

UC Davis

UC Davis Previously Published Works

Title

Repeated manure inputs to a forage production soil increase microbial biomass and diversity and select for lower abundance genera

Permalink

<https://escholarship.org/uc/item/3t05854w>

Authors

Sayre, Jordan M
Wang, Daoyuan
Lin, Jonathan Y
[et al.](#)

Publication Date

2023-09-01

DOI

10.1016/j.agee.2023.108567

Peer reviewed



Repeated manure inputs to a forage production soil increase microbial biomass and diversity and select for lower abundance genera

Jordan M. Sayre^{a,1}, Daoyuan Wang^{a,2}, Jonathan Y. Lin^{a,3}, Rachel E. Danielson^{a,4},
Kate M. Scow^{a,5}, Jorge L. Mazza Rodrigues^{a,b,*,6}

^a Department of Land, Air, and Water Resources, University of California Davis, Davis, CA 95616, USA

^b Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

ARTICLE INFO

Keywords:

Agriculture
Dairy farm
Microbial diversity
Microbial biomass
Carbon
Soil
Manure
Waste management

ABSTRACT

Adding manure to croplands restores carbon and nutrients in depleted soils, while addressing a waste disposal need. This practice depends on the abundance and activity of microbial communities to break down manure inputs, which provide carbon for microbial growth and release nutrients for plant and microbial uptake. In a 2-year field study, we measured changes to soil physicochemical properties and microbial community composition in a conventional rotation of summer silage corn and winter wheat. Three ratios of manure to mineral N fertilizer were used: all manure (100% of plant N from manure), mixed (50% plant N from by manure and 50% from mineral fertilizer), and all mineral (100% plant N from mineral fertilizer). Yields of corn and wheat were similar across treatments. Soil microbial community dissimilarity increased with higher ratios of application, suggesting a dose-dependent relationship between manure application and microbial community composition. Both microbial biomass and dissolved organic carbon showed the greatest response immediately following manure application, then decreased until the next application. The Shannon index increased shortly after manure application, with the largest increase observed in soils receiving manure input. Many of the taxa that increased in relative abundance following manure application were specifically taxa known to be present in manure and not common or present in soil before manure application. Microbial community changes following manure application were transitory, indicating that without continual inputs of resources, these alterations are not maintained. This was in contrast to microbial biomass which slowly increased in size over the 2-year study period. Better understanding of how manure interacts with microbial communities will help in developing better nutrient management practices and help reduce our reliance on mineral fertilizers.

1. Introduction

Manure application can provide nutrients to crops while simultaneously addressing a waste disposal problem by recycling nutrients into the production of forage crop and reducing reliance on chemical fertilizers. The inputs of carbon and other nutrients from manure application greatly impact the underlying soil microbial community (Nicol et al.,

2016) resulting in an increase in soil microbial biomass and diversity (Caban et al., 2018). In one study, after 30 years of using only mineral fertilizers, adding animal manure slurry as a soil amendment resulted in increased bacterial community diversity and microbial biomass compared to the mineral fertilizer control (van der Bom et al., 2018). Similar findings were described in a meta-analysis of 103 peer-reviewed publications where microbial biomass was found to be higher in

* Corresponding author at: Department of Land, Air, and Water Resources, University of California Davis, Davis, CA 95616, USA.

E-mail addresses: jmsayre@ucdavis.edu (J.M. Sayre), dyuwang@ucdavis.edu (D. Wang), Johlin@ucdavis.edu (J.Y. Lin), redanielson@ucdavis.edu (R.E. Danielson), kmscow@ucdavis.edu (K.M. Scow), jmrodrigues@ucdavis.edu (J.L. Mazza Rodrigues).

¹ 0000-0001-6358-8671.

² 0000-0002-1683-0530.

³ 0000-0003-4977-2506.

⁴ 0000-0001-7044-0747.

⁵ 0000-0002-2649-1122.

⁶ 0000-0002-6446-6462.

manure-applied systems relative to those receiving only chemical fertilizer. Furthermore, the longer the practice occurred, the larger were the increases in microbial biomass (Ren et al., 2019).

Soil microbial communities are drivers of many processes involved in carbon cycling, both decomposition and organic matter formation. Organic inputs such as manure provide readily available carbon, in addition to mineral nutrients, that can promote the maintenance and sometimes growth of soil microbial populations (Diacono and Montemurro, 2011). To study this, assessments of dissolved organic carbon (DOC) can be used to determine how pools of microbially accessible carbon change following organic matter input (Mentges et al., 2019). Determining the extent organic carbon pools are related to microbial diversity could provide insight into the link between organic matter application and microbially mediated decomposition. A reduction in microbial diversity can reduce the decomposition of organic carbon, suggesting a link between changes in microbial diversity to changes in the soil carbon cycle (Maron et al., 2018). Models of soil carbon dynamics indicate that soil organic matter is created by inputs of carbon compounds, their metabolism by soil microbial communities, and physical protection of these metabolic by-products (Grandy and Neff, 2008). The abundance of copiotrophs and specific organotrophs in manure may stimulate the ability of the soil to metabolize a wide variety of carbon compounds and potentially increase soil organic carbon (Toju et al., 2018; Wang et al., 2021). Manure amendment was shown to induce higher activity of enzymes associated with carbon metabolism compared to soils with no manure applied (Chowdhury et al., 2021). In conclusion, the stimulation of microbial communities brought about by increases in readily available organic material, in turn, appears to help facilitate the formation of soil organic matter (Cotrufo et al., 2015).

Long-term applications of solely mineral fertilizers are often associated with decreases in bacterial biomass and diversity (Könninger et al., 2021), most likely due to absence of organic inputs. A primary attention on plant productivity in crop production systems has led to a focus on just mineral nutrients and neglects the needs of soil biological communities for carbon and energy sources (Sun et al., 2015). Even in systems receiving manure as a source of fertilizer, management practices are usually focused entirely on crops and not on soil communities and thus may not be as beneficial as they could. In a recent review of published papers and legislation related to manure management in the European Union, it was found that manure management practices often neglect the potential role of manure application on soil microbial biomass and biodiversity. This is notable because nutrients in manure feed soil microorganisms, promoting greater soil biodiversity which can detoxify the soil, increase carbon stabilization and improve soil nitrogen retention. As such, positive ecosystem characteristics and the soil microbial community are linked and should therefore be considered holistically (Könninger et al., 2021). Other potential benefits to soil that could result from optimally managed manure amendments include increased moisture retention, soil aggregation, biodegradation capacity, and nutrient cycling (Martínez-García et al., 2018; Reganold and Wachter, 2016; Wang et al., 2020; Könninger et al., 2021), all of which are mediated to some degree by microbial activity (Cobo et al., 2002).

