Biology and Fertility of Soils (2019) 55:565–576
https://doi.org/10.1007/s00374-019-01371-w

ORIGINAL PAPER

Robustness of the autochthonous microbial soil community after amendment of cattle manure or its digestate

Sabine Marie Podmirseg[1] · Sebastian Waldhuber[1] · Brigitte Amalia Knapp[1] · Heribert Insam[1] · Marta Goberna[2]

Received: 23 February 2019 / Revised: 14 May 2019 / Accepted: 31 May 2019 / Published online: 19 June 2019
© The Author(s) 2019

Abstract

In the last two decades, a change in land use has taken place in Europe. Manures that used to be applied to agricultural soils are now used in biogas reactors, and instead, digestate is applied. Here, we simulated soil amendment with either fresh or anaerobically digested cattle manure. The aim was to investigate the resilience of the resident microbiota and detect differences in the microbial biomass and activity after fertilizer amendment. Furthermore, the physiological community profile and the role of the indigenous microbial community was elucidated. In a microcosm experiment, two kinds of agricultural soil (γ-irradiated versus non-irradiated) were amended with either treatment. The effect of amendments on the community composition and physiological activity was tested immediately, after 1 and 3 months of incubation through 16S rRNA gene amplicon sequencing (initial soil community), genetic and physiological profiling (PCR-DGGE and MicroResp™), and measurement of basal respiration and microbial biomass. Either fertilizer did not affect the community composition of dominant fungi and bacteria in non-irradiated soils. This indicates the ability of the indigenous microbiota to outcompete allochthonous microorganisms. Soil microbial biomass was not changed, whereas basal respiration was significantly higher after amendment, especially when using fresh manure. MicroResp™ revealed slightly higher respiration for some substrates after 1 month; this finding was, however, not persistent and similar for manure and digestate. Generally, after 1 month, treatments returned to control levels for all parameters. In conclusion, amendment with anaerobically digested manure did not have a greater impact on soil microbial properties.

Keywords Indigenous soil microbiota · Community level physiological profile · Land-use change · Allochthonous microbes · Anaerobic digestion · Resilience

Introduction

Agricultural soils are subjected to recurring external disturbances such as tillage, crop cultivation, harvest, and fertilization (e.g., application of manures). These events are continuously changing the ecological succession stages of soil and also the microbial activity through varying nutrient availability and physical soil properties (Altieri 1999). Such disturbances can generally have very different effects on microbial community composition, activity, or abundance. They either lead to a completely altered structure and performance, to a novel but functionally redundant community, or, on the other hand, to resilience to original parameters or even resistance to changed environmental conditions (Allison and Martiny 2008).

In the last two decades, small- and mid-scale biogas reactors have thrived in Europe and led to a new way in dealing with agricultural wastes from livestock husbandry (Tabajdi 2007). Manures that used to be applied to agricultural soils are now used as a substrate for biogas plants. Besides a sanitation aspect, reduction of the organic pollutant load and malodors, this process also represents a renewable energy source through the combustion of generated biogas (Goberna et al. 2011; Insam et al. 2015). In this survey, we took a case study that

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00374-019-01371-w) contains supplementary material, which is available to authorized users.

✉ Sabine Marie Podmirseg
Sabine.Podmirseg@uibk.ac.at

[1] Institute of Microbiology, University of Innsbruck, Technikerstraße 25d, Bauteil V, 6020 Innsbruck, Austria

[2] Department of Environment and Agronomy, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Carretera de La Coruña km 7.5, 28040 Madrid, Spain

exemplifies the change in land use that is taking place in many farms and agricultural cooperatives across Central and Northern Europe. The case presented here can be considered representative of a now established land-use strategy that has been encouraged through the European promotion of anaerobic digestion (Nkoa 2014; Tabajdi 2007; Weiland 2010).

At the agricultural school in Rotholz (Austria), cattle manure used to be applied to arable soils three times per year at a maximum rate of 80 kg N ha^{-1} for each application. But with the installation and start-up of a full-scale plant that was built with the BIO4GAS® technology (Podmirseg et al. 2016; Wett and Insam 2010), cattle manure was no longer applied to the fields but used as a substrate for biogas production. An equivalent amount of digestate was used to be applied to the school's agricultural soils instead. Anaerobically digested manure shows some specific characteristics compared to fresh cattle manure, such as a reduced chemical oxygen demand and total solids, but increased electrical conductivity and higher concentrations of mineral N, especially in the form of NH_4^+-N (Goberna et al. 2011; Gomez-Brandon et al. 2016). This implies that the use of digestates as organic amendments can change the soil habitat and thus the microbial activity and community composition in ways that are not fully understood.

The aim of this study was to (i) investigate the resistance and resilience of the resident microbial community following the application of anaerobically digested as compared to fresh cattle manure, (ii) detect effects on the microbial biomass, activity and substrate preferences after fertilizer administration, and (iii) evaluate the role of the indigenous soil microbiota. We applied a single administration of fresh cattle manure or biogas digestate to an agricultural soil in a microcosm experiment. Prior to administration, soils were either treated using γ-irradiation (i.e., indigenous soil microbiota was devitalized) or non-irradiated (i.e., indigenous soil microbiota was kept intact). According to the literature, γ-irradiation is an effective way to sterilize soil samples having the least impact on general soil properties (Skulcova et al. 2018; Trevors 1996). The initial, indigenous microbial community and the potential changes due to fresh manure or digestate application were characterized by a 16S rRNA gene amplicon sequencing approach, targeting archaea and bacteria, the main microbial groups introduced by the amendment of soil with organic fertilizer, such as fresh cattle manure or its digestate. The effect of the amendments on the bacterial, fungal, and archaeal community composition was tested immediately and after 1 and 3 months of incubation by denaturing gradient gel electrophoresis (DGGE) and community-level physiological profiling (MicroResp™). In addition, basal respiration and microbial biomass were monitored for the same period.

Material and methods

Sampling and experimental setup

The sampling of soils and substrates, the chemical and physical properties, and experimental setup are described in Goberna et al. (2011). Half of the soil was reduced in vital microbes through γ-irradiation at a dose of 25 kGy with Co 60 as radiation source (Mediscan, Kremsmünster, Austria). An overview of the six treatments and three incubation times is given in Table 1. In short, 90 soil-filled columns (11-cm diameter, 20-cm depth) were filled with 2 kg of sieved (4 mm) topsoil (wet weight) and humidity adjusted to 50% water-holding capacity (WHC) (2 irradiation levels × 3 amendment levels × 3 incubation times × 5 replicates). The amendment of soil columns with organic material (fresh manure (M) or anaerobically digested manure (D)) was performed at a dose to simulate an amendment equivalent to 80 kg N ha^{-1} for a single application. After an equilibration phase of 4 days at 4 °C, the experiment was started (0 m) and microcosm columns were incubated at 20 °C under constant control of soil humidity for a period of 90 days. Destructive sampling was carried out at 0, 30, and 90 days (30 columns each).

DNA extraction

Soil DNA was extracted from ca. 0.30 g soil using the PowerSoil DNA isolation kit (MO BIO Laboratories, Carlsbad, California). Extracted DNA was checked for quality by electrophoresis in 1% agarose gels run in 0.5 × TAE buffer.

