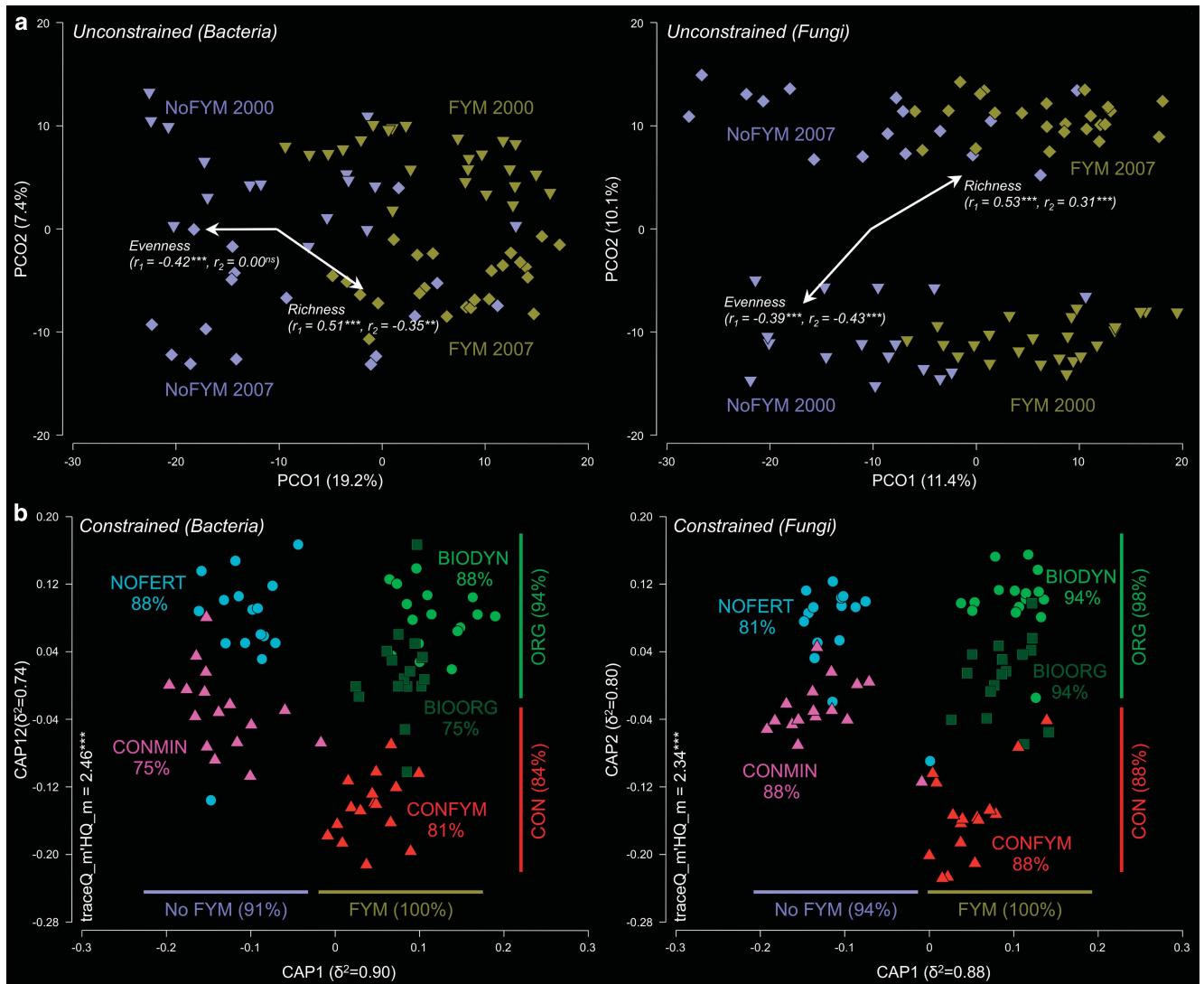


Figure 1 Management effects on bacterial and fungal community structures. **(a)** PCO ordinations of Bray–Curtis similarities calculated based on relative OTU abundances showing major differences induced by farmyard manure application, that is, FYM (brown symbols) versus NoFYM (purple symbols), and year of sampling, that is, 2000 (triangles) versus 2007 (diamonds). The variance explained by each PCO axis is given in parentheses. Joint biplots show the correlation between richness or evenness and the ordinations scores on each PCO axis. Correlation coefficient r and level of significance ($***P < 0.001$ and $^{ns} P > 0.05$) are provided. **(b)** CAP ordinations of bacterial and fungal communities maximizing discrimination among the different farming systems, that is, NOFERT (blue circles), CONMIN (pink triangles), BIOORG (dark green squares) and CONFYM (red triangles). These symbols (same symbol reflects same crop protection strategy) and colours (different farming systems) are used throughout the article where applicable. The canonical correlation (δ^2) of each CAP axis, indicating the association strength between the multivariate data cloud and the hypothesis of differences between farming systems, is given in parentheses. The third axes (not shown) further separate BIOORG and BIODYN with $\delta^2 = 0.52$ and 0.55 for bacteria and fungi, respectively. The CAP reclassification rates (in percent) for each farming system are given in parentheses next to each cluster. The reclassification rate of the CAP model provides a quantitative estimate of the degree of discrimination among the systems achieved by the canonical axes. The traceQ_m'HQ_m statistic (sum of canonical eigenvalues) given in the plots tests the null hypothesis of no significant differences in multivariate location among farming systems and represents an overall test of rejecting the null hypothesis.

to 94% that, in contrast to PERMANOVA and ANOSIM, also discriminated NOFERT from CONMIN for bacteria. FYM application exerted the strongest effect on β -diversity and separated the data on the first CAP axis with high canonical correlations and reclassification rates between 91% and 100%. The FYM application effect on β -diversity was confirmed by ANOSIM with R -values of 0.54 and 0.71 ($P < 0.001$) for bacteria and fungi, respectively (Supplementary Table 1). Organic versus conventional

systems separated the data on the second CAP axis with high reclassification rates between 84% and 98%. This effect was also confirmed by ANOSIM with R -values of 0.24 and 0.53 ($P < 0.001$) for bacteria and fungi, respectively.