Amendment of soil with manure is potentially a source of microorganisms from the manure itself and it may therefore modify the composition of the recipient soil microbial community (Chen et al., 2017). Concurrently, the addition of manure also has the potential to stimulate resident soil microorganisms by the addition of carbon and other nutrients (Mohammadi et al., 2011). Recently, a meta-analysis of 37 studies investigating the effects of organic vs mineral fertilizers on soil microbial diversity found that functional as well as taxonomic diversity is higher in organic systems using manure (Bebber and Richards, 2022). High amounts of manure may lead to increases in pathogenic bacteria concentrations (Hruby et al., 2016) and those carrying antimicrobial resistance genes (He et al., 2019). Thus, it is crucial to identify the amounts and timing of manure inputs that support positive outcomes and reduce the risk of negative impacts.

The objectives of our study were to: 1) determine the relationship between manure application ratio and DOC, microbial biomass, microbial community composition, and crop yields; 2) identify manure-associated microbial taxa that change in abundance in soil following manure application; and 3) describe interactions between soil microbial communities and physical-chemical properties as related to manure inputs. Our field study was conducted in a conventionally managed corn-wheat forage rotation where samples were collected and analyzed 22 times over a two-year period.

2. Material and methods

2.1. Experiment design and soil sampling

In August 2017, an experimental field was established at the University of California Davis Russell Ranch (Davis, CA) as corn-wheat rotation on a field previously used as fallow. This field was divided into twelve treatment plots (4.57 m by 36.58 m) with each treatment plot being comprised of three beds (1.52 m by 36.58 m). In total, three treatments of varying manure to mineral fertilizer application ratios were used with four replicates per treatment type, resulting in twelve total treatment plots. Treatments were randomly distributed within blocks in a randomized block design (Supplemental Fig. 1). Plots received manure (2.24 kg/m², n = 4), mixed (1.12 kg/m², n = 4), or mineral (n = 4) ratios of solid dairy manure amendment at the start of each growing season for a total of four growing seasons, where it was applied to the topsoil before being mixed in during bed construction. Application ratios were determined based off assumed N availability to meet crop nutrient demands and crop yields were measured after each season to determine if ratios were sufficient in maintaining growth and productivity. One treatment received 100% of its N from manure inputs (manure), a second treatment received 50% of their N from manure and 50% N from chemical urea fertilizer (mixed), and a third treatment consisted of 100% N with chemical urea fertilizer inputs (mineral) to meet crop demands.

The crop rotation consisted of corn in summer (SC1), wheat in winter (WW1), corn in summer again (SC2) and wheat winter again (WW2). Corral scraped manure was collected from a local dairy farm located approximately 20 km from the field site and transported to the experimental location by truck. Management on this farm followed conventional practices used in dairy farming. However, the nutrient content of dairy manure can vary even within the same farm. For instance, the total nitrogen content of manure can range from 1.65% wt. to 2.49% wt. (60-degree dried manure), but the manure used in this experiment typically had a high nitrogen content. The cattle on this farm were treated with antibiotics, including neomycin sulfate, chlortetracycline, oxytetracycline, and sulfamethazine. These are the most common antimicrobial drugs used in California dairy farms, as reported in a 2017 survey of Grade A milk-producing dairies and calf ranches (Okello et al., 2021). To ensure that all treatments received the targeted amount of nitrogen, a representative manure sample was taken before application. The total nitrogen content, mineral nitrogen contents, and manure moisture content were analyzed to determine the amount of manure to be applied in each growing season.

Soil samples were collected using twist augers from the center bed of each treatment plot (7 cm diameter by 15 cm depth) on an approximate bi-monthly basis, with sampling dates designed to align with agricultural milestones such as tillage, planting, irrigation events, harvesting, and fertilizer application. In total, 22 sampling dates were recorded spanning the range of 665 days. For each sampling event, 10 soil cores per treatment plot were collected from bulk soil in the center bed at randomly dispersed intervals 15 m away from the edge of the field. Soil collected from each treatment plot was mixed in the field before transport back to the laboratory on ice, with a portion subsampled from each mixture and stored at - 80 °C to be used for DNA extraction and molecular analysis. In total, 12 representative samples with 4

corresponding to each ratio of manure application (manure, mixed, mineral) were generated from each sampling event, corresponding to each of the 12 treatment plots established at the start of the field trial. In addition, manure was collected from the manure spreader as it was applied to the field then returned to the laboratory and stored at the same conditions as soils samples.

2.2. DNA extraction, PCR amplification and sequence analysis

Total DNA was extracted from 0.25 g of soils and 0.3 g of manure using the DNeasy PowerLyzer PowerSoil kit (Qiagen Inc., Germantown, MD) following the manufacturer's protocol. Gel electrophoresis was used to assess quality of DNA after each extraction, to ensure minimal degradation. Yields were assessed with a Qubit 3 fluorometer (ThermoFisher, Waltham, MA) and extractions producing > 15.0 ng/ μ L DNA were used to construct 16 S rRNA gene libraries.

Libraries were prepared using a standard protocol using the 16 S rRNA gene primer pair 515-F (GTGCCAGCMGCCGCGGTAA) and 806-R (GGACTACHVGGGTWTCTAAT) targeting the V4 hypervariable region (Caporaso et al., 2011). PCR was performed in duplicate using Phusion Hot Start II high-Fidelity PCR Master Mix (Thermo Scientific Inc., Waltham, MA). Reactions were conducted using a modified version of the manufacturers protocol, with 1 μ L DNA template (15 ng/ μ L), 1 μ L of each primer (10 pMol), 10 μ L Master Mix, and 7 μ L water to reach a final volume of 20 μ L/reaction. Negative controls were used in each batch of PCR substituting 1 μ L DNA template with 1 μ L water and a unique reverse barcode to remove any possible contaminating DNA following sequencing analysis. All reactions were conducted using the C1000 Touch Thermocycler from Bio-Rad Laboratories, Inc. (Hercules, CA). PCR cycles included a 30 s initial denaturation at 98 °C, followed by 27 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, extension at 72 °C for 15 s, and a 7 min final extension at 72 °C before being held at 4 °C. Following PCR, a 3 μ L aliquot of each reaction was assessed on an agarose gel to ensure specific and successful amplification. Duplicate reactions were mixed and assessed for concentration using the Qubit 3 fluorometer (Thermo Fisher Scientific Inc., Waltham, MA). Next, 100 ng of each successful reaction was pooled and purified using the QIAGEN's QIAquick PCR Purification Kit (Qiagen, Inc., Germantown, MD) according to the manufacturer's protocol. Completed libraries were sequenced on the Illumina MiSeq PE250 system at the UC Davis DNA Technologies Core. Here, 11 million (28% PhiX) sequencing reads were generated with an overall Q30 > 90.1%. The Dada2 platform (version 1.18) was used in the R environment (www.r-project.org) to process demultiplexed reads using the conventional pipeline recently described (Parsons et al., 2020). Dada2 uses error modeling to correct sequencing errors and group sequences into exact sequence variants (ESVs) that were used in conjunction with the Silva database (version 128) to determine phylotype counts (Callahan et al., 2016). After processing on the Dada2 pipeline, ESVs corresponding to mitochondrial and chloroplast taxonomy were removed and samples were rarefied to 12,000 reads per sample.