16S rRNA gene amplicon sequencing of the initial microbial soil community

In order to identify the most important soil prokaryotic community members and to reveal potential groups introduced through manure or digestate administration, a rRNA gene sequencing approach was chosen. The five replicate DNA

Table 1 Abbreviations used for different treatments and time points of this study

Label	Treatment/time points
C	Control soil (no amendment)
M	Soil + fresh cattle manure
D	Soil + anaerobically digested cattle manure
γC	γ-irradiated control soil (no amendment)
γM	γ-irradiated soil + fresh cattle manure
γD	γ-irradiated soil + anaerobically digested cattle manure
0d	Sampled at the start of the incubation
30d	Sampled after 1 month of incubation
90d	Sampled after 3 months of incubation

extract samples of each treatment (C, M, and D) at time point 0 m were pooled and DNA quality (260/280 nm and 260/230 nm ratios) and quantity were verified with a microvolume spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples were 16S rRNA gene amplicon sequenced (Microsynth AG, Balgach, Switzerland) on an Illumina MiSeq device. A 250-bp paired-end approach targeting the prokaryotic V4 region of the 16S SSU rRNA gene was chosen with the following forward and reverse primers 515f (GTGCCAGCMGCCGCGGTAA) and 806r (GGACTACHVGGGTWTCTAAT) (Caporaso et al. 2011), respectively. Data processing was performed with the CoMA pipeline (Hupfauf et al. 2017). Briefly, paired-end reads were merged and barcodes and primers trimmed and only sequences with an average quality score of ≥ 25 and median length of 250 bp were used for further analysis. Sequence alignment and taxonomic assignment were based on the blast algorithm with SILVA SSU (release 123) as primary database and Greengenes (release 13_5) as backup database at a 97% similarity level. Datasets were subsampled according to the sample with the lowest read number (i.e., 68,493 reads), singletons and doubletons removed, and datasets split into subsets encompassing all archaeal or bacterial reads, respectively. Finally, for a general overview at each phylogenetic level, only OTUs ≥ 1% of the total abundance (excluding non-assigned reads) were considered. SRA files were deposited to NCBI database under BioProject PRJNA508612 and BioSamples SAMN10844943-SAMN10844945 with their respective SRA numbers SRR8529684-SRR8529686.

PCR amplification and denaturing gradient gel electrophoresis

Partial sequences of the small subunit rRNA genes of bacteria, archaea, and fungi were PCR amplified using the specific primer sets given in Table 2. PCR reactions were performed in 25 μL volumes, with each standard reaction mix containing a final concentration of 1 × reaction buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 0.01% Tween 20], 200 μM each dNTP, 0.2 μM each primer, 1 mM $MgCl_2$, 0.4 mg mL^{-1} bovine serum albumin, 0.63 U BioTherm™ DNA polymerase (GeneCraft, Germany), sterile water and 1 μL template (i.e., ≥ 2 ng DNA). Bacterial cycling consisted of the following steps: 94 °C for 3 min, 30 cycles (94 °C for 1 min, 62 °C for 1 min and 72 °C for 2 min), concluded with a final elongation at 72 °C for 15 min. Archaeal rRNA gene fragment amplification consisted of a nested PCR approach with the following conditions: the first PCR included a step of 95 °C for 5 min, 31 cycles at 95 °C for 1 min, 55 °C for 1 min and 72 °C for 3 min, with a final extension at 72 °C for 15 min. Nested PCR was the same as above, but included 40 cycles, annealing temperature was 40 °C, and each elongation step had a length of 2 min. And finally, thermal cycling with universal fungal primers was started at 94 °C for 8 min and followed by 35 cycles including 94 °C for 30 s, 48 °C for 45 s and 72 °C for 2 min, concluded with a final elongation at 72 °C for 10 min. PCR products were checked for quality and length in 1% agarose gel and quantified with the Quant-iT™ PicoGreen® dsDNA Kit (Invitrogen, Carlsbad, USA) and Lambda DNA as standard.

Denaturing gradient gel electrophoresis (DGGE) was performed using an INGENY phorU® system (Ingeny Int., The Netherlands) and is a reproducible, rapid, and ideally suited method to perform a genetic profiling of the most dominant community members for a larger set of samples (Gelsomino et al. 1999; Pimentel et al. 2017). A total amount of 60 ng PCR amplicon of each sample was loaded onto 8% acrylamide-bisacrylamide gels at different urea-formamide gradients (40–70% for bacteria, 45–60% for archaea, and 30–60% for fungi, respectively; 100% denaturant corresponding to 7 M urea plus 40% *w*/*v* deionized formamide). Gels were run in 1 × TAE buffer (60 °C) at 100 V for 16 h, silver-stained (Sanguinetti et al. 1994) using the Hoefer Automated Gel Stainer (Amersham Pharmacia Biotech, Germany) and scanned for subsequent image analysis.

Table 2 Oligonucleotide primers used in this study to target the bacterial 16S rRNA and fungal 18S rRNA gene

Primer	Target	Sequence 5′-3′	Reference
FR1†	Fungi	AICCATTCAATCGGTAIT‡	(Vainio and Hantula 2000)
FF390		CGATAACGAACGAGACCT	
984f†	Bacteria	AACGCGAAGAACCTTAC	(Nübel et al. 1999)
1378r		CGGTGTGTACAAGGCCCGGGAACG	(Heuer et al. 1997)
109f	Archaea (PCR 1)	ACKGCTCAGTAACACGT	(Grosskopf et al. 1998)
934r		GTGCTCCCCCGCCAATTCCT	
A357f†	Archaea (PCR 2)	CCCTACGGGGCGCAGCAG	(Yu et al. 2008)
A693r		GGATTACARGATTTC	

† GC-clamp (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG) attached to the 5′-end of the primer (Muyzer et al. 1993), ‡ *I*, inosine

Three independent gels were loaded to compare all five replicates from each treatment and time point. Banding patterns among replicates were highly reproducible (see Fig. A5 and Fig. A6); thus, two replicates were randomly selected for their final comparison in a single gel to avoid inter-gel variation. In this way, all treatments and time points could be loaded onto one final gel for the analysis of bacterial, archaeal, and fungal communities, respectively.

Gel pictures were analyzed with the GelCompar® II software (version 4.0, Applied Maths, Belgium). Banding patterns were normalized and cluster analysis was performed using the Ochiai coefficient-based, pair-wise similarities and the UPGMA algorithm. The program settings were set at 1.0% optimization and 1.0% position tolerance.

Substrate-induced respiration

Soil basal respiration (BR) [μg CO_2 g^{-1} soil (dry weight) h^{-1}] was measured as the CO_2 production from moist (50% WHC) soil samples (50 g) at 20 °C, using a continuous flow infrared gas analyzer (IRGA MK3, The Analytical Development Co. LTD, Hoddesdon, UK) (Heinemeyer et al. 1989). Microbial biomass (C_{mic}) [μg C g^{-1} soil (dry weight)] was determined by substrate-induced respiration [SIR; (Anderson and Domsch 1978)] after the addition of 0.5 g glucose (1% of soil wet weight).

Community level physiological profile (MicroResp™)

MicroResp™ was used to determine the metabolic diversity and activity of soil microbial communities (Campbell et al. 2003), following the manufacturer's instructions except for the preparation of the indicator solution, which contained 10 mM instead of 1 mM $NaHCO_3$. Thirteen substrates of different lability, known to be common root exudates were chosen for the assay to simulate soil nutrient availability encountered during crop cultivation periods: six carbohydrates (D-fructose, D-galactose, L-arabinose, D-glucose, D-raffinose, and D-maltose), three amino acids (D-alanine, γ-aminobutyric acid, and L-proline), and four organic acids (citric acid, DL-malic acid, oxalic acid, and propionic acid). Nine of these substrates are coincident with those used by Campbell et al. (2003). Others (D-Raffinose, D-Maltose, and L-proline and propionic acid) were selected as known exudates of maize and wheat (Kozdrój 2000; Kraffczyk et al. 1984; Rovira and McDougall 1967), which are important crops in the study area (Josef Norz, personal communication). All substrates were prepared at a concentration equivalent to 30 mg glucose g^{-1} soil water (or 11 mg C g^{-1} soil water), except for D-Alanine, DL-aspartic acid and oxalic acid, which were prepared at 7.5 mg glucose g^{-1} soil water (5.5 mg C g^{-1} soil water) due to their lower solubility (Campbell et al. 2003). In total 15 plates were prepared corresponding to 5 replicate plates × 3 time points. Each plate held all six treatments that were analyzed for 13 substrates and water as control, i.e., $14 \times 6 \geq 84$ wells. Plates were incubated at 20 °C and absorbance was measured at 590 nm at times 0 h and 6 h with the plate reader (Anthos Zenyth 3100, HVD, Grödig, Austria). Obtained data were calculated according to Campbell et al. (2003) and are given as μg CO_2-C dry weight (DW) g^{-1} h^{-1}.