Differences in β -diversity between farming systems as detected by PERMANOVA and ANOSIM can arise from differences in similarity, differences in dispersion or both. A separate test of dispersion using PERMDISP revealed that differences among farming

Table 2 Effects of agricultural management effects on bacterial and fungal β -diversity

| Main test ^a | Bacteria | | | Fungi | | |
|---|------------|---------------------|-------------|-------------|---------------------|-------------|
| | F | P | VC | F | P | VC |
| Management (F _{4,30}) | 3.6 | <0.001 | 10.3 | 3.9 | <0.001 | 13.2 |
| Crop (F _{1,30}) | 1.9 | 0.023 | 3.7 | 6.1 | <0.001 | 11.0 |
| Management × crop (F _{4,30}) | 0.8 | 0.914 | Neg | 1.1 | 0.172 | 3.6 |
| Plot (F _{30,30}) | 1.4 | <0.001 | 9.4 | 1.3 | <0.001 | 11.1 |
| Time (F _{1,30}) | 7.4 | <0.001 | 8.4 | 13.0 | <0.001 | 14.5 |
| Time × management (F _{4,30}) | 1.3 | 0.003 | 4.3 | 1.5 | <0.001 | 6.6 |
| Time × crop (F _{1,30}) | 1.4 | 0.031 | 3.1 | 3.1 | <0.001 | 8.7 |
| Time × management × crop (F _{4,30}) | 1.0 | 0.610 | Neg | 1.2 | 0.065 | 5.6 |
| Farming systems ^b | t | P _{adjust} | Øsim | t | P _{adjust} | Øsim |
| NOFERT vs CONMIN | 1.2 | 0.142 | 63.4 | 1.5 | 0.010 | 50.1 |
| NOFERT vs BIODYN | 2.5 | 0.003 | 59.8 | 2.1 | 0.001 | 48.9 |
| NOFERT vs BIOORG | 1.8 | 0.013 | 62.4 | 1.8 | 0.001 | 49.3 |
| NOFERT vs CONFYM | 1.7 | 0.013 | 63.2 | 2.3 | <0.001 | 48.0 |
| CONMIN vs BIODYN | 2.8 | 0.002 | 59.3 | 2.4 | <0.001 | 49.7 |
| CONMIN vs BIOORG | 2.1 | 0.003 | 61.9 | 2.0 | <0.001 | 50.4 |
| CONMIN vs CONFYM | 1.7 | 0.013 | 64.0 | 2.0 | <0.001 | 51.7 |
| BIODYN vs BIOORG | 1.4 | 0.013 | 66.3 | 1.4 | 0.002 | 55.1 |
| BIODYN vs CONFYM | 2.0 | 0.002 | 64.6 | 2.3 | <0.001 | 52.7 |
| BIOORG vs CONFYM | 1.3 | 0.032 | 66.4 | 1.7 | <0.001 | 54.3 |

Abbreviations: BIODYN, manured biodynamic; BIOORG, manured bioorganic; CONFYM, manured conventional; CONMIN, mineral fertilized conventional; Neg, negative; NOFERT, unfertilized biodynamic.

^aEffects of main factors and their interactions as assessed by multivariate permutational analysis of variance (PERMANOVA; degrees of freedom for each factor and the corresponding error term are given in brackets). Main factors represent agricultural management system (NOFERT, CONMIN, BIODYN, BIOORG and CONFYM), crop (winter wheat and grass-clover), plot (nested in management and crop) and time (year of sampling, that is, 2000 and 2007). Values represent the pseudo-F ratio (F), the permutation-based level of significance (P) and the estimation of the variance component (VC). Values at $P < 0.05$ are shown in bold. Negative variance components (neg) can result from underestimations of small or zero variances; therefore, variance components of the remaining factors were estimated according to Fletcher and Underwood (2002) by sequentially removing factors with negative components from the model.

^bPairwise comparisons between farming systems. Values represent the univariate *t*-statistic (*t*) and the average between-group Bray–Curtis similarity (Øsim). The permutation-based level of significance was adjusted for multiple comparisons using the Benjamini–Hochberg procedure (P_{adjust}). Values at $P < 0.05$ are shown in bold.

systems were at least partially driven by different within-system heterogeneities, whereas the factors crop and sampling time had little effect on dispersion (Supplementary Table 1). FYM application significantly reduced dispersion in both bacteria and fungi, with the highest dispersion observed in the unfertilized system (data not shown). Dispersion for BIODYN, BIOORG and CONFYM was similar, suggesting that differences in β -diversity between FYM-based systems were largely driven by dissimilarity rather than dispersion (Supplementary Table 1).

Management effects on α -diversity were statistically less robust, although significant shifts were still detected (Table 3 and Supplementary Table 2). In contrast, the crop type had no significant effect on α -diversity. Joint biplot correlations revealed trends of increasing richness and decreasing evenness in FYM-amended soils (Figure 1). ANOSIM tests confirmed this observation for bacteria but only partially for fungi (Supplementary Table 2). CONMIN showed the lowest richness and highest evenness among all farming systems, whereas BIODYN showed the opposite trend (Table 3). All parameters except bacterial evenness were also significantly

influenced by the temporal component (Table 3), and the significant ‘time × management’ interaction for bacterial richness calls for a cautious interpretation of these results. Differences in α -diversity among FYM-amended soils were small and not significant.

In order to investigate treatment effects on α -diversity at greater sequencing depth, we pooled all sequences from the same farming system and generated rarefaction curves of the observed richness including confidence intervals (Supplementary Figure 1). The bacterial and fungal rarefaction curves revealed higher α -diversity in the fertilized organically managed systems and lower α -diversity in the conventional systems (BIODYN > BIOORG > CONFYM > CONMIN). Differences were less supported for fungi than for bacteria. Interestingly, the unfertilized system NOFERT revealed low bacterial but the highest fungal α -diversity.

Soil chemical properties and relationship with community structure

As part of the regular data collection in the DOK experiment, the parameters pH, C_{org}, N_{tot}, P, K and Mg

Table 3 Effects of agricultural management on bacterial and fungal α -diversity

| Main test ^a | Bacteria | | Fungi | |
|---|---------------------------------|----------------------------------|---------------------------------|---------------------------------|
| | Richness (S_{obs}) F (P) | Evenness (E_{var}) F (P) | Richness (S_{obs}) F (P) | Evenness (E_{var}) F (P) |
| Management ($F_{4,30}$) | 8.2 (<0.001) | 6.6 (<0.001) | 5.4 (0.002) | 2.6 (0.055) |
| Crop ($F_{1,30}$) | 0.3 (0.589) | 0.6 (0.445) | 0.0 (0.831) | 0.2 (0.696) |
| Management \times crop ($F_{4,30}$) | 1.4 (0.258) | 0.3 (0.862) | 0.7 (0.586) | 0.3 (0.853) |
| Plot ($F_{30,30}$) | 1.5 (0.149) | 1.3 (0.212) | 1.0 (0.453) | 1.3 (0.263) |
| Time ($F_{1,30}$) | 32.7 (<0.001) | 0.8 (0.384) | 6.7 (0.015) | 20.5 (<0.001) |
| Time \times management ($F_{4,30}$) | 3.3 (0.024) | 1.4 (0.243) | 0.9 (0.474) | 0.3 (0.850) |
| Time \times crop ($F_{1,30}$) | 0.8 (0.378) | 0.0 (0.945) | 8.5 (0.007) | 18.0 (<0.001) |
| Time \times management \times crop ($F_{4,30}$) | 0.7 (0.569) | 0.4 (0.832) | 0.5 (0.731) | 1.1 (0.368) |
| Farming system ^b | Mean \pm s.e. | Mean \pm s.e. | Mean \pm s.e. | Mean \pm s.e. |
| NOFERT | 522 \pm 7 ^{A,B} | 0.247 \pm 0.005 ^{A,B} | 282 \pm 9 ^{A,B} | 0.385 \pm 0.005 ^A |
| CONMIN | 517 \pm 5 ^A | 0.251 \pm 0.003 ^A | 265 \pm 7 ^A | 0.393 \pm 0.004 ^A |
| CONFYM | 541 \pm 6 ^{B,C} | 0.232 \pm 0.003 ^{B,C} | 288 \pm 6 ^{A,B} | 0.391 \pm 0.004 ^A |
| BIOORG | 544 \pm 8 ^{B,C} | 0.228 \pm 0.004 ^C | 290 \pm 8 ^{A,B} | 0.382 \pm 0.004 ^A |
| BIODYN | 560 \pm 7 ^C | 0.230 \pm 0.004 ^{B,C} | 308 \pm 6 ^B | 0.379 \pm 0.003 ^A |