2.3. Soil physicochemical analysis

Fresh soils collected from the field trial were also used to evaluate the impacts of manure application on DOC and microbial biomass-C (MBC). A representative bulk soil sample (8 g) was mixed with 40 mL 0.5 mol L⁻¹ K₂SO₄ in polypropylene tubes and centrifuged at a force of 7969g for 15 min to remove suspended solids. Supernatant solutions were retained for DOC concentrations (mg L⁻¹). The MBC was measured by chloroform fumigation (Joergensen, 1996; Yang et al., 2016). Both DOC and MBC were measured using a Total Organic Carbon analyzer (Shimadzu TOC-VCSH analyzer, Kyoto, KYT, Japan). Reactive carbon was assessed as permanganate oxidizable carbon (POXC) was measured as outlined elsewhere (Culman et al., 2012). In brief, 2 g of soils were weighed into 50 mL polypropylene tubes prior to oxidation. The

reaction was initiated by adding 18 mL of deionized water and 2 mL of 0.2 mol L⁻¹ KMnO₄ (final reaction concentration = 0.02 mol L⁻¹ MnO₄⁻) to each tube containing pre-weighed soil, followed by mixing for exactly 2 min on a reciprocal shaker and then allowed samples to settle for exactly 10 min. The reaction was terminated by transferring 0.5 mL of supernatant into a fresh 50 mL tube with 49.5 mL of deionized water, which was inverted to mix thoroughly, resulting in a homogenized 1:100 dilution. To minimize variability in reaction termination time, the five analytical replicates were run in sequence. The 1:100 dilution was transferred to microcuvettes and analyzed by UV-Vis spectrophotometry utilizing the Genesys™ spectrophotometer to quantify MnO₄⁻ remaining in solution by absorbance at 550 nm. A separate 8 g of fresh soil was used to determine NO₃⁻ and NH₄⁺ concentrations via standard spectrophotometric methods (Doane and Horwath, 2003; Verdouin et al., 1978). Potentially mineralizable nitrogen (PMN) was measured using an anaerobic incubation method with extraction by KCl (Griffin et al., 2017; Waring and Bremner, 1964). Electrical conductivity (EC) was measured according to Sudduth et al. (2005), specific ultraviolet absorbance (SUVA) was measured using UV absorbance to determine the aromaticity of DOC (Weishaar et al., 2003).

2.4. Plant analysis

Corn and wheat plant samples were dried at 60 °C until constant weight after harvest, then weighed to determine relative yields between treatment plots and then ground. The processed plant samples were sent to SoilTest Farm Consultants (Moses Lake, WA) for quantification of nutrient contents (Marten et al., 1989).

2.5. Statistical analysis

All statistical analysis was performed using the R environment (version 4.1.2). The Shannon index diversity was determined using the vegan package (version 2.5–7) (vegan.r-forge.r-project.org), and community patterns were assessed by using the Bray-Curtis dissimilarity distance, represented by use of non-metric multidimensional scaling (NMDS). To determine the dates where changes to microbial diversity, DOC and MBC between manure, mixed and mineral were significant, unpaired t-tests were performed on manure and mineral, mixed and mineral and manure and mixed at each time point. Genera found to be significantly increased or decreased in abundance within the manure vs. mineral plots throughout the field trial were determined by Wilcoxon test. In each season (SC1, WW1, SC2, WW2) the timepoints with the most and least genera significantly changed in abundance were used to determine what percentage of those genera were also found in the source manure used throughout the field trial. The role of different ratios of manure application on the relationships between soil microbial diversity, taxonomy, and their relationship to soil chemical and physical characteristics over time was assessed using Spearman rank-order correlations. Finally, a two-way ANOVA with block as a random factor was used to assess changes in plant yield by comparing yields between treatment plots with 4 replicates per treatment (manure, mixed, mineral).

3. Results

3.1. Higher ratios of manure application resulted in increased differences in microbial communities

Microbial similarity patterns varied greatly between manure and mineral application treatments (PERMANOVA, $df = 2$, $r^2 = 0.05$, $p < 0.001$). Mixed and manure treatment plots had increasing amounts of dissimilarity with microbial communities in manure treatment plots being more dissimilar. Conversely, plots that received mineral fertilizer had microbial community compositions that resembled one another throughout this experiment (Fig. 1).

665 (MEAN = 112.89 mg kg⁻¹, SD = 23.32 mg kg⁻¹), the end of the field trial ($t = -4.55$, $df = 5.99$, $p = 0.003$). The differences between mixed ratios of manure application and mineral application were less pronounced and notably, non-significant at the end of the field trial. Nevertheless, similar responses to manure application were observed on day 17 where mixed ratios (MEAN = 89.43 mg kg⁻¹, SD = 14.19 mg kg⁻¹) had a significantly higher MBC than mineral plots (MEAN = 56.23 mg kg⁻¹, SD = 6.79 mg kg⁻¹) ($t = 4.22$, $df = 4.31$, $p = 0.01$). The difference between mixed (MEAN = 113.95 mg kg⁻¹, SD = 15.18 mg kg⁻¹) and mineral (MEAN = 69.74 mg kg⁻¹, SD = 5.61 mg kg⁻¹) plots was highest on day 343 ($t = 5.46$, $df = 3.81$, $p = 0.006$) although the difference between mixed and mineral plots never exceeded the difference between manure and mineral. In manure plots, MBC was significantly higher (MEAN = 109.61 mg kg⁻¹, SD = 11.95 mg kg⁻¹) than mixed (MEAN = 82.25 mg kg⁻¹, SD = 14.41 mg kg⁻¹) ($t = 2.92$, $df = 5.81$, $p = 0.03$) starting on day 120. A significant difference between manure and mixed plots remained for most of the experiment culminating at the conclusion of the field trial on day 665 where manure plots maintained significantly higher MBC (MEAN = 112.89 mg kg⁻¹, SD = 23.32 mg kg⁻¹) than mixed (MEAN = 65.22 mg kg⁻¹, SD = 7.07 mg kg⁻¹) ($t = 3.92$, $df = 3.55$, $p = 0.02$) (Fig. 2C).