Statistical analysis

Data were tested for normal distribution with the Shapiro-Wilk test and dependent on results further analyzed with ANOVA or Kruskal-Wallis tests. Post hoc analysis was performed with the Tukey HSD test. Soil columns represented the subjects, and treatments (control C, cattle manure M, and digestate D), sterilization (γ-irradiated or non-sterilized), and time (0 day, 30 days, and 90 days) were fixed as categorical predictors (factors). For investigation of multivariate effects (i.e., MicroResp™-data) three-way MANOVA was conducted on rank transformed data. All statistical tests were performed either with the PAST 3.13 software (Hammer et al. 2001) or R (R-Core-Team 2018; Version 3.5.0).

Results and discussion

Initial microbial community characterization

In total, 68,493 quality-checked reads of each sample (C, M, and D at 0 day) were phylogenetically assigned and the relative abundance of the most important families can be described as follows. The autochtonous archaeal soil microbiota was essentially constituted by members of the family Nitrosophaeraceae (Thaumarchaeota) and a marginal presence of Methanomassiliicoccaceae, Crenarchaeaceae (Crenarchaeota), and Methanosarcinaceae. At the genus level, the most dominant soil archaea were *Candidatus Nitrososphaera* and eventually a small population of *Methanosarcina*. Thus, the arable soil used in this study clearly reflected an archaeal soil microbiota that is dominated by ammonia-oxidizing archaea of the phylum Thaumarchaeota (Nitrososphaeraceae) (He et al. 2012; Pester et al. 2011) and exhibits minor populations of methanogens that already point out recurrent events of soil fertilization through manure or digestate. Managing both manure or digestate, the soil microbiota is potentially increased with methanogens, where manure application suggests an increase in Methanocorpusculaceae and Methanobacteriaceae abundance while digestate seems to enrich the soil mostly with Methanosarcinaceae. These different groups, related to either fresh cattle manure or digestate, represent a general community evolution occurring during anaerobic digestion of cattle manure that is characterized by a shift from mostly

hydrogenotrophic methanogens towards a mixture of hydrogenotrophic and acetoclastic members (Podmirseg et al. 2016).

Looking at the bacterial domain, the top eight phyla of the indigenous bacterial soil community were Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Planctomycetes, Nitrospirae, and Verrucomicrobia and thus comparable to other studies describing arable soil communities (Fierer et al. 2012; Gkarmiri et al. 2017). The three most dominant genera were *Rhodoplanes*, *Nitrospira*, and *Flavobacterium*. These three genera are capable of very specific microbial processes and are important players in N-fixation (Buckley et al. 2007), nitrification (NOB) (Hayatsu et al. 2008), and during degradation of lignocellulosic biomass, respectively (Jimenez et al. 2016). Data are summarized in Fig. A1 and a detailed list of the relative abundances of the top 50 OTUs per sample at the family level is given in Table A2 in the ESM.

Characterization of the fungal consortium was not performed; however, it can be expected that different soil treatments (especially in the presence of an autochthonous soil community) have less impact than plant cover and root exudates (Mamet et al. 2017; Sommermann et al. 2018). This concept is also corroborated by the cluster analysis of non-irradiated soil samples that is not influenced by the different amendments but rather by incubation time (see "Microbial community evolution").

Microbial community evolution

Obtained DNA concentrations ranged from 182 ± 42.1 ng DNA g^{-1} soil FW (fresh weight) for non-irradiated to 24.2 ± 12.7 ng DNA g^{-1} soil FW for γ-irradiated soil samples and was thus significantly different ($H = 21.7$; $p < 0.001$). Management type did not significantly alter DNA concentrations in non-irradiated soils, but did in γ-irradiated soils ($H = 11.6$; $p < 0.01$). Non-irradiated soils showed no temporal difference, which could, however, be noticed in γ-irradiated soils ($H = 8.67$; $p < 0.01$) with a slight increase of DNA concentrations, indicating microbial proliferation or recolonization over time. A slight bias through DNA contamination via the extraction kit cannot be excluded, as discussed by Scholer et al. (2017) or Vestergaard et al. (2017); this is, however, rather the case for samples yielding very low DNA amounts and would be equally spread over all sample types.

The amplification of archaeal gene fragments disclosed a very low diversity with an average band number of 3.36 ± 2.4 SD. This fact made validation of trends difficult, but highlighted the dominance of a few genera, supporting the sequencing results (see "Initial microbial community characterization" above) where only two to eight OTUs reached abundance levels > 1% of the archaeome. No clear clustering of time points but a grouping of treatments could be noticed, with digestate-amended samples exhibiting significantly more bands than manure-amended ones ($p < 0.01$; M 1.5 ± 1 SD versus D 5.5 ± 2 SD). For more details, refer to the cluster analysis of archaeal 16S rRNA gene fragments in Fig. A2 in the ESM.

The number of detectable bands in the DGGEs ranged from 24 (γD_0d) to 53 (γD_90d) bands with a mean ± SD of 41.6 ± 6.27 for bacteria (Fig. 1a), and from 5 (γC_0d) to 23 (M_30d) with a mean of 15.9 ± 5.31 for fungi (Fig. 1b), respectively (Table 3). There were significantly less bacterial and fungal bands in sterilized compared to non-sterilized soil columns, independent from amendment ($H = 7.71$; $p < 0.005$ and $H = 17.5$; $p < 0.001$). These findings were underlined by the cluster analyses of the bacterial and fungal DGGEs, which, apart from the bacterial γC_0d-sample, separated the fingerprinting patterns into a non-sterilized and sterilized (γ-irradiated) cluster. A reduction of band numbers up to 42.1% for bacteria and even up to 72.4% for fungi could be achieved due to γ-radiation. However, a fraction of living cells and maybe also of extractable DNA, targeted by the primers used in this study, was not completely disrupted. The higher number of bacterial 16S rRNA gene fragments (34 ± 1 SD bands $sample^{-1}$; Fig. 1a) compared to 5.3 ± 0.6 SD fungal 18S rRNA gene fragments (Fig. 2b) in treatment γC_0d further indicated a higher sterilization efficiency of γ-irradiation for fungi.

This finding is in accordance with the work of Jackson et al. (1967) and Mclaren (1969), who stated that—compared to fungi—two or even three times of irradiation energy was necessary to kill all bacterial cells. They further postulated that the bigger the cells the less ionizing radiation was needed. Interestingly, according to a study by Muehe et al. (2015), although γ-irradiation strongly disturbs the soil microbiota, plant growth was unaffected and comparable crop yields were obtained.

