Short- and long-term effects of continuous compost amendment on soil microbiome community

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Abstract

Organic amendment, especially use of composts, is a well-accepted sustainable agricultural practice. Compost increases soil carbon and microbial biomass, changes enzymatic activity, and enriches carbon and nitrogen soil reservoirs. However, relatively little is known about the immediate and long-term temporal dynamics of agricultural soil microbial communities following repeated compost applications. Our study was conducted at two field sites: Newe Ya'ar (NY, Mediterranean climate) and Gilat (G, semi-arid climate), both managed organically over four years under either conventional fertilization (0, zero compost) or three levels of compost amendment (20, 40 and 60 m^3/ha or 2, 4, 6 l/m^2). Microbial community dynamic in the soils was examined by high- and low-time-resolution analyses. Annual analyses community composition in compost-amended soils was significantly affected by compost amendment levels in G (first, second and third years) and in NY (third year). Repeated sampling at high resolution (9 -10 times over 1 year) showed that at both sites, compost application initially induced a strong shift in microbial communities, lasting for up to one month, followed by a mild response. Compost application significantly elevated alpha diversity at both sites, but differed in the compost-dose correlation effect. We demonstrate higher abundance of taxa putatively involved in organic decomposition and characterized compost related indicator taxa and compost derived core microbiome at both sites. Overall, this study describes changes of the ecology of soil microbiome