3.3. Microbial genera that increased in abundance following manure application were genera present in manure

The most abundant genera found in manure differed from those found in soil (a breakdown of the most represented manure microorganisms can be found in Supplemental Fig. 3). In manure-applied plots, genera found in the source manure showed the most significantly increases throughout the study. On day 1 following manure application, 27 genera significantly increased in abundance in plots receiving manure compared to mineral ($p < 0.05$). This trend was maintained over the first summer corn growing season. In day 50, the date with the lowest number of genera ($n = 11$), 64% of those genera that showed significant increases were also found in the source manure. In the following wheat season on day 120, of the 16 genera that increased in abundance in manure, but not mineral, 75% were found in the source manure. By day 280, only 2 of the original 16 genera could still be detected, of which only 1 had been detected in the source manure. Early in the second corn growing season, 31 genera increased in abundance in manure vs. mineral plots of which 39% were also found in the source manure. Later in the season, 57% of the genera that had significantly increased in abundance in manure-applied plots were found in the source manure. At day 469 (final wheat season) and at the end of the experiment, 100% and 49%, respectively, of the genera that increased in

abundance in the manure plots had also been detected in the source manure.

Conversely, genera that were not detected in the manure appeared to significantly decrease in abundance in manure compared to mineral plots. During the initial corn growing season, day 1 represented the date with the most genera were significantly impacted by manure application, on this date 22 genera were found to be in significantly lower abundance in plots receiving manure vs. those that received mineral. On day 50, 60% of the genera decreased in abundance were found in manure, but in total only 5 genera were significantly decreased in abundance at this date, whereas 11 were found to be in increased abundance. In the first winter wheat growing season a majority (78%) of the genera found to be in decreased abundance were those not found in the source manure. On day 280, no genera were found to be in significantly lower abundance in plots receiving manure vs. those not receiving manure. Throughout the second year of the field trial, a majority of genera that decreased in abundance were not found in the source manure. On days 323, 431, 469 and 665, genera not found in manure constituted 92%, 100%, 84%, and 100% of the genera that decreased in abundance in plots receiving manure vs. mineral (Fig. 3). Furthermore, these trends are broadly reflected when considering the totality of this field trial. When manure application is examined alone without taking into consideration unique sampling events, 140 genera increased in abundance in manure vs. mineral plots with a majority (58.6%) of those genera being found to be in manure. Conversely, 51 genera had lower abundance in manure vs. mineral plots and a majority (84.4%) of those genera are absent from the manure microbial community (Supplemental Fig. 4).

3.4. Strength of associations between soil physicochemical and biological characteristics increased in manure-applied soils

Positive correlations between soil physicochemical and biological factors increased with the ratio of manure application (Fig. 4). In manure plots, there was no significant relationship between MBC and microbial diversity. There was, however, a relationship between those factors in mixed ($r_s = 0.27$) and manure ($r_s = 0.33$). Furthermore, there was no relationship between DOC and *Bacillus* in mineral manure plots, but in mixed ($r_s = 0.35$) and manure ($r_s = 0.42$) manure plots, the relationship was increasingly pronounced with higher ratios of manure application. Not all the relationships between microbial genera and soil C were positive in manure-applied soils. In manure plots, *Sphingomonas* ($r_s = -0.16$), *Psychroglaciecola* ($r_s = -0.21$), *Gemmatimonas* ($r_s = -0.22$), *Ramlibacter* ($r_s = -0.27$), and *Mycobacterium* ($r_s = -0.39$) were all negatively associated with DOC. The negative association between genera and DOC was only reflected in *Ramlibacter* ($r_s = -0.25$) in

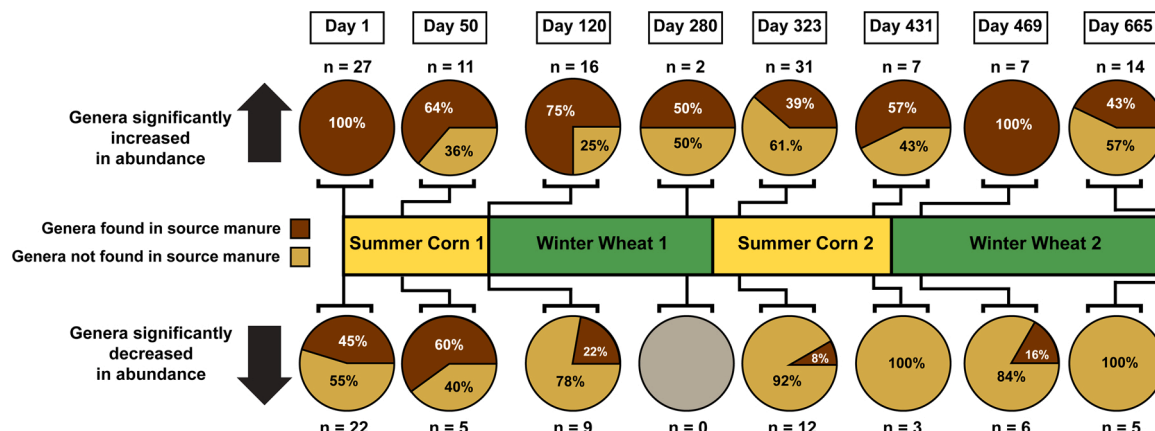


Fig. 3. Microbial genera found to be significantly different between manure and mineral treatments. The percentage of genera that increased in manure compared to mineral fertilizer soils are on top and those that decreased in manure compared to mineral fertilizer soils are on the bottom. Differences between the treatments are shown in each growing season (summer corn 1, winter wheat 1, summer corn 2, winter wheat 2). Genera are either detected in source manure (brown), or not (tan).