Looking at the community pattern of the most dominant members, no differences were found in the number of bacterial or fungal bands in amended (M and D) compared to control (*C*) soils. However, as regards γ-irradiated soils, those amended with manure (γM) showed significantly more fungal bands than γC and γD ($H = 18.8$; $p < 0.001$). In general, a constant increase of band number could be detected with the incubation time for bacteria ($H = 26.2$; $p < 0.001$), but not for fungi. For both domains, samples belonging to the non-sterilized cluster were grouped into three subclusters, corresponding to the three sampling times. Therefore, the incubation time, and not the treatment, was the main factor discriminating the non-sterilized samples. Similarities above 90% were reached among banding patterns within the same incubation time in any case. On the contrary, the banding profiles of sterilized (γ-irradiated) samples mostly clustered based on the treatment, rather than incubation time. This pattern was evident for fungi already from the very start, when γC was clearly separated from γD and γM. For bacteria, the amended

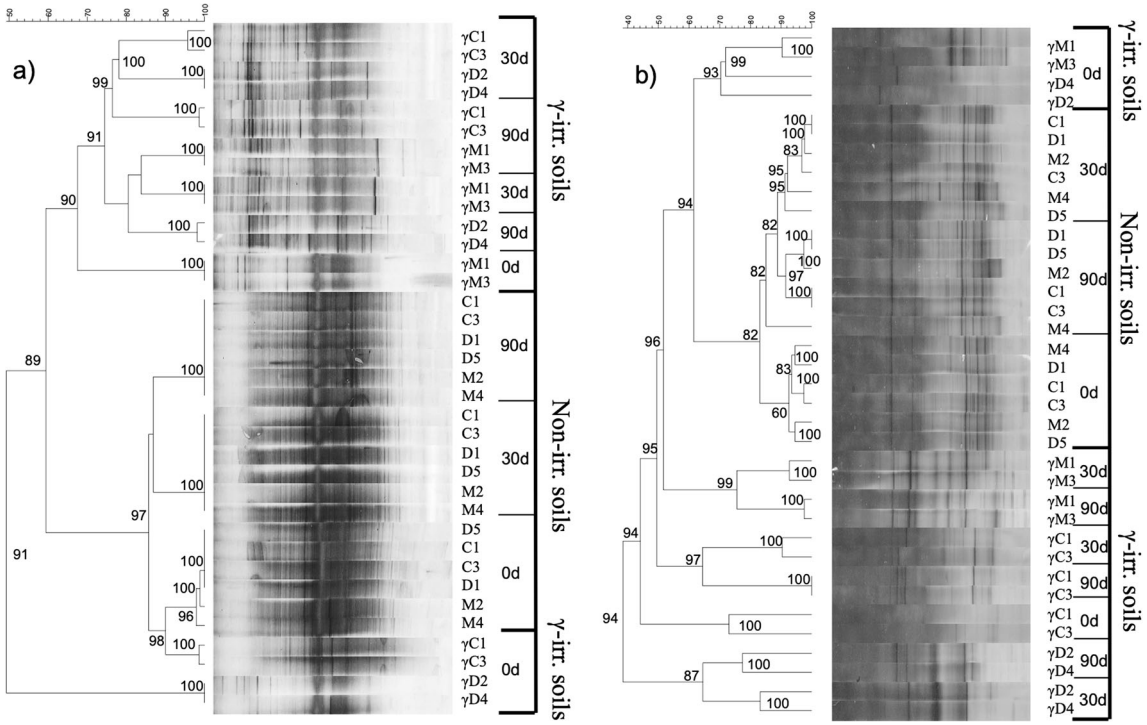

Fig. 1 Cluster analysis of **a** bacterial DGGE fingerprints based on the 16S rRNA gene fragments amplified with the 984f-GC/1378r primer pair and **b** fungal DGGE fingerprints based on the 18S rRNA gene fragments amplified with the FF390/FR1-GC primer pair, respectively. Two parallels per treatment and time point were loaded. Values at nodes indicate the cophenetic correlation coefficients and the scale bar the Ochiai coefficient-based similarity; irr, irradiated

soils (γD and γM) and the control (γC) showed very characteristic patterns, and the community in each treatment evolved differently over time.

The fact that the dominant microbial community composition of the sterilized soils differed depending on the treatment confirms that the microbiota of soils supplied with manure or digestate were different between each other and from the control soil. Furthermore, it suggests that the microbial communities applied to the soils were able to proliferate in the soil environment in the absence of an equilibrated indigenous microbiota. It also is, however, to be considered that the amendments could have changed the soil environment distinctly. This could have promoted different zymogenous soil microbes, which are able to proliferate in the presence of energy-rich substrates (Langer et al. 2004) like organic fertilizers. A further fact that could have boosted fast colonization of γ-irradiated soils is the irradiation treatment itself, rendering killed microbial cells available to other microorganisms.

Recall that the fingerprint patterns, representing the most abundant microbiota in the non-sterilized soils, were very homogeneous and equal to the control samples independent of the amendment. This led to the conclusion that the autochthonous microbiota that prevails in the soil is outcompeting and partially inhibiting the proliferation of allochthonous microorganisms. Microbial competitive interactions leading to the exclusion of allochthonous microbes by indigenous soil inhabitants has been proven in laboratory experiments using co-cultures, e.g., Buchanan and Bagi (1999). Such interactions have

Table 3 Evolution of microbial richness, based on bacterial and fungal DGGE band numbers; mean ± SD ($n = 3$)

		C	D	M	γC	γD	γM
0 day	BB[†]	42 ± 0	42 ± 0	42 ± 1	34 ± 1.0	24.3 ± 0.6	33 ± 0
	FB[‡]	19.3 ± 0.6	17.7 ± 1.2	17.7 ± 1.2	5.3 ± 0.6	11 ± 2.6	15.7 ± 0.6
30 days	BB	43 ± 0	43 ± 0	43 ± 0	44.7 ± 0.6	40 ± 0	41 ± 0
	FB	20.7 ± 0.6	20 ± 1	22.3 ± 0.6	10.3 ± 1.2	11 ± 2	20.3 ± 0.6
90 days	BB	47 ± 0	47 ± 0	47 ± 0	37.3 ± 0	52 ± 0	47 ± 0
	FB	20 ± 0.6	19 ± 0.6	18 ± 0.6	8.3 ± 0.6	8.3 ± 0.6	21 ± 0.6

[†] *BB*, bacterial band number (DGGE); [‡] *FB*, fungal band number (DGGE)

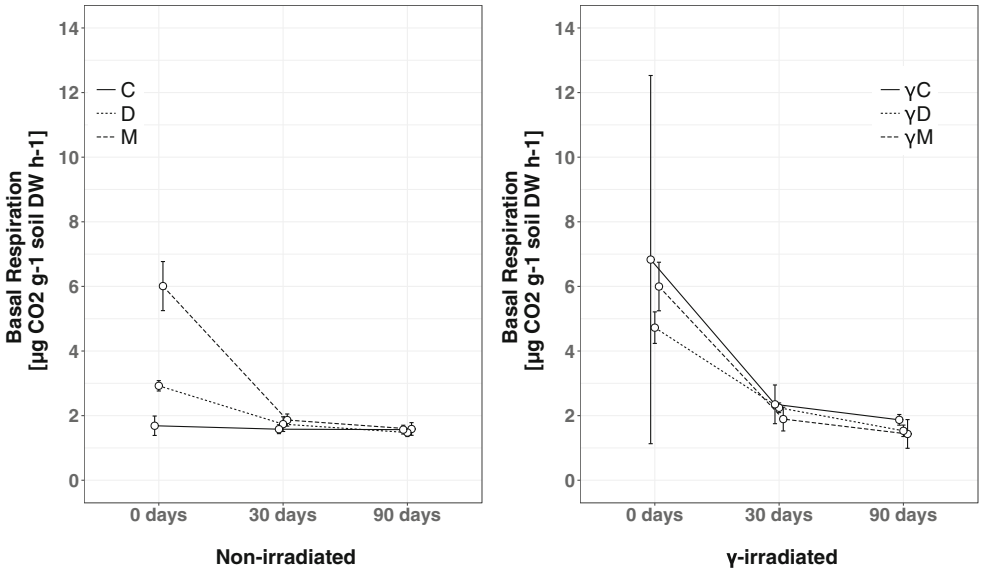

Fig. 2 Evolution of basal respiration [µg CO_2-C g^{-1} soil (dry weight) h^{-1}] over time, comparing non-irradiated and γ-irradiated soil columns. 0d, 30d, 90d refer to 0, 30, and 90 days of incubation, respectively. C, D, M refer to control, anaerobically digested cattle manure (sludge), and fresh cattle manure, respectively

been particularly well studied to understand the suppression of pathogens by soil microbes. This is known to be mediated through the production of antibiotics, the deprivation of nutrients due to the liberation of chelators and the interference with pathogenicity factors (Haas and Défago 2005), among others. Pathogen suppression, more precisely the reduction in cultivable *Escherichia coli* and *Salmonella* and pathogenic *Listeria* after application of fresh and digested manure, was confirmed in an additional study (Goberna et al. 2011), where selective media and specific primers targeting pathogenicity genes were used. Soils amended with fresh instead of anaerobically digested manure bore higher pathogen levels, especially *E.coli* and *Salmonella*. No pathogenic, that is to say hlyA-positive, *Listeria* could be detected in samples amended with anaerobically digested manure. In both experimental lines (M and D), pathogen abundances reached control levels after 3 months if an autochthonous microbiota was present. General pathogen levels remained higher in γ-irradiated soils.