Abbreviations: BIODYN, manured biodynamic; BIOORG, manured bioorganic; CONFYM, manured conventional; CONMIN, mineral fertilized conventional; E_{var} , Smith–Wilson evenness index; NOFERT, unfertilized biodynamic; S_{obs} , observed richness.

^aEffects of main factors and their interactions were assessed by univariate permutational analysis of variance (PERMANOVA; degrees of freedom for each factor and the corresponding error term are given in brackets). Main factors represent agricultural management system (NOFERT, CONMIN, BIODYN, BIOORG and CONFYM), crop (winter wheat and grass-clover), plot (nested in management and crop) and time (year of sampling, that is, 2000 and 2007). Values represent the pseudo-F ratio (F) and the level of significance (P). Values at $P < 0.05$ are shown in bold.

^bAverage richness and evenness (mean \pm s.e.; $n = 16$) for each agricultural management system. Estimates are based on rarefied data sets (that is randomly subsampled to the same number of sequences per sample, that is, 2812 bacterial and 3292 fungal sequences). Different letters represent significant differences at $P < 0.05$ with P -values adjusted for multiple comparisons using the Benjamini–Hochberg method.

were measured biannually between 2000 and 2008. Overall, soil chemistry showed the most pronounced differences between the unfertilized system NOFERT and all other systems (Figure 2), but ANOSIM tests significantly differentiated all farming systems (Supplementary Table 3). Although the management regime accounted for the largest part of the variance, crop type and the spatiotemporal component also introduced significant variability and often interacted with the management effect, calling for careful interpretation of the results (Table 4). The ‘time \times management’ interaction was not strong in NOFERT, BIOORG and BIODYN, but more pronounced in CONMIN and CONFYM (Supplementary Table 3). NOFERT showed the lowest values for all soil chemical properties (Table 4), leading to a separate clustering (Figure 2). Among the fertilized systems, soil pH, C_{org} and N_{tot} were significantly higher in BIODYN, but similar among CONMIN, CONFYM and BIOORG. In contrast, soil P was higher in CONFYM and soil Mg was higher in CONMIN when compared with all other fertilized systems. All six chemical parameters were significantly co-correlated (data not shown), with the strongest correlations between C_{org} and N_{tot} as well as between P and K (Figure 2).

Because of the biannual sampling scheme, soil chemical data were available for 2000 but not 2007. Thus, microbial and chemical data were not exactly comparable. However, despite the significant ‘time \times management’ interaction observed for most

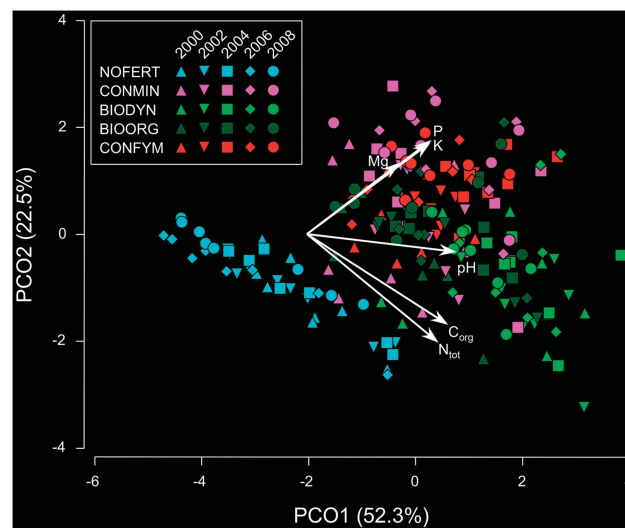


Figure 2 Management effects on soil chemistry measured biannually between 2000 and 2008. PCO ordinations of Euclidean distances calculated based on z-transformed soil chemical parameters, that is, pH, C_{org} , N_{tot} , P, K and Mg. Joint biplots show the correlation between the soil chemical parameters and the ordinations scores on each PCO axis.

chemical properties, we may use the data from 2006 and 2008 as approximation for 2007, as soil chemistry did not change significantly in that period as assessed by ANOSIM (Supplementary Table 3). Accordingly, distance-based multivariate regression between β -diversity and soil chemistry gave very

Table 4 Soil chemical properties between 2000 and 2008 and the relationship between soil chemistry and bacterial or fungal β -diversity