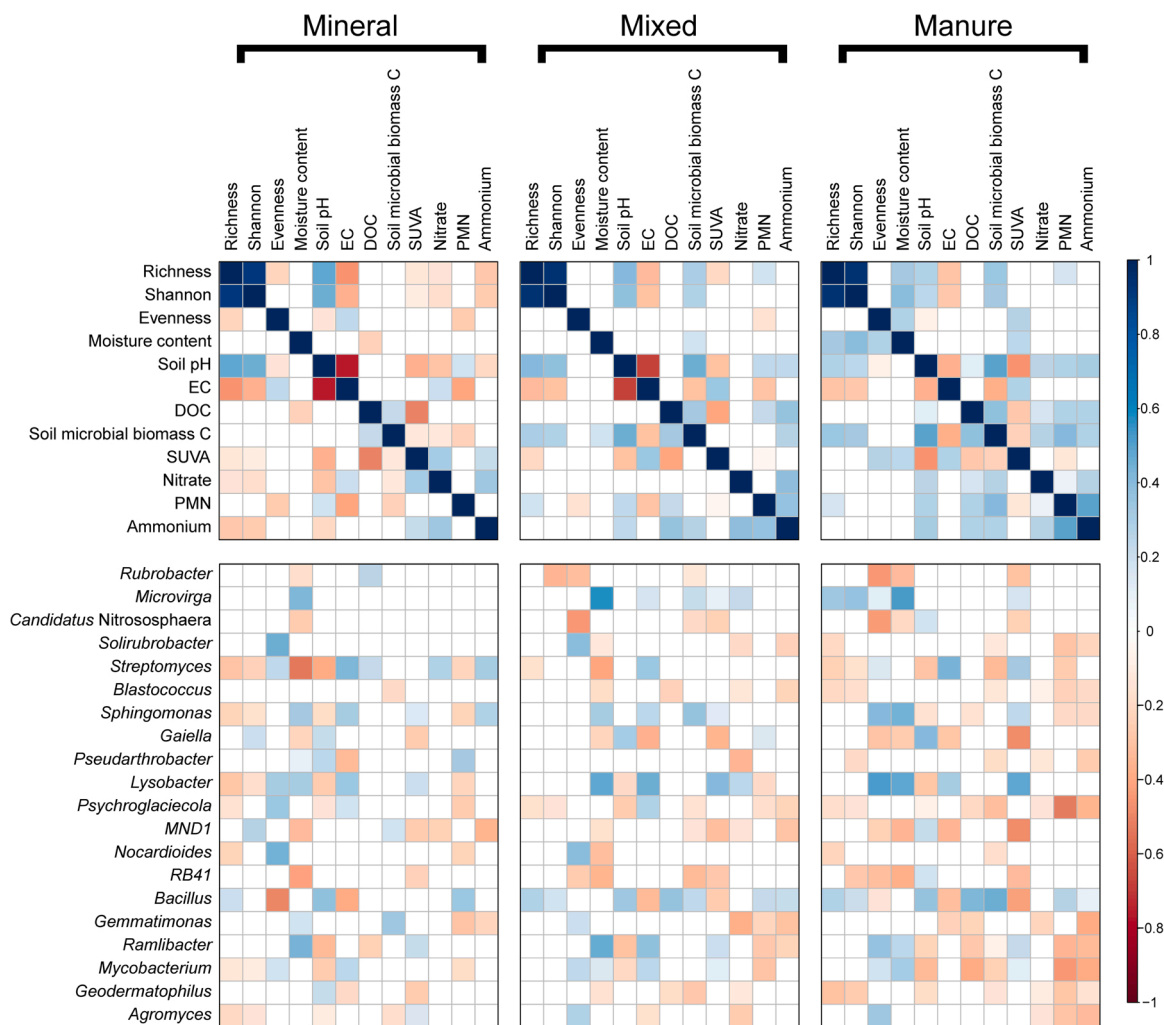


Fig. 4. Spearman's correlations between physicochemical and biological characteristics in soils treated with mineral, mixed and manure treatments. Stronger positive correlations are indicated by increasing blue color intensity, negative correlations are indicated by increasing red color intensity and non-significant correlations are left blank. Richness, Shannon diversity, microbial evenness, soil moisture content, soil pH, electrical conductivity (EC), dissolved organic carbon (DOC), soil microbial biomass, specific ultraviolet absorbance (SUVA), potentially mineralizable nitrogen (PMN), and ammonium are grouped together on top and the top 20 most abundant genera are grouped on the bottom in order of decreasing abundance.

mineral plots. *Rubrobacter* ($r_s = -0.44$) and *Nitrososphaera* ($r_s = -0.43$), were negatively correlated under manure application. These trends in mineral plots were absent.

3.5. Plant yields did not differ between fertilizer and manure amended plots

Silage corn yield did not vary significantly between treatments in either growing season. In Season 1 it was $3.80 \pm 0.25 \text{ MG ha}^{-1}$ in mineral, $4.03 \pm 0.27 \text{ MG ha}^{-1}$ in low, and $3.87 \pm 0.20 \text{ MG ha}^{-1}$ in manure plots. In season 3 it was $2.77 \pm 0.18 \text{ MG ha}^{-1}$ in mineral, $2.78 \pm 0.13 \text{ MG ha}^{-1}$, in low, and $2.68 \pm 0.13 \text{ MG ha}^{-1}$ in manure plots. Similarly, wheat yields did not significantly vary between treatments with season 2 silage wheat yield being $2.68 \pm 0.60 \text{ MG ha}^{-1}$ in mineral, $2.51 \pm 0.33 \text{ MG ha}^{-1}$ in low, and $2.35 \pm 0.29 \text{ MG ha}^{-1}$ in manure plots. In season 4 silage wheat yield was $2.18 \pm 0.13 \text{ MG ha}^{-1}$ in mineral, $2.15 \pm 0.23 \text{ MG ha}^{-1}$ in low, and $1.94 \pm 0.06 \text{ MG ha}^{-1}$ in mineral, mixed and manure plots respectively. Therefore, partially, or completely replacing mineral nitrogen fertilizer with dairy manure maintained silage corn and wheat yields for all four growing seasons (Table 1).

Table 1

Silage corn and wheat yields for each growth season (dry weight) with mineral, mixed and manure treatments. The numbers to the right of each value represent the standard error of the mean. Significant differences based on Tukey test between all four treatments are indicated by different letters in parentheses to the right of each value.

Treatment	Season 1 silage corn yield Mg ha^{-1}	Season 2 silage wheat yield	Season 3 silage corn yield	Season 2 silage wheat yield
Mineral	$3.80 \pm 0.25 \text{ a}$	$2.68 \pm 0.60 \text{ a}$	$2.77 \pm 0.18 \text{ a}$	$2.18 \pm 0.13 \text{ a}$
Mixed	$4.03 \pm 0.27 \text{ a}$	$2.51 \pm 0.33 \text{ a}$	$2.78 \pm 0.13 \text{ a}$	$2.15 \pm 0.23 \text{ a}$
Manure	$3.87 \pm 0.20 \text{ a}$	$2.35 \pm 0.29 \text{ a}$	$2.68 \pm 0.13 \text{ a}$	$1.94 \pm 0.06 \text{ a}$

4. Discussion

Dairy farms are often associated with nearby forage crop soils which receive the vast quantities of manure produced by cows. Nutrients from the manure can feed the crops, which in turn can feed cows, improving the nutrient use efficiency of the system. Soil microorganisms are critical as they help convert manure into crop available nutrients. We found that manure increased total soil microbial biomass over our study (Fig. 2).

and likely increased overall microbial activity. With regard to microbial diversity, however, changes with manure were temporary. Specific taxa associated with manure increase immediately following application, yet declined rapidly until the next application (Fig. 3), providing evidence that their presence was temporary in soil. Our findings suggest that nutrients from manure feed the soil microbial community, and regular inputs are necessary to maintain the changes brought about by application.