A study on the effectiveness of fungal bioaugmentation of either fumigated or non-irradiated soil (Federici et al. 2007) indicated that the competitiveness of allochthonous microorganisms was not only dependent on the presence of an indigenous microbiota but also on the species that was applied to the soil. In our study, lower fungal band numbers were detected in the experimental lines γC and γD, while manure-amended samples (γM) had a similar diversity compared to all non-irradiated treatments (Fig. 1b). This might indicate that the fungal consortium present in fresh cattle manure is more competitive in populating the irradiated soil than fungal communities that underwent anaerobic digestion in a biogas plant. Fungal groups that can be found in fresh cattle manure are the monophyletic group of strictly anaerobic Neocallimastigomycota that greatly promotes degradation of lignocellulose-rich biomass in their hosts (Griffith et al. 2010). Their survival under aerobic conditions is however very limited (Leis et al. 2014; Nagler et al. 2019). Thus, it is unlikely that the detected fungal sequences at later time points were affiliated with Neocallimastigomycota. On the other hand, Schnürer and Schnürer (2006) studied the survival of specific fungal species during mesophilic and thermophilic AD and detected some species were able to survive these processes (e.g., via generation of heat-resistant ascospores) and even tolerated prior sanitation treatments (70 °C, 1 h). Interestingly, in their study, it was clearly the subsequent aerobic storage of digestate that reduced fungal counts (Schnurer and Schnurer 2006). These findings are reflected in our results with significantly lower fungal band numbers in γD compared to γM. An explanation of higher band numbers in the latter treatment might most probably be attributable to a fast recolonization of the fresh manure via airborne fungal spores. Further studies on the qualitative community composition in γM and γD, but also γC would be necessary to determine which allochthonous bacteria and fungi are the most competitive in colonizing irradiated soil.

Non-sterilized samples showed very few variances irrespective of the application of an organic amendment, suggesting a dominant and highly competitive autochthonous soil community. Allochthonous communities were very diverse and different to those in the soil; however, they were not able to proliferate in the presence of an indigenous microbiota. It is thus unlikely that the allochthonous microorganisms that could also be identified at 0 day are thriving in the soil habitat, especially as fertilization events are performed in longer intervals, when initial effects are not noticeable anymore.

It needs to be stated that genetic profiling performed via PCR-DGGE is only reflecting the evolution of most dominant

community members and that the fate of rare but potentially important microorganisms that might have been amended with manure or digestate remains unresolved. However, it is also this persistency of main microbial guilds, irrespective of the amendment type, that stresses the ability of the autochthonous microbiota to cope with this external disturbance such as an organic fertilizer amendment.

A study on the effect of different doses of manure application to red soils in subtropical China indicated an increase in bacterial abundance after amendments and with increased doses of manure application also a reduction of bacterial diversity. This reduced diversity, however, also had a negative effect on peanut yield, and a general recommendation for moderate manure application was given (Yang et al. 2017). Short-time effects were not studied. In another experiment, dealing with long-term effects of manure application (Zhang et al. 2018), distinct microbial communities could be detected, if soils were treated with cattle manure as compared to chemical N fertilizers. To our knowlegde, no long-term studies exist on the effect of digestate management to agricultural soils, our results suggest, however, that effects are comparable to manure management. One major reason supporting this fact and explaining why the core microbial community was so resistant towards the disturbance factor "fertilizer application" and resilient regarding microbial activity (as discussed in the next sections) might be that the soil used for this microcosm experiment was already adapted to recurrent amendments with cattle manure and that a distinct consortium had already established in the soil. Such general adaptation of soil communities to fertilizer is often observed and its effect very long lasting (Zhang et al. 2018). Nevertheless, in this study, the focus was laid on the comparison of two different fertilizer types and the single application of either amendment led to highly similar results.

Basal respiration and biomass

Results of basal respiration and biomass measurements are summarized in Table A1 and can be described as follows.

Basal respiration was significantly different between non-sterilized and γ-irradiated soil ($H = 4.46$; $p < 0.03$) and over time ($H = 27.7$; $p < 0.001$). Initially, all treatments, apart from treatment C, showed increased values and stabilized at a significantly lower level within 1 month (Fig. 2). In contrast to this, control samples (C) showed low basal respiration (1.69 ± 0.3 μg CO_2 g^{-1} soil (dry weight) h^{-1}) from the beginning and further remained constant. This stability is generally encountered in soils that have experienced longer phases of similar agricultural use (Insam and Haselwandter 1989) and also indicates that the manipulation of soil samples during the experimental setup of the microcosm study did not further destabilize the microbial community.

In fact, a study on soil manipulation, as typically performed for microcosm setups, states a significant effect on organic carbon mineralization after changing soil structure (Juarez et al. 2013). This effect was, however, equally pronounced for microbial consortia of different levels of diversity (Juarez et al. 2013). This suggests that part of the metabolic activity measured in our experiment is due to the soil manipulation itself. Since it seems to be independent of species richness (γ-irradiated versus non-irradiated soils, or control versus manure/digestate), this bias should be equal for all treatments and thus allow for inter-treatment comparison.

Manure-amended soil (M) exhibited a double BR compared to soil amended with anaerobically digested manure (D). These differences were significant (C, M, and D; $H = 7.2$; $p < 0.03$), however evened out within 1 month. A decrease in the basal respiration signifies that the initial pool of easily degradable substrates, applied with the amendments, was depleted and thus microorganisms became metabolically less active. The initially higher basal respiration in soils amended with fresh manure could be due to the higher availability of organic matter compared to a digested manure. Similarly, γ-irradiated samples had higher BR than non-irradiated soils, which is attributed to immediate recolonization and microbial consumption of dead cells. Eno and Popenoe (1964) observed that γ-radiation can increase nutrient availability. Also, Tuominen et al. (1994) found out that γ-irradiation leads to an increased breakup of carbohydrates such as cellulose and dead microbial cells.

In our study, the main physical and chemical soil properties, including the concentrations of most macro- and micronutrients, remained unchanged after γ-irradiation (Goberna et al. 2011; and Fig. A7). Detailed monitoring of cellulose content or degradation products was not performed. However, NH_4^+-N concentrations increased 14-fold in γ-irradiated compared to non-irradiated soils (Goberna et al. 2011). This could have favored nitrifying bacteria and archaea, as well as heterotrophs using NH_4^+-N as N source. The initial burst in microbial respiration in irradiated samples, however, stabilized progressively during the course of the experiment.