| Main test ^a | pH | C _{org} | N _{tot} | P | K | Mg |
|---|--------------------------|--------------------------|------------------------------|-------------------------|-------------------------|-------------------------|
| | F (P) | F (P) | F (P) | F (P) | F (P) | F (P) |
| Management (F _{4,30}) | 36.5 (<0.001) | 28.8 (<0.001) | 20.0 (<0.001) | 78.6 (<0.001) | 72.0 (<0.001) | 87.8 (<0.001) |
| Crop (F _{4,30}) | 1.9 (0.112) | 4.0 (0.005) | 6.6 (<0.001) | 3.0 (0.217) | 11.7 (<0.001) | 8.8 (<0.001) |
| Management × crop (F _{16,30}) | 1.4 (0.138) | 0.4 (0.975) | 0.1 (1.000) | 1.7 (0.063) | 2.4 (0.005) | 7.1 (<0.001) |
| Plot (F _{135,30}) | 12.3 (<0.001) | 2.4 (0.004) | 26.0 (<0.001) | 1.6 (0.088) | 1.1 (0.376) | 7.7 (<0.001) |
| Time (F _{2,30}) | 6.2 (0.004) | 25.7 (<0.001) | 52.6 (<0.001) | 4.6 (0.010) | 5.3 (0.008) | 48.9 (<0.001) |
| Time × management (F _{8,30}) | 26.8 (<0.001) | 0.8 (0.574) | 5.0 (<0.001) | 2.4 (0.029) | 4.2 (0.001) | 36.4 (<0.001) |
| Pairwise test ^b | (% soil) | | (% soil) | (mg per kg soil) | (mg per kg soil) | (mg per kg soil) |
| NOFERT | 5.94 ± 0.06 ^A | 1.09 ± 0.03 ^A | 0.150 ± 0.004 ^{A,B} | 19 ± 1 ^A | 3.9 ± 0.1 ^A | 60 ± 2 ^A |
| CONMIN | 6.27 ± 0.05 ^B | 1.22 ± 0.03 ^B | 0.160 ± 0.003 ^{B,C} | 59 ± 2 ^B | 11.5 ± 0.7 ^B | 129 ± 6 ^C |
| CONFYM | 6.32 ± 0.03 ^B | 1.24 ± 0.02 ^B | 0.162 ± 0.002 ^C | 87 ± 3 ^C | 11.4 ± 0.4 ^B | 94 ± 2 ^B |
| BIOORG | 6.36 ± 0.03 ^B | 1.30 ± 0.03 ^B | 0.167 ± 0.003 ^C | 60 ± 3 ^B | 10.9 ± 0.4 ^B | 95 ± 1 ^B |
| BIODYN | 6.71 ± 0.03 ^C | 1.52 ± 0.03 ^C | 0.193 ± 0.003 ^D | 64 ± 3 ^B | 12.2 ± 0.5 ^B | 95 ± 3 ^B |
| DISTLM ^f | VC (P) | VC (P) | VC (P) | VC (P) | VC (P) | VC (P) |
| Bacteria (marginal test) | 9.1 (<0.001) | 9.3 (<0.001) | 9.0 (<0.001) | 4.9 (<0.001) | 4.4 (<0.001) | 1.6 (0.139) |
| Bacteria (sequential test) | 3.3 (<0.001) | 9.3 (<0.001) | 1.2 (0.229) | 1.5 (0.044) | 2.0 (0.003) | 3.1 (<0.001) |
| Fungi (marginal test) | 5.3 (<0.001) | 5.6 (<0.001) | 6.4 (<0.001) | 4.6 (<0.001) | 3.9 (<0.001) | 2.1 (0.016) |
| Fungi (sequential test) | 2.6 (<0.001) | 1.5 (0.080) | 6.4 (<0.001) | 4.2 (<0.001) | 1.8 (0.011) | 2.6 (<0.001) |

Abbreviations: BIODYN, manured biodynamic; BIOORG, manured bioorganic; Ca, calcium; CONFYM, manured conventional; CONMIN, minerally fertilized conventional; C_{org}, organic carbon; DISTLM, distance-based linear modelling; K, potassium; Mg, magnesium; NOFERT, unfertilized biodynamic;

N_{tot}, total nitrogen; P, phosphorus.

^aEffects of main factors and their interactions assessed by univariate permutational analysis of variance (PERMANOVA; degrees of freedom for each factor and the error term are given in brackets). Main factors represent agricultural management system (NOFERT, CONMIN, BIODYN, BIOORG and CONFYM), crop (winter wheat and grass-clover), plot (nested in management and crop) and time (year of sampling, that is, 2000 and 2007). Because of the temporally shifted crop rotation, crop types were different in the different years, leading to a nonfactorial design. Therefore, interactions 'Time × crop' and 'Time × management × crop' could not be analysed and terms were pooled into the residuals. Values represent the pseudo-F ratio (F) and the level of significance (P). Values at P < 0.05 are shown in bold.

^bAverage soil chemical properties (mean ± s.e.; n = 40) for each agricultural management system. Different letters indicate significant differences assessed by PERMANOVA at P < 0.05 with P-values adjusted for multiple comparisons using the Benjamini–Hochberg method.

^fDistance-based linear modelling examining the relationship between soil chemistry and microbial β -diversity. Soil chemical data were derived from 2000 and 2006 (as proxy for 2007). The marginal test examines the relationship between β -diversity and each predictor variable individually, whereas the sequential test examines the relationship by sequentially fitting all predictors into the most parsimonious model. The sequential modelling was performed using a stepwise selection procedure and the adjusted R² selection criterion. Values in table represent the estimation of the variance component (VC) and the level of significance (P).

similar results when used with data from 2006 or 2008 (data not shown). Most soil chemical properties revealed significant relationships with both bacterial and fungal β -diversity when examined separately (Table 4, 'marginal test'). C_{org}, N_{tot} and pH were the strongest predictors of community structure, explaining between 5% and 9% of the variance. Because of the significant co-correlations among the chemical properties, fitting all parameters into one model can add additional information by removing contributions from co-correlations and simultaneously unravelling underlying relationships with other parameters. When all parameters were fitted into one model (Table 4, 'sequential test'), C_{org} was the strongest predictor of bacterial β -diversity, whereas N_{tot} was the strongest predictor of fungal β -diversity. pH explained an additional 3% in both data sets. P added only little additional information in the case of bacteria, but another 4% in the case of fungi. Notably, whereas Mg was a weak predictor of community structure when considered separately, it explained an additional 3% when the effects of the other variables were removed in the

combined model. In total, the six soil chemical properties explained ~19–20% of the variance in β -diversity (Table 4).

Taxonomic composition and management-sensitive taxa

The overall taxonomic complexity of the community is visualized in Figure 3 and a complete list of all detected bacterial and fungal taxa, summarized from phylum to OTU level, is provided in Supplementary Data 2. Among the 3877 bacterial OTUs, 2840 (representing 97% of all sequences) were assigned at the phylum level. At lower taxonomic levels, 2504 (94%), 1912 (83%), 1104 (53%), 484 (25%) and 61 (6%) bacterial OTUs were assigned at the levels class, order, family, genus and species, respectively. Equivalently, among the 2554 fungal OTUs, 1962 (89%), 1507 (80%), 1223 (78%), 1087 (75%), 897 (65%) and 361 (33%) were assigned at the levels phylum, class, order, family, genus and species, respectively. Thus, the assignment success at lower taxonomic levels such as genus or species was higher for the fungal pyrotags, whereas the bacterial