Manure inputs increased the variability of the microbial community composition and increased microbial diversity with the greatest change happening in plots applied with only manure (Fig. 1). The carbon and other nutrients provided by manure inputs likely created opportunities for new microbial growth; similar findings were reported in a low-organic matter soil (Bastida et al., 2021). Ultimately, fluctuating diversity seen in manure-applied plots drove changes in community composition because microbial diversity increased approximately two weeks after application, but not in mineral fertilizer plots (Fig. 2A). In this instance manure-applied plots arrived at different community compositions, while mineral plots remained similar. In a study of impacts of swine manure inputs on the microbial community in bulk soil, it was found that compositional changes lasted up to 28 days following manure addition and then returned to a stable composition more similar to that observed in untreated soil (Jechalke et al., 2014). Therefore, the delay between manure application and changes in the soil microbial community can contribute to a wider range of fluctuating community compositions in manure-applied soil.

DOC was highly responsive to manure application where even mixed plots had significantly higher DOC when compared to mineral plots and these differences were maintained throughout the experiment (Fig. 2B). Increases in DOC were detected prior to increases in microbial diversity, suggesting that manure provides labile carbon to the soil which feeds the microbial community, contributing to improvements in microbial composition and diversity. In this case, organic matter provides an initially robust amount of labile carbon, which then transitions to a steady source of carbon, because labile pools of carbon can be continuously restored through decomposition of the organic matter in manure (Nelson and Wear, 2014). These increases to DOC through manure application drives changes to soil carbon processing by heterotrophic microorganisms just as it has in marine ecosystems (Hansell, 2013).

Furthermore, MBC incrementally increased over the course of the experiment, with small increases following each round of manure application (Fig. 2C). In a study of the response of soil microbial communities to pig slurry, manure, and biochar in soils with variable soil organic matter (SOM), soil receiving a mixture of organic inputs was more prone to increased microbial biomass and activity (Yanardağ et al., 2017). This taken with our results suggests that changing community composition is more difficult than increasing the overall abundance of soil microorganisms. Furthermore, most of the taxa that did increase in abundance following manure application were also present in manure itself. Most manure-associated microorganisms diminished in abundance over the growing season until the next round of application (Fig. 3). It is therefore likely that few microorganisms from the manure itself are added to the soil following manure application, and those that are added are dependent on the resources in manure to persist. In a 9-year field study of annual manure applications to maize, potato, and white mustard, increases in the abundance of manure-associated genera occurred closely following application; however, they did not survive in the soil after a few months (Semenov et al., 2021). This suggests that frequent applications of manure are necessary to ensure a persistent cohort of manure-associated microorganisms and that increases to diversity may be maintained by fresh applications of manure.

Our study suggests that application of manure increases the relative abundance of taxa that were rare in soil before application. Of the top 20 most represented genera in our experimental plots, only *Lysobacter* significantly increased in abundance for multiple growing seasons, with *Mycobacterium* and *Streptomyces* increasingly temporarily (Supplemental

Fig. 2). Increases to the soil nitrate and PMN in manure-applied plots were seen in tandem with decreased relative abundances of the most represented soil microbial genera (Supplemental Fig. 2). This resulted in more negative correlations between soil nitrate, PMN and ammonium and represented microbial genera in manure plots, a trend not seen in mineral plots except for *MND1* and *Gemmatimonas*. As such, it seems that microorganisms already present in manure, such as *Lysobacter*, can best utilize the nutrient pools of manure, where microorganisms already abundant in soil do not utilize the temporary increases to nutrients that follow application to the same extent. Indeed, increases to MBC were negatively correlated with the relative abundances of the previously most represented genera (Fig. 4).

Microbial invasion from poultry manure-compost, also at Russell Ranch, was compared in soils with a 20-year history of poultry manure compost application vs. no compost additions (Gravuer et al., 2021). The non-composted soil was susceptible to invasion of compost-derived taxa; however, the abundance of the compost-derived taxa dropped substantially by a month after application. Our study found continual inputs of manure over our two-year study were needed to maintain the relative abundance of manure-associated organisms, suggesting that their persistence may also be short-lived unless manure continues to be added. It is likely challenging, for taxa adapted to a manure environment to be competitive and establish themselves in the distinctly different environment of soil (e.g. lower carbon, nutrients), which itself has already colonized by highly adapted soil organisms (Attwood et al., 2019). In a 3 year study of manure application to grasslands, repeated manure applications increased the abundance of antibiotic resistance genes in soil, indicating that manure-induced changes to the microbial community may persist over long periods of time (Shawver et al., 2021). Together, changes to the microbial community brought about by manure likely need to be maintained by continual application.

The increase in microbial diversity and biomass after manure application can be due to the addition of microbial groups from the rumen or manure environment, or from the influx of manure-derived nutrients that provide resources for the existing soil microbial community. The primary contributor is likely the addition of manure nutrients, which feed the resident soil community, as the rumen environment differs significantly from soil. Therefore, rumen microorganisms that persist in soil after manure addition are likely only temporary (Attwood et al., 2019). Although we observe a temporary increase in manure-associated microorganisms after application, many of these microbial groups were also present in the soil before, but in lower abundances. Microbial evenness increased in manure and mixed compared to mineral fertilizer plots, providing additional evidence that less abundant soil microorganisms benefit most from manure application (Supplemental Fig. 2). The frequency of our sampling allowed us to detect the dynamics of rapidly increased DOC after manure application, followed by increases in MBC and diversity. If the direct addition of microorganisms from manure was primarily responsible for increases in diversity after application, we would expect to see an immediate increase in diversity following application, as we do with DOC.

Future studies should aim to disentangle the sources of microorganisms that increase community diversity following manure application. Stable isotope probing (SIP) is a technique used to track the flow of carbon through radiolabeled substrates to specific members of the soil microbial community (Barnett et al., 2021). This approach could provide insight into which taxa incorporate various forms of labile carbon, providing more detailed information on key organisms involved in carbon metabolism. Using the 16 S rRNA gene to determine microbial community taxonomy limited our ability to assess functional changes that may occur in the soil following manure application. Metagenomic assessments of functional gene diversity could help address this limitation in the future (Jansson and Hofmockel, 2018). Finally, our study considered only dry manure from animal corrals. Studies of impacts of other forms such as slurries and liquid manure from lagoons on soil microbial communities are also important and needed.

In conclusion, we found that continuous applications of manure modify soil microbial community composition and higher manure inputs were associated with greater changes. Microbial diversity showed short-term increases immediately following manure application and then dropped down to pre-input levels. In contrast, MBC incrementally increased with each manure application with the highest biomass being reached in the last season of the experiment. Many of the taxa that increased immediately after manure application were taxa found in manure and, for the most part, not important members of the soil microbial community before manure addition. Our findings highlight the necessity of continual applications of manure to maintain changes in the soil microbial community and can help guide waste management practices to create both more efficient waste streams and healthier soils.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank Israel Herrera, Nicole E Tautges and staff for logistical support in managing the simulated forage farm at Russell Ranch. We thank Julio Cezar Fornazier, Albert Barberan, Fernanda Mancini Nakamura, Eloi Parladé, Maria do Carmo Catanho, Daniel Rath, Caio Augusto Yoshiura, Carlotta Eliza Sainato and Sung Won for assistance in sampling and processing throughout the field experiment; and Jorge Rodriguez for insight into common practices employing the use of manure as fertilizer.