The analysis of the microbial biomass (MB) showed significant differences over time ($H = 7.04$; $p < 0.03$) (Fig. 3), although not discriminable at the start of the experiment. After 3 months of incubation, however, the microbial biomass was distinctly higher in non-irradiated soil with a mean of 486 ± 30.1 μg C g^{-1} soil (dry weight) compared to 146 ± 24.1 μg C g^{-1} γ-irradiated soil (dry weight), respectively ($H = 12.8$; $p < 0.001$). There was only a non-significantly higher biomass in manure-amended samples with regard to treatment D. These results highlight that digestate does not have a greater impact on the microbial biomass than manure and that a quick return to original values is achieved.

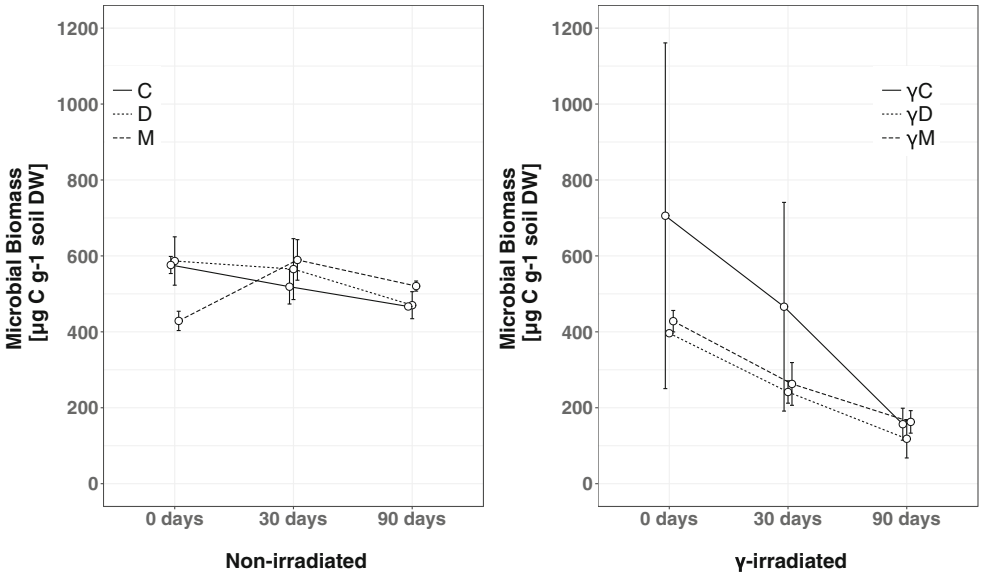

Fig. 3 Evolution of the microbial biomass [µg C g^{-1} soil (dry weight)] over time, comparing non-irradiated and γ-irradiated soil columns. 0d, 30d, 90d refer to 0, 30, and 90 days of incubation, respectively. C, D, M refer to control, anaerobically digested cattle manure (sludge) and fresh cattle manure, respectively

Microbial substrate utilization pattern

The γ-irradiation had a strong influence on community-level physiological profiling (CLPP). At each time point, non-irradiated soil samples could easily be discriminated from γ-irradiated ones. It needs to be stated, however, that the general pattern for each substrate utilization (independent of amendments) was similar, but despite the respiratory pulse in non-irradiated soil, samples exhibited higher substrate use. Parekh et al. (2005) found that the type of soil and the composition of the indigenous microbiota is determining to which extent γ-irradiation is affecting microbial activity and community composition. In that study, γ-irradiation also affected the number of utilized substrates, a finding that was not observed in our experiment and which suggests that the indigenous or establishing microbiota after γ-irradiation still managed to occupy the same metabolic niches.

CLPPs in γ-irradiated soil (Fig. A4) reacted differently to manure and digestate amendment. This trend was not as pronounced at 0 day, but at 30 days, amended soils (γD and γM) could clearly be discriminated from γC samples. After 3 months of incubation, the catabolic fingerprint of all three treatments had aligned again.

In contrast to this pattern, the autochthonous microbiota of non-irradiated soil samples (Fig. A3) was not affected by either amendment. Comparing the overall substrate utilization (13 substrates), the overall utilization pattern was not significantly different among C, M, and D at any time point, which is further corroborated by the constant overlap of convex hulls of C, M, and D at each time point (Table 4 and Fig. 4). Only after 1 month of incubation, a (marginally) significant higher turnover of D-fructose ($p = 0.06$), D-galactose ($p = 0.03$) (both D compared to C), and D-alanine ($p = 0.04$) (amendments M and D versus C) could be found. The amendments with digestate and manure did never trigger different substrate utilization patterns. Irrespective of treatments, over time, there was a general switch from a relatively balanced utilization of all 13 substrates with slightly higher organic acid (citric, malic and oxalic acid) turnover towards a microbial community that showed increased utilization of the latter mentioned organic acids. This is also demonstrated by the time shift of subjects from left to right on the first principal component in Fig. 4.

Table 4 Multivariate analysis of variance (MANOVA) for MicroResp™ results including all 13 test substrates and categorical predictors (factors) sterilization (non-irradiated, γ-irradiated), amendment (C, M, D), and time (0 day, 30 days, 90 days)

	Df	Pillai approx	*F*	num Df	den Df	Pr(>*F*)
Sterilization	1	0.77	15.24	13	60	< 0.001
Amendment	2	0.83	3.36	26	122	< 0.001
Time	2	1.16	6.50	26	122	< 0.001
Sterilization × amendment	2	1.03	4.94	26	122	< 0.001
Sterilization × time	2	0.89	3.74	26	122	< 0.001
Amendment × time	4	0.97	1.55	52	252	0.02
Sterilization × amendment × time	4	0.95	1.50	52	252	0.02
Residuals	72	NA	NA	NA	NA	NA

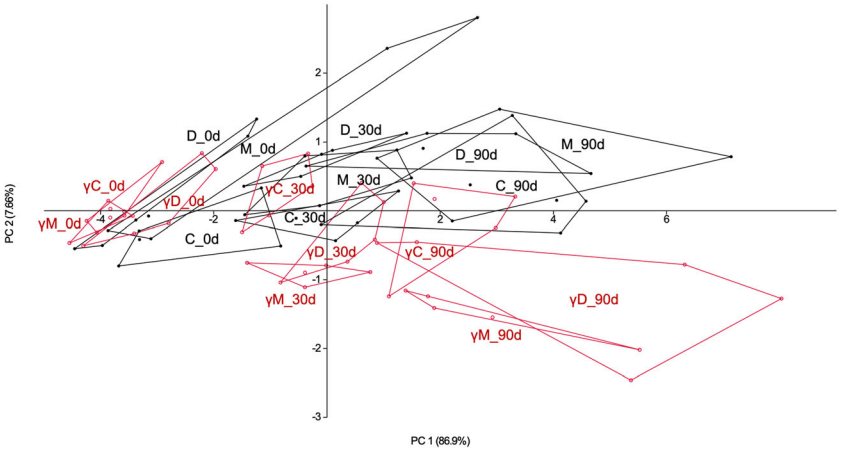

Fig. 4 Principal component analysis plot of MicroResp™ data (mean ± SE; $n = 5$) for all three time points 0d, 30d and 90d, substrates (C, M, and D) and sterilization levels (γ-irradiated or non-irradiated), resulting in 18 different treatments. Single subjects and convex hulls around each treatment are given. The two first axes explain 94.56% of the variance

This component is essentially correlated with the utilization of citric-, oxalic- and malic acids (Fig. 5). Although comparison with other studies is delicate, as experimental setups and tested substrates vary from study to study, major trends were comparable. The contributions of substrate groups to the overall metabolic activity could be ranked as follows: carboxylic acids > carbohydrates > amino acids (Andruschkewitsch et al. 2014). And respiration rates of specific substrates were all found within the range described in a comprehensive study investigating forest, grassland, and arable soils throughout Europe (Creamer et al. 2016).