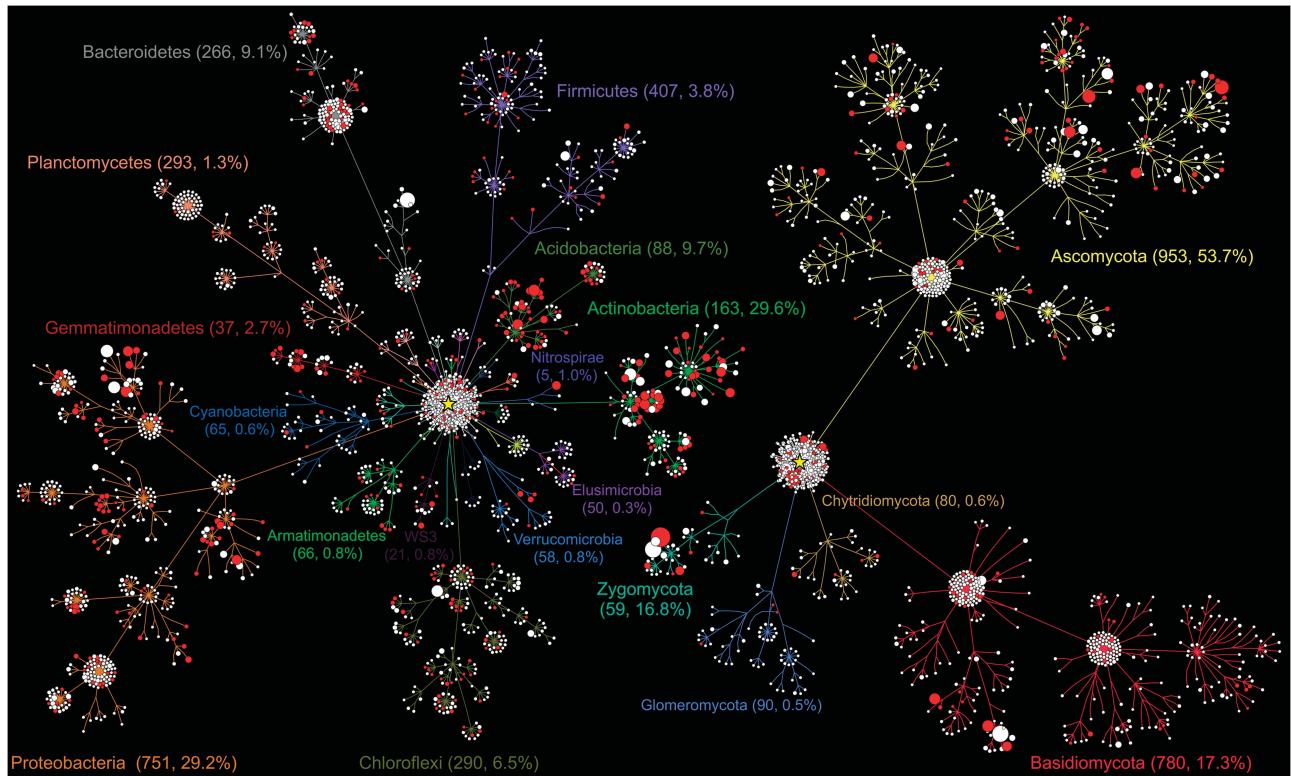


Figure 3 Taxonomic dendrograms of the detected bacterial and fungal communities showing the OTU distribution (excluding OTUs with $<0.001\%$ relative abundance) across the different taxonomic branches (colour coded by phylum). Nodes correspond to OTUs and node sizes correspond to their relative abundances (square root) in the data set. Edges (that is, lines connecting the nodes) represent the taxonomic path from the root, that is, bacteria or fungi (marked by yellow asterisks), to OTU level, and OTUs were placed at the level of the lowest possible assignment. The most abundant phyla are labelled including the total OTU number and relative abundance in parentheses. Red nodes correspond to OTUs that significantly ($q < 0.05$) differed among farming systems, whereas white nodes represent insensitive OTUs. Supplementary Figure 2 shows the same taxonomic dendrograms with only the significant OTUs colour coded according to the system association information.

pyrotags had higher assignment success at higher taxonomic levels such as phylum or class. A total of 1037 (2.6%) bacterial and 592 (11%) fungal OTUs were unclassified at the phylum level. Overall, 44 phyla, 113 classes, 203 orders, 345 families, 602 genera and 329 species were identified.

In the following, we focus only on OTUs that differed significantly among the different farming systems, but point out that, given the main effects on the overall community structure (Figure 1), there were certainly other OTUs that solely differed between crop types or sampling years. We provide the indicator statistics for all 6431 detected OTUs in Supplementary Data 2. A total of 452 (12%) bacterial and 176 (7%) fungal OTUs were significantly ($q < 0.05$) associated with specific farming systems or system combinations; notably however, these $\sim 10\%$ OTUs represented 51% and 43% of the pyrotags in the bacterial and fungal data sets, respectively. These 628 bacterial and fungal OTUs were broadly distributed across the taxonomic tree (Figure 3, red nodes). However, certain abundant phyla such as Actinobacteria and Acidobacteria showed a clear accumulation of these management-sensitive OTUs.

A bipartite association network was used to visualize the associations between OTUs and the different farming systems or system combinations (Figure 4). The bipartite network strongly resembled the constrained CAP ordination plots (Figure 1b) by recovering the major discriminative gradients related to FYM application (horizontally) and organic versus conventional management (vertically). The association strength (that is, correlation coefficient R) of the 628 significant OTUs varied between 0.30 and 0.82 (indicated by the different edge lengths in the network of Figure 4). Of these OTUs, 49% were most strongly associated with only one system (Figure 4, clusters 1–5), confirming the basic distinctness of the communities in all five systems. Approximately 23% of the significant OTUs were most strongly driven by FYM application (clusters 6, 7, and 9), whereas another 17% were associated with combinations of organic or conventional farming systems (clusters 8, 10–12). Only 11% of the significant OTUs were associated with cross-combinations (Figure 4, white nodes). All 628 management-sensitive OTUs occurring in these clusters are marked in the Supplementary Data 2 (column

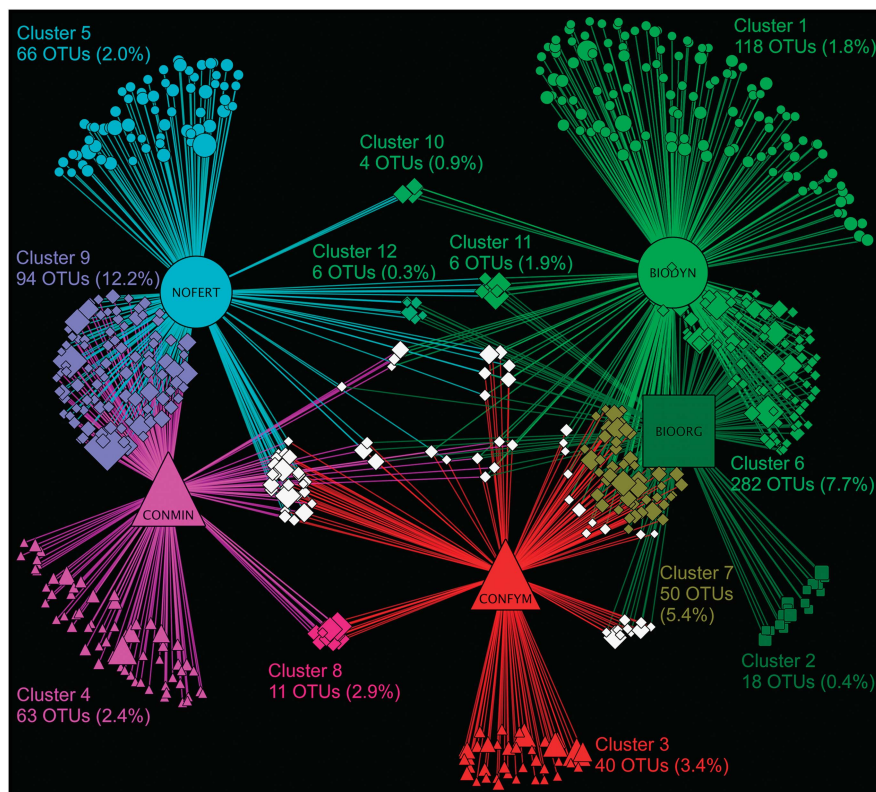


Figure 4 Bipartite association network showing positive associations between the farming systems and the 628 significantly ($q < 0.05$) associated OTUs. Node sizes represent relative abundance (square root) of the OTUs in the data sets. Edges represent the association patterns of individual OTUs with the farming systems. The edge-weighted spring-embedded algorithm pulled together OTUs with similar associations and systems with similar structure. OTUs associated with only one farming system are symbol and colour coded according to Figure 1. Diamond-shaped nodes represent OTUs associated with multiple farming systems. White nodes represent multisystem cross-combinations not falling into the same category with respect to either FYM application (FYM or no FYM) or farming regime (conventional or organic). Clusters are labelled as discussed in the text and marked in the Supplementary Data 2. Number of OTUs and relative abundances are provided for each cluster.