This research was supported by the US Department of Agriculture – National Institute of Food and Agriculture (2016-67003-24991). JMS also recognizes support from the UC Davis Henry A. Jastro Research Award.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2023.108567](https://doi.org/10.1016/j.agee.2023.108567).

References

- Attwood, G.T., Wakelin, S.A., Leahy, S.C., Rowe, S., Clarke, S., Chapman, D.F., Muirhead, R., Jacobs, J.M.E., 2019. Applications of the soil, plant and rumen microbiomes in pastoral agriculture. *Front. Nutr.* 6, 107. <https://doi.org/10.3389/FNUT.2019.00107/BIBTEX>.
- Barnett, S.E., Youngblut, N.D., Koechli, C.N., Buckley, D.H., 2021. Multisubstrate DNA stable isotope probing reveals guild structure of bacteria that mediate soil carbon cycling. *e2115292118 Proc. Natl. Acad. Sci. USA* 118. <https://doi.org/10.1073/PNAS.2115292118>.
- Bastida, F., Eldridge, D.J., García, C., Kenny Png, G., Bardgett, R.D., Delgado-Baquerizo, M., 2021. Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. *ISME J.* 15 (7), 2081–2091. <https://doi.org/10.1038/s41396-021-00906-0>.
- Bebber, D.P., Richards, V.R., 2022. A meta-analysis of the effect of organic and mineral fertilizers on soil microbial diversity. *Appl. Soil Ecol.* 175, 104450 <https://doi.org/10.1016/j.apsoil.2022.104450>.
- Caban, J.R., Kuppusamy, S., Kim, J.H., Yoon, Y.E., Kim, S.Y., Lee, Y.B., 2018. Green manure amendment enhances microbial activity and diversity in antibiotic-contaminated soil. *Appl. Soil Ecol.* 129, 72–76. <https://doi.org/10.1016/j.apsoil.2018.04.013>.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13 (7), 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA Suppl.* 108, S4516–S4522. <https://doi.org/10.1073/pnas.1000080107>.

- Chen, Q.L., An, X.L., Li, H., Zhu, Y.G., Su, J.Q., Cui, L., 2017. Do manure-borne or indigenous soil microorganisms influence the spread of antibiotic resistance genes in manured soil. *Soil Biol. Biochem.* 114, 229–237. <https://doi.org/10.1016/J.SOILBIO.2017.07.022>.
- Chowdhury, S., Bolan, N., Farrell, M., Sarkar, B., Sarker, J.R., Kirkham, M.B., Hossain, M. Z., Kim, G.H., 2021. Role of cultural and nutrient management practices in carbon sequestration in agricultural soil. *Adv. Agron.* 166, 131–196. <https://doi.org/10.1016/BS.AGRON.2020.10.001>.
- Cobo, J.G., Barrios, E., Kass, D.C.L., Thomas, R.J., 2002. Decomposition and nutrient release by green manures in a tropical hillside agroecosystem. *Plant Soil* 240, 331–342.
- Cotrufo, M., Francesca, Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D. H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat. Geosci.* 8, 776–779. <https://doi.org/10.1038/ngeo2520>.
- Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirsky, S.B., Spargo, J.T., Wander, M.M., 2012. Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. *Soil Sci. Soc. Am. J.* 76, 494–504. <https://doi.org/10.2136/SSAJ2011.0286>.
- Diacono, M., Montemurro, F., 2011. Long-term effects of organic amendments on soil fertility. *Sustain. Agric.* 2, 761–786. https://doi.org/10.1007/978-94-007-0394-0_34.
- Doane, T.A., Horwath, W.R., 2003. Spectrophotometric determination of nitrate with a single reagent. *Anal. Lett.* 36, 2713–2722. <https://doi.org/10.1081/AL-120024647>.
- Grandy, A.S., Neff, J.C., 2008. Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. *Sci. Total Environ.* 404, 297–307. <https://doi.org/10.1016/J.SCITOTENV.2007.11.013>.
- Gravuer, K., Scow, K.M., 2021. Invader-resident relatedness and soil management history shape patterns of invasion of compost microbial populations into agricultural soils. *Appl. Soil Ecol.* 158. <https://doi.org/10.1016/J.APSOIL.2020.103795>.
- Griffin, D.E., Wang, D., Parikh, S.J., Scow, K.M., 2017. Short-lived effects of walnut shell biochar on soils and crop yields in a long-term field experiment. *Agric. Ecosyst. Environ.* 236, 21–29. <https://doi.org/10.1016/j.agee.2016.11.002>.
- Hansell, D.A., 2013. Recalcitrant dissolved organic carbon fractions. *Annu. Rev. Mar. Sci.* 5, 421–445. <https://doi.org/10.1146/ANNUREV-MARINE-120710-100757>.
- He, L.Y., He, L.K., Liu, Y.S., Zhang, M., Zhao, J.L., Zhang, Q.Q., Ying, G.G., 2019. Microbial diversity and antibiotic resistance in swine farm environments. *Sci. Total Environ.* 685, 197–207. <https://doi.org/10.1016/J.SCITOTENV.2019.05.369>.
- Hruby, C.E., Soupir, M.L., Moorman, T.B., Shelley, M., Kanwar, R.S., 2016. Effects of tillage and poultry manure application rates on Salmonella and fecal indicator bacteria concentrations in tiles draining Des Moines Lobe soils. *J. Environ. Manag.* 17, 60–69. <https://doi.org/10.1016/J.JENVMAN.2016.01.040>.
- Jansson, J.K., Hofmockel, K.S., 2018. The soil microbiome—from metagenomics to metaproteomics. *Curr. Opin. Microbiol.* 43, 162–168. <https://doi.org/10.1016/j.mib.2018.01.013>.
- Jechalke, S., Focks, A., Rosendahl, I., Groeneweg, J., Siemens, J., Heuer, H., Smalla, K., 2014. Structural and functional response of the soil bacterial community to application of manure from difloxacin-treated pigs. *FEMS Microbiol. Ecol.* 87, 78–88. <https://doi.org/10.1111/1574-6941.12191>.
- Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the kEC value. *Soil Biol. Biochem.* 28, 25–31. [https://doi.org/10.1016/0038-0717\(95\)00102-6](https://doi.org/10.1016/0038-0717(95)00102-6).
- Königer, J., Lugato, E., Panagos, P., Kochupillai, M., Orgiazzi, A., Briones, M., 2021. Manure management and soil biodiversity: towards more sustainable food systems in the EU. *Agric. Syst.* 194, 103251. <https://doi.org/10.1016/j.agsy.2021.103251>.
- Maron, P.A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., Karimi, B., Bernard, L., Dequiedt, S., Terrat, S., Chabbi, A., Ranjard, L., 2018. High microbial diversity promotes soil ecosystem functioning. *Appl. Environ. Microbiol.* 84. https://doi.org/10.1128/AEM.02738-17/SUPPL_FILE/ZAM009188469S1.PDF.
- Marten, G.C., Shenk, J.S., Barton, F.E., 1989. Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality.
- Martínez-García, L.B., Korthals, G., Brussaard, L., Jørgensen, H.B., de Deyn, G.B., 2018. Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. *Agric. Ecosyst. Environ.* 263, 7–17. <https://doi.org/10.1016/j.agee.2018.04.018>.
- Mentges, A., Feenders, C., Deutsch, C., Blasius, B., Dittmar, T., 2019. Long-term stability of marine dissolved organic carbon emerges from a neutral network of compounds and microbes. *Sci. Rep.* 9 (1), 1–13. <https://doi.org/10.1038/s41598-019-54290-z>.
- Mohammadi, K., Heidari, G., Khalesro, S., Sohrabi, Y., 2011. Soil management, microorganisms and organic matter interactions: a review. *Afr. J. Biotechnol.* 10, 19840–19849. <https://doi.org/10.5897/AJBX11.006>.
- Nelson, C.E., Wear, E.K., 2014. Microbial diversity and the lability of dissolved organic carbon. *Proc. Natl. Acad. Sci. USA* 111, 7166. <https://doi.org/10.1073/PNAS.1405751111>.
- Nicol, G.W., Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., Reitz, T., 2016. Mineral vs. organic amendments: microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. *Front. Microbiol.* <https://doi.org/10.3389/fmicb.2016.01446>.
- Okello, E., Williams, D.R., ElAshmawy, W.R., Adams, J., Pereira, R.V., Lehenbauer, T.W., Aly, S.S., 2021. Survey on antimicrobial drug use practices in California preweaned dairy calves. *Front. Vet. Sci.* 8. <https://doi.org/10.3389/fvets.2021.636670>.
- Parsons, L.S., Sayre, J., Ender, C., Rodrigues, J.L.M., Barberán, A., 2020. Soil microbial communities in restored and unrestored coastal dune ecosystems in California. *Restor. Ecol.* 28, S311–S321. <https://doi.org/10.1111/rec.13101>.