Conclusion

The γ-irradiation treatment helped to confirm the role of the indigenous soil microbiota to attenuate manure or digestate effects. In the presence of indigenous microbiota, no significant effects were noticeable, at least for major microbial players, demonstrating a clear resistance of the main members of the autochthonous soil community after nutrient and microbe input. Furthermore, fast consumption of organic substances within an interval that is clearly shorter than the commonly used fertilizer application interval of this agricultural region (two to three times a year), allows for the conclusion that land spreading of anaerobically digested sludge, a by-product from biogas production, instead of fresh cattle manure seems to be an adequate management alternative, leading to a fast resilience of microbial activity parameters towards original values. Still, the excess nitrate liberation (2-fold compared to M) should be considered and applied amounts thoroughly matched with crop cultivation approaches and plant demands to avoid excess leaching to groundwater or increased denitrification and thus gaseous emission to the atmosphere.

A follow-up microcosm experiment using marker gene amplicon sequencing could elucidate if there are significant effects on the microbial community composition at least for rare OTUs, which might have been overlooked by the DGGE approach. Furthermore, a focus should not only be laid on relative but also on absolute changes of microbial populations after manure or digestate amendment via qPCR measurement for the most important groups. Resistance and resilience of the

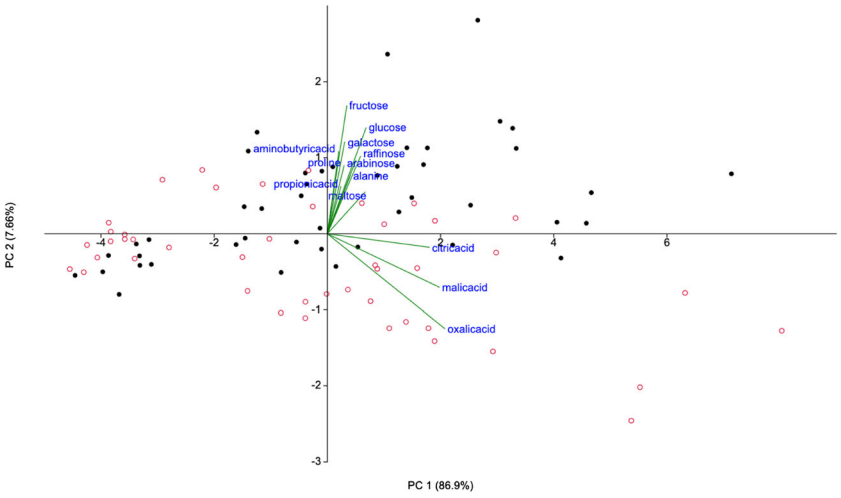

Fig. 5 Principal component analysis plot showing combined subject factor and variable map of MicroResp™ data (mean ± SE; $n = 5$) for all three time points 0d, 30d, and 90d, substrates (C, M, and D) and sterilization levels (γ-irradiated or non-irradiated), resulting in 18 different treatments. The two first axes explain 94.56% of the variance

main microbial players and activity need to be tested for recurrent fertilizer events.

Acknowledgments We would like to thank the staff of the agricultural school Rotholz for their kind supply of fresh cattle manure, anaerobically digested slurry and arable soil sample material that was used in the course of this microcosm experiment. Furthermore, we would like to thank the anonymous reviewers for their helpful and very constructive comments on this manuscript.

Funding information Open access funding provided by University of Innsbruck and Medical University of Innsbruck. Financial support was provided by the EU Marie Curie Programme (MEIF-CT-2006-041034) to MG and HI. SMP was further supported by the doctoral fellowship (LFU Doktoratsstipendium) of the University of Innsbruck, Austria.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. PNAS 105:11512–11519

Altieri MA (1999) The ecological role of biodiversity in agroecosystems Agric. Ecosys Environ 74:19–31

Anderson JPE, Domsch KH (1978) A physical method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221

Andruschkewitsch M, Wachendorf C, Sradnick A, Hensgen F, Joergensen RG, Wachendorf M (2014) Soil substrate utilization pattern and relation of functional evenness of plant groups and soil microbial community in five low mountain NATURA 2000. Plant Soil 383:275–289. https://doi.org/10.1007/s11104-014-2167-9

Buchanan RL, Bagi LK (1999) Microbial competition: effect of Pseudomonas fluorescens on the growth of Listeria monocytogenes. Food Microbiol (Lond) 16:523–529

Buckley DH, Huangyutitham V, Hsu SF, Nelson TA (2007) Stable isotope probing with 15N2 reveals novel noncultivated diazotrophs in soil. Appl Environ Microbiol 73:3196–3204. https://doi.org/10.1128/AEM.02610-06

Campbell CD, Chapman SJ, Cameron CM, Davidson MS, Potts JM (2003) A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl Environ Microbiol 69:3593–3599

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. P Natl Acad Sci USA 108:4516–4522. https://doi.org/10.1073/pnas.1000080107

Creamer RE, Stone D, Berry P, Kuiper I (2016) Measuring respiration profiles of soil microbial communities across Europe using MicroResp (TM) method. Appl Soil Ecol 97:36–43. https://doi.org/10.1016/j.apsoil.2015.08.004

Eno CF, Popenoe H (1964) Gamma radiation compared with steam and methyl bromide as a soil sterilizing agent. Soil Sci Soc Am J 28:533–535

Federici E, Leonardi V, Giubilei MA, Quaratino D, Spaccapelo R, D'Annibale A, Petruccioli M (2007) Addition of allochthonous fungi to a historically contaminated soil affects both remediation efficiency and bacterial diversity. Appl Microbiol Biotechnol 77:203–211. https://doi.org/10.1007/s00253-007-1143-1

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J 6:1007–1017. https://doi.org/10.1038/ismej.2011.159

Gelsomino A, Keijzer-Wolters AC, Cacco G, van Elsas JD (1999) Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. J Microbiol Methods 38:1–15

Gkarmiri K, Mahmood S, Ekblad A, Alstrom S, Hogberg N, Finlay R (2017) Identifying the active microbiome associated with roots and rhizosphere soil of oilseed rape. Appl Environ Microbiol 83. https://doi.org/10.1128/AEM.01938-17

Goberna M, Podmirseg SM, Waldhuber S, Knapp BA, Garcia C, Insam H (2011) Pathogenic bacteria and mineral N in soils following the land spreading of biogas digestates and fresh manure. Appl Soil Ecol 49:18–25. https://doi.org/10.1016/j.apsoil.2011.07.007

Gomez-Brandon M, Juarez MF, Zangerle M, Insam H (2016) Effects of digestate on soil chemical and microbiological properties: a comparative study with compost and vermicompost. J Hazard Mater 302:267–274. https://doi.org/10.1016/j.jhazmat.2015.09.067

Griffith GW, Baker S, Fliegerova K, Liggenstoffer A, Van der Giezen M, Voigt K, Beakes G (2010) Anaerobic Fungi: Neocallimastigomycota. IMA Fungus 1:181–185

Grosskopf R, Janssen PH, Liesack W (1998) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl Environ Microbiol 64:960–969

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Hammer Ø, Harper DAT, Ryan PD (2001) Paleontological Statistics Software Package for Education and Data Analysis 4:9–18

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci Plant Nutr 54:33–45

He JZ, Hu HW, Zhang LM (2012) Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils. Soil Biol Biochem 55:146–154. https://doi.org/10.1016/j.soilbio.2012.06.006

Heinemeyer O, Insam H, Kaiser EA, Walenzik G (1989) Soil microbial biomass and respiration measurements: an automated technique on infra-red gas analysis. Plant Soil 116:191–195

Heuer H, Krsek M, Baker P, Smalla K, Wellington EM (1997) Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl Environ Microbiol 63:3233–3241

Hupfauf S, Etemadi M, Podmirseg S, Insam H (2017) CoMA – Comparative Microbiome Analysis. https://www.uibk.ac.at/microbiology/services/coma.html