‘BipartiteCluster’) in order to facilitate individual inspection.

A complete discussion of all statistically significant observations is beyond the scope of this study and we attempt to focus on the most salient patterns. At a first glance, however, OTUs responding to a specific management regime were heterogeneously distributed across the taxonomic tree with largely no taxonomic clades responding uniformly (Supplementary Figure 2). Thus, we used a two-step strategy to retrieve more specific information. First, we examined co-correlation patterns among the 628 statistically significant OTUs in order to elucidate general trends at the phylum level. Second, as OTUs only classified at higher taxonomic levels carry little information to infer the putative ecological role of the taxon, we extracted all OTUs that were at least assigned to genus level.

Co-correlation networks can identify groups where members respond uniformly to a specific influence. Among the 10 most populated networks at the phylum level, network density was highest for Acidobacteria, Actinobacteria, Gemmatimonadetes and Bacteroidetes, whereas Basidiomycota and Ascomycota were more dispersed (Figure 5).

Many of the denser networks showed a bimodal distribution reflecting the strong effect of FYM application, but largely no other influences (Supplementary Figure 3). In contrast, the more dispersed networks constructed for Proteobacteria, Ascomycota and Basidiomycota showed additional influences. For example, Ascomycota and Basidiomycota appeared to contribute most in separating CONFYM from BIODYN and BIOORG (Figure 5 and Supplementary Figure 3), in agreement with the higher farming system reclassification rates observed for fungi (Figure 1). The Firmicutes network constituted an exception to the contrasting distributions of the other networks. Although the overall network density for Firmicutes was only intermediate, it represented almost exclusively positive correlations (Figure 5). All Firmicutes OTUs except one were associated with farming systems receiving FYM (Supplementary Figure 3). At lower taxonomic levels, the three networks containing the highest numbers of positive correlations included the acidobacterial genus *Candidatus Solibacter*, the ascomycete family Lasiosphaeriaceae and the firmicute class Clostridia (Figure 5, white boxes).

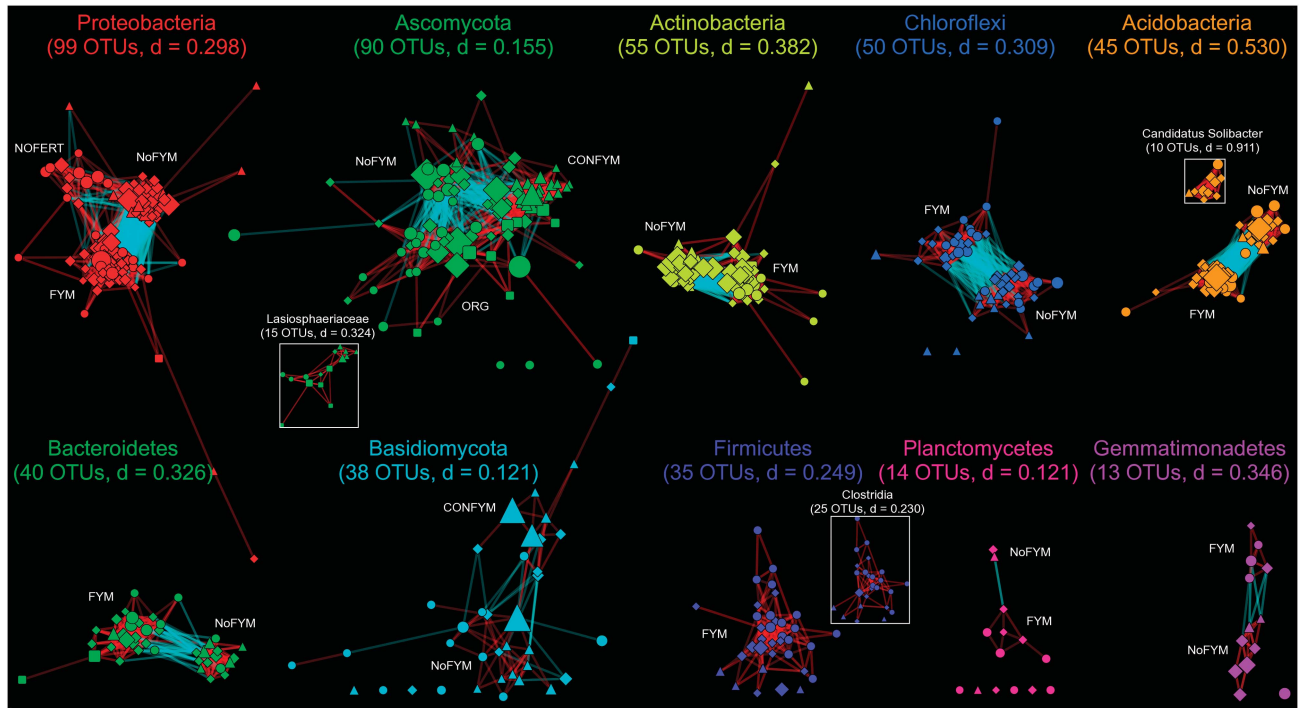


Figure 5 Co-correlation networks calculated for the significantly ($q < 0.05$) associated OTUs of the 10 most populated phyla (coded with different colours). Nodes correspond to OTUs and node sizes correspond to their relative abundances (square root) in the data set. Edges represent significant ($q < 0.01$) negative (blue) or positive (red) Spearman's correlations between pairs of OTUs. The edge-weighted spring-embedded algorithm pulled together OTUs that were strongly co-correlated. Dense co-correlation networks indicate that all or most OTUs in this cluster showed either a similar (= positive correlations) or contrasting (= negative correlations) response. Network density (d) calculated for each network represents the number of significant co-correlations divided by all possible co-correlations, that is, higher density represents more uniform response. Symbol coding indicates the association with the different farming systems as provided in Figure 4. Clusters are labelled with the approximate association information with respect to the management regime (that is, farming systems or system combinations such as FYM or NoFYM). For closer inspection, the same network but OTUs colour coded with the system association information is provided in Supplementary Figure 3.