- Reganold, J.P., Wachter, J.M., 2016. Organic agriculture in the twenty-first century. *Nat. Plants*. <https://doi.org/10.1038/NPLANTS.2015.221>.
- Ren, F., Sun, N., Xu, Meng, Zhang, X., Wu, L., Xu, Minggang, 2019. Changes in soil microbial biomass with manure application in cropping systems: a meta-analysis. *Soil Tillage Res.* 194, 104291 <https://doi.org/10.1016/J.STILL.2019.06.008>.
- Semenov, M. v, Krasnov, G.S., Semenov, V.M., Ksenofontova, N., Zinyakova, N.B., van Bruggen, A.H.C., 2021. Does fresh farmyard manure introduce surviving microbes into soil or activate soil-borne microbiota. *J. Environ. Manag.* 294, 113018 <https://doi.org/10.1016/J.JENVMAN.2021.113018>.
- Shawver, S., Wepking, C., Ishii, S., Strickland, M.S., Badgley, B.D., 2021. Application of manure from cattle administered antibiotics has sustained multi-year impacts on soil resistome and microbial community structure. *Soil Biol. Biochem.* 157, 108252 <https://doi.org/10.1016/J.SOILBIO.2021.108252>.
- Sudduth, K.A., Kitchen, N.R., Wiebold, W.J., Batchelor, W.D., Bollero, G.A., Bullock, D. G., Clay, D.E., Palm, H.L., Pierce, F.J., Schuler, R.T., Thelen, K.D., 2005. Relating apparent electrical conductivity to soil properties across the north-central USA. *Comput. Electron. Agric.* 46, 263–283. <https://doi.org/10.1016/j.compag.2004.11.010>.
- Sun, R., Zhang, X.X., Guo, X., Wang, D., Chu, H., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biol. Biochem.* 88, 9–18. <https://doi.org/10.1016/j.soilbio.2015.05.007>.
- Toju, H., Peay, K.G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., Kiers, E.T., 2018. Core microbiomes for sustainable agroecosystems. *Nat. Plants* 4, 247–257. <https://doi.org/10.1038/s41477-018-0139-4>.
- van der Bom, F., Nunes, I., Raymond, N.S., Hansen, V., Bonnicksen, L., Magid, J., Nybroe, O., Jensen, L.S., 2018. Long-term fertilisation form, level and duration affect the diversity, structure and functioning of soil microbial communities in the field. *Soil Biol. Biochem.* 122, 91–103. <https://doi.org/10.1016/J.SOILBIO.2018.04.003>.
- Verdouw, H., van Echteld, C.J.A., Dekkers, E.M.J., 1978. Ammonia determination based on indophenol formation with sodium salicylate. *Water Restor.* 12, 399–402. [https://doi.org/10.1016/0043-1354\(78\)90107-0](https://doi.org/10.1016/0043-1354(78)90107-0).
- Wang, D., Felice, M.L., Scow, K.M., 2020. Impacts and interactions of biochar and biosolids on agricultural soil microbial communities during dry and wet-dry cycles. *Appl. Soil Ecol.* 152, 103570 <https://doi.org/10.1016/j.apsoil.2020.103570>.
- Wang, H., He, X., Zhang, Z., Li, M., Zhang, Q., Zhu, H., Xu, S., Yang, P., 2021. Eight years of manure fertilization favor copiotrophic traits in paddy soil microbiomes. *Eur. J. Soil Biol.* 106, 103352 <https://doi.org/10.1016/J.EJSOBL.2021.103352>.
- Waring, S.A., Bremner, J.M., 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature*. <https://doi.org/10.1038/201951a0> [49].
- Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* 37, 4702–4708. <https://doi.org/10.1021/ES030360X>.
- Yanardağ, I.H., Zornoza, R., Bastida, F., Büyükkılıç-Yanardağ, A., García, C., Faz, A., Mermut, A.R., 2017. Native soil organic matter conditions the response of microbial communities to organic inputs with different stability. *Geoderma* 295, 1–9. <https://doi.org/10.1016/J.GEODERMA.2017.02.008>.
- Yang, L., Zhang, L., Geisseler, D., Wu, Z., Gong, P., Xue, Y., Yu, C., Juan, Y., Horwath, W. R., 2016. Available C and N affect the utilization of glycine by soil microorganisms. *Geoderma* 283, 32–38. <https://doi.org/10.1016/J.GEODERMA.2016.07.022>.