Insam H, Gomez-Brandon M, Ascher J (2015) Manure-based biogas fermentation residues - friend or foe of soil fertility? Soil Biol Biochem 84:1–14. https://doi.org/10.1016/j.soilbio.2015.02.006

Insam H, Haselwandter K (1989) Metabolic quotient of the soil microflora in relation to plant succesion. Oecologia 79:174–178

Jackson NE, Corey JC, Frederick LR, Picken JC Jr (1967) Gamma irradiation and the microbial population of soils at two water contents. Soil Sci Soc Am J 31:491–494

Jimenez DJ, de Lima Brossi MJ, Schuckel J, Kracun SK, Willats WG, van Elsas JD (2016) Characterization of three plant biomass-degrading microbial consortia by metagenomics- and metasecretomics-based approaches. Appl Microbiol Biotechnol 100:10463–10477. https://doi.org/10.1007/s00253-016-7713-3

Juarez S, Nunan N, Duday A-C, Pouteau V, Chenu C (2013) Soil carbon mineralisation response to alterations of microbial diversity and soil structure. Biol Fertil Soils 49:939–948

Kozdrój J (2000) Microflora of technogenous wastes characterised by fatty acid profiling. Microbiol Res 155:149–156

Kraffczyk I, Trolldenier G, Beringer H (1984) Soluble root exudates of maize: influence of pottassium supply and rhizosphere microorganisms soil. Biol Biochem 16:315–322

Langer U, Böhme L, Böhme F (2004) Classification of soil microorganisms based on growth properties: a critical view of some commonly used terms. J Plant Nutr Soil Sci 167:267–269. https://doi.org/10.1002/jpIn.200421362

Leis S, Dresch P, Peintner U, Fliegerova K, Sandbichler AM, Insam H, Podmirseg SM (2014) Finding a robust strain for biomethanation: anaerobic fungi (Neocallimastigomycota) from the alpine ibex (Capra ibex) and their associated methanogens. Anaerobe 29:34–43. https://doi.org/10.1016/j.anaerobe.2013.12.002

Mamet SD, Lamb EG, Piper CL, Winsley T, Siciliano SD (2017) Archaea and bacteria mediate the effects of native species root loss on fungi during plant invasion. ISME J 11:1261–1275. https://doi.org/10.1038/ismej.2016.205

Mclaren AD (1969) Radiation as a technique in soil biology and biochemistry. Soil Biol Biochem 1:63–73

Muehe EM, Weigold P, Adaktylou IJ, Planer-Friedrich B, Kraemer U, Kappler A, Behrens S (2015) Rhizosphere microbial community composition affects cadmium and zinc uptake by the metal-hyperaccumulating plant Arabidopsis halleri. Appl Environ Microbiol 81:2173–2181. https://doi.org/10.1128/AEM.03359-14

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Nagler M, Kozjek K, Etemadi-Shalamzari M, Insam H, Podmirseg S (2019) Simple yet effective: microbial and biotechnological benefits of rumen liquid addition to lignocellulose-degrading biogas plants. J Biotechnol 300:1–10. https://doi.org/10.1016/j.jbiotec.2019.05.004

Nkoa R (2014) Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron Sustain Dev 34:473–492. https://doi.org/10.1007/s13593-013-0196-z

Nübel U, Garcia-Pichel F, Kühl M, Muyzer G (1999) Quantifying microbial diversity: morphotypes, 16S rRNA genes, and carotenoids of oxygenic phototrophs in microbial mats. Appl Environ Microbiol 65:422–430

Parekh N, Potter E, Poskitt J, Dodd B, Beresford N (2005) Detection of irradiation induced changes on the activity and diversity of soil microbial communities: The effect of soil type Radioprotection S1 40:S939-S944

Pester M, Schleper C, Wagner M (2011) The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. Curr Opin Microbiol 14:300–306. https://doi.org/10.1016/j.mib.2011.04.007

Pimentel T, Marcelino J, Ricardo F, Soares A, Calado R (2017) Bacterial communities 16S rDNA fingerprinting as a potential tracing tool for cultured seabass Dicentrarchus labrax. Sci Rep 7:11862. https://doi.org/10.1038/s41598-017-11552-y

Podmirseg SM, Gadermaier M, Franke-Whittle IH, Wett B, Insam H, Goberna M (2016) Prokaryotic community dynamics during the start-up of a full-scale BIO4GAS digester. J Environ Eng 142:04015055. https://doi.org/10.1061/(Asce)Ee.1943-7870.0001011

R-Core-Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL http://www.R-project.org

Rovira AD, McDougall B (1967) Microbiological and biochemical aspects of the rhizosphere. In: McLaren A, Petersen GG (eds) Soil biochemistry, vol 1. Marcel Dekker, Inc, New York, pp 417–463

Sanguinetti CJ, Dias Neto E, Simpson AJ (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. BioTechniques 17:914–921

Schöler A, Jacquiod S, Vestergaard G, Schulz S, Schloter M (2017) Analysis of soil microbial communities based on amplicon sequencing of marker genes. Biol Fertil Soils 53:485–489

Schnurer A, Schnurer J (2006) Fungal survival during anaerobic digestion of organic household waste. Waste Manag 26:1205–1211. https://doi.org/10.1016/j.wasman.2005.09.007

Skulcova L, Scherr KE, Chrast L, Hofman J, Bielska L (2018) Influence of soil gamma-irradiation and spiking on sorption of p,p'-DDE and soil organic matter chemistry. Ecotoxicol Environ Saf 155:125–132. https://doi.org/10.1016/j.ecoenv.2018.02.061

Sommermann L, Geistlinger J, Wibberg D, Deubel A, Zwanzig J, Babin D, Schlüter A, Schellenberg I (2018) Fungal community profiles in agricultural soils of a long-term field trial under different tillage, fertilization and crop rotation conditions analyzed by high-throughput ITS-amplicon sequencing. PLoS One 13:e0195345. https://doi.org/10.1371/journal.pone.0195345

Tabajdi CS (2007) Draft report on sustainable agriculture and biogas: a need for review of EU-legislation (2007/2107 INI) vol. Committee on Agriculture and Rural Development, Brussels

Trevors JT (1996) Sterilization and inhibition of microbial activity in soil. J Microbiol Meth 26:53–59

Tuominen L, Kairesalo T, Hartikainen H (1994) Comparison of methods for inhibiting bacterial activity in sediment. Appl Environ Microbiol 60:3454–3457

Vainio EJ, Hantula J (2000) Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycol Res 104:927–936

Vestergaard G, Schulz S, Schöler A, Schloter M (2017) Making big data smart-how to use metagenomics to understand soil quality. Biol Fertil Soils 53:479–484

Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biotechnol 85:849–860. https://doi.org/10.1007/s00253-009-2246-7

Wett B, Insam H (2010) Biogas technology - controlled gas flow for enhanced mixing, heating, and desulfurization. In: Insam H, Franke-Whittle I, Goberna M (eds) Microbes at work - from wastes to resources. Springer, Heidelberg, Germany, pp 79–91

Yang Y, Li X, Liu J, Zhou Z, Zhang T, Wang X (2017) Bacterial diversity as affected by application of manure in red soils of subtropical China. Biol Fertil Soils 53:639–649

Yu Z, Garcia-Gonzalez R, Schanbacher FL, Morrison M (2008) Evaluations of different hypervariable regions of archaeal 16S rRNA genes in profiling of methanogens by Archaea-specific PCR and denaturing gradient gel electrophoresis. Appl Environ Microbiol 74:889–893. https://doi.org/10.1128/AEM.00684-07

Zhang Y, Hao X, Alexander TW, Thomas BW, Shi X, Lupwayi NZ (2018) Long-term and legacy effects of manure application on soil microbial community composition. Biol Fertil Soils 54:269–283

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.