Co-correlation networks are well suited to detect general patterns in highly populated taxonomic groups, but they lack power to examine taxonomic groups with only very few OTUs, and this is intrinsically the case at lower taxonomic levels (for example, many genera are represented by only one OTU). On the other hand, OTUs classified at lower taxonomic levels contain more information relevant to inferring their putative ecological role in the system. Therefore, and in combination with the observations in the co-correlation networks, we examined the individual response of all genus-level OTUs in order to find clades where multiple genus-level OTUs responded similarly. The response of these genus-level indicators is visualized in Supplementary Figure 4 followed by a more detailed discussion in the context of the existing literature in the Discussion section below.

Discussion

The DOK field experiment represents a unique system to evaluate the influence of management strategies under near-practical conditions including different crop types. More than two decades of continuous organic and conventional farming

altered soil microbial diversity (Figure 1 and Tables 2 and 3). The long-term effect of agricultural management revealed a greater impact than the short-term effects of the cultivated crop, in particular on bacteria. The spatiotemporal variability was substantial, demonstrating the importance of thoroughly replicated, temporally monitored long-term field studies to measure robust effects. Application and quality of the fertilizer appeared to be the major factor shaping the soil microbiota, whereas the plant protection measures, applied at moderate and targeted levels, were of subordinate importance (Figures 1 and 4). In general, management-sensitive taxa were heterogeneously distributed across the taxonomic tree (Figure 3 and Supplementary Figure 2); however, some consistent patterns, for example among members of the Acidobacteria and Firmicutes (Figure 5), were observed.

Long-term agricultural management drives soil microbial community structure

All five farming systems harboured structurally distinct microbial communities, and both bacteria and fungi showed a very similar response (Figure 1). Despite the significant spatiotemporal variability common to field studies, our approach revealed

consistent underlying management effects, indicating that the spatiotemporal variation, although high, did not confound these effects. While these observations are largely in agreement with earlier assessments in the DOK experiment using first-generation molecular techniques, the pyrotag approach offered improved resolution of the management effects in terms of explained variance and discrimination power (see Supplementary Results for detailed evaluation). Overall, FYM application appeared to be the major driver of microbial diversity by altering composition, reducing dispersion, increasing richness and decreasing evenness of the soil microbiota (Figure 1, Tables 2 and 3 and Supplementary Tables 1 and 2). The observation that conventional (CONFYM and CONMIN) or organic (BIODYN and NOFERT) systems under the same plant protection regime share less similarity in community structure than systems with similar nutritional status but different plant protection regimes (for example, NOFERT and CONMIN) suggest that the plant protection component is likely of subordinate significance in the DOK experiment (Figures 1 and 4). It is, however, important to understand that the DOK experiment compares management regimes at the system level rather than evaluating the impact of individual management factors; therefore, the impact of the plant protection strategies cannot be completely isolated from fertilization effects. Although it can be expected that plant protection strategies affect microbial diversity, either directly by means of fungicides or indirectly by changing above- and below-ground communities through herbicide and insecticide application (Bünemann *et al.*, 2006), the rather small plant protection effect in the DOK experiment is not necessarily surprising as herbicides, fungicides and insecticides have been applied according to the Swiss standards of integrated farming that largely corresponds to a moderate and targeted application of these chemicals (Fließbach *et al.*, 2007; Mäder *et al.*, 2007).

While research has long focussed on the effect of agricultural management on biodiversity of higher organisms, assessing microbial diversity has only recently become more accurate in the light of high-resolution sequencing. Based on the response of richness, evenness and dispersion, it could be hypothesized that the high availability of a rich substrate like FYM increased richness by promoting copiotrophic organisms, whose predominance in turn reduced evenness. Furthermore, the consistent availability of the same substrate in all these plots streamlined the community and therefore reduced across-sample dispersion. In contrast, the absence of FYM led to a less eutrophic environment and a likely more variable distribution of nutrients, leading to reduced richness while increasing evenness and dispersion potentially by favouring various slow-growing oligotrophic organisms. We can conclude that organic farming significantly altered the soil microbiota when compared with

conventionally managed soils under exclusively mineral fertilization; however, these effects were largely attributed to the use and quality of organic fertilizer, as differences became smaller when conventionally managed soils under an integrated fertilization scheme were compared.

Reports on the effects of organic farming on microbial diversity are often ambiguous, in particular because the experimental systems and management definitions vary widely. Although Ge *et al.* (2008) observed the same countertrend between richness and evenness, other studies reported an increase in richness being accompanied by either positive (Parham *et al.*, 2003; Jangid *et al.*, 2008) or no effect (Sun *et al.*, 2004) on evenness after manure amendment. More recent high-throughput sequencing studies reported an increase in microbial evenness in organic systems (Sugiyama *et al.*, 2010; Chaudhry *et al.*, 2012), but have not detected significant effects on richness (Sugiyama *et al.*, 2010; Li *et al.*, 2012). Hence, it seems difficult to draw a robust conclusion on the effect of conventional and organic farming on bulk diversity parameters, in part because these metrics have often little power in resolving differences in community structure (Hartmann and Widmer, 2006), but most importantly because the conclusion drawn strongly depend on the methods used, on the metric itself and, largely, on the experimental design. As an example for the latter, it has been reported that bacterial evenness under organic farming only increased in the first few years and then decreased in the long term (van Diepeningen *et al.*, 2006), highlighting the importance of the temporal component for evaluating management effects.

Soil chemistry appeared to be a statistically significant determinant of the soil microbial community structure, but it explained only ~20% of the variance (Table 4), and this could largely be attributed to the consistent differences between the unfertilized and all other systems (Figure 2). The consistently lowest values in the unfertilized system could indicate poor sustainability of this farming system. At the other end of the spectrum, the biodynamic system showed significantly higher C_{org} , N_{tot} and pH, all of which are factors known to influence the soil microbiota (Lauber *et al.*, 2008, 2009). The higher degree of organic matter stability in composted FYM could be one explanation for the higher C_{org} content (Fließbach *et al.*, 2007). The strongest differences were observed for soil P and Mg (Table 4). It could be hypothesized that arbuscular mycorrhizal fungi changed in abundance and/or composition in soils with lower P concentrations (Antunes *et al.*, 2012); however, we observed only few Glomeromycota, probably in part because of limited coverage by the primers used (Kohout *et al.*, 2014; Stockinger *et al.*, 2010), and their response to the management regimes was minor. Overall, given the rather small differences in soil chemistry among

the other farming systems, for example, 0.4 units of pH, 0.3% C or 0.03% N, it must be acknowledged that these differences, although statistically robust, are likely of minor biological significance. These small differences in soil chemistry, despite the large differences in carbon and nutrient inputs among the farming systems, suggest that substrate amendments had likely a more direct effect on the community structure than indirectly by altering the soil chemical status.

Management-sensitive microbial taxa

One of the most important attributes of the high-throughput sequencing approach is the potential to identify microbial taxa responsible for shifts in community structure. A considerably large fraction of the community, representing 10% of the OTUs that accounted for 50% of the pyrotags, responded significantly to the management regimes (Figure 4). In general, OTUs associated with the same farming system or system combination were scattered across the taxonomic tree and only very few taxonomic groups responded uniformly (Supplementary Figure 2). This is not necessarily surprising. Whereas a severe environmental impact such as soil compaction can affect entire clades of the soil microbiota by changing fundamental factors such as oxygen and water availability (Hartmann *et al.*, 2014), more moderate changes introduced by agricultural management such as differences in the nutritional status likely cause more subtle shifts in community composition.

The construction of co-correlation networks demonstrated that many of the abundant phyla revealed a strongly bimodal response to FYM application instead of favouring one condition (Figure 5). Acidobacteria showed the strongest bimodal response, but different acidobacterial groups were found to occupy different clusters. OTUs assigned to the genus *Candidatus Solibacter* (and one *Candidatus Koribacter*) revealed the most tightly correlated cluster in the complete network and were associated with systems not receiving FYM (Supplementary Figure 4). Members of this genus have been suggested to be slow-growing oligotrophs adapted to nutrient-limited environments (Ward *et al.*, 2009). Therefore, an increased abundance of these taxa in farming systems not receiving manure, where nutrients inputs are either low (NOFERT) or directly accessible to plants (CONMIN), is in agreement with this putative lifestyle. In contrast, the cluster tightly associated with FYM-based systems was mainly characterized by OTUs assigned to the classes Chloracidobacteria and RB25, whose lifestyles are largely unknown. Our observations therefore partially confirm the hypothesis that Acidobacteria generally prefer soil environments of low resource availability (Fierer *et al.*, 2007) and higher acidity (Jones *et al.*, 2009), but are also in agreement with the contrasting

behaviour of individual acidobacterial subgroups reported previously (Rousk *et al.*, 2010).

The Firmicutes clade appeared to be the only abundant phyla responding in the same direction (Figure 5). All OTUs assigned to this phylum, with one exception (*Paenibacillus chondroitinus*), were associated with systems receiving FYM; however, the rather dispersed co-correlation network indicates very different preferences for the different FYM systems. Among these 35 Firmicutes OTUs, 12 were assigned at genus level and included the genera *Bacillus*, *Clostridium*, *Epulopiscium*, *Paenibacillus*, *Solibacillus*, *Symbiobacterium*, *Tepidimicrobium*, *Thermobacillus* and *Ureibacillus* (Supplementary Figure 4). Many of these genera have been found during meso- and thermophilic degradation processes of organic materials such as manure or compost (Ryckeboer *et al.*, 2003) and are known to be capable of degrading various complex organic materials (Watanabe *et al.*, 2007; Charbonneau *et al.*, 2012). Similar observations were made for fungi. OTUs assigned to coprophilous taxa such as *Coprinellus*, *Coprinopsis*, *Preussia*, *Psathyrella* and *Mortierella*, including members of the family Lasiosphaeriaceae such as *Cercophora*, *Cladorrhinum*, *Podospora*, *Schizothecium* and *Zopfiella* (Krug *et al.*, 2004; Bills *et al.*, 2013), were tightly associated with FYM-based systems (Supplementary Figure 4). Indeed, co-correlation analysis identified the family Lasiosphaeriaceae as a largely uniform cluster associated with FYM (Figure 5).

It is important to understand that we can only speculate on the ecological role of the detected taxa based on what has been previously described in other systems. Furthermore, we discovered several management-sensitive bacterial and fungal taxa for which we have little or no information about their lifestyle or for which we were not able to get taxonomic information at lower levels. It therefore remains challenging to infer the ecological role for many community members simply from phylogenetically based surveys, and additional information on the distribution of functional genes can now shed more light on our overarching observations. Therefore, our data should not be overgeneralized and the statistically significant observations need to be confirmed in other agricultural systems. It seems, however, that many of the OTUs associated with FYM-based farming systems are related to bacterial and fungal taxa that have been frequently described in manure and similar substrates. It remains to be determined whether manure served as inoculum for introducing novel taxa to the soil, or whether manure mainly served as substrate for indigenous taxa. As a next step, it would therefore be interesting to analyse the microbiota of the different manure types and evaluate how soil communities that have been unfertilized for a long time would respond to manure amendments over an extended period of time.

With the novel sequencing technologies, we have tools at hand to monitor soil microbial taxa at higher throughput and resolution than previously possible. This offers the potential to evaluate success of agricultural soil management at the level of individual taxa and, potentially, their attributed function. For example, we can look specifically for known beneficial or pathogenic taxa that are promoted or suppressed by different management strategies. Members of the fungal order Hypocreales, for instance, are of vast economic importance in agricultural systems as they include many plant pathogens as well as potential biocontrol agents (Rossman, 1996). In this study, several members of this group responded to the different management strategies (Supplementary Figure 4). One of the most abundant OTUs (1.8%) assigned to the hypocrealean genus *Bionectria* was strongly ($R=0.6$) associated with all organic systems, suggesting a negative influence of fungicide application or other plant protection measures. The necro- and biotrophic *Bionectria* are known plant, insect and mycoparasites that have found use as biocontrol agents in agriculture (Schroers, 2001). Another common agricultural biocontrol agent, the entomopathogenic fungus *Beauveria bassiana* (Feng *et al.*, 1994), was also positively associated with one of the organic systems. Conversely, several members of the common plant pathogen *Fusarium* were associated with conventional systems or systems not receiving manure (Supplementary Figure 4). As another example, members of the potential plant pathogens *Phoma* and *Ascochyta* (Davidson *et al.*, 2009) were particularly associated with the unfertilized system. These observations demonstrate that specific management strategies can select for beneficial or detrimental organisms. In the light of these examples, the novel technologies offer new ways to monitor the presence and absence of different beneficial and pathogenic taxa and thereby managing the soil microbiome for improving sustainable agricultural production (Chaparro *et al.*, 2012).

Conclusion

Agricultural soils under long-term organic and conventional farming harbour distinct microbiomes. The response of microbial diversity to agricultural management is, however, highly complex and simplistic statements like 'higher biodiversity under low-input farming' fall short of this complexity. Under the exclusion of other fundamental factors often common to agricultural management such as differential soil tillage or monocropping systems, our study demonstrated that the fertilization scheme, in particular the application and quality of organic fertilizers, is the major determinant of microbial diversity. The impact of an integrated pest management regime, characterized by moderate and targeted application of pesticides, appears to be of

subordinate importance, although some effects may be attributed to this factor. It can be assumed that differences in microbial diversity between organic and conventional farming would have been even more substantial at more intense pesticide applications and soil tillage operations as well as with cropping systems lacking soil-replenishing crops such as legumes, all traits that are common to many conventional farming systems. Long-term agricultural management in the DOK experiment appeared to select for system-specific community patterns that are consistent with the existing knowledge of individual taxonomic groups, but the limited functional information provided by phylogenetic surveys also precludes more definite conclusions. However, the ability to observe specific structural shifts at the level of individual microbial taxa now offers novel insights into the potential of managing the soil microbiome for sustainable agricultural productivity and plant protection.

Conflict of Interest

The authors declare no conflict of interest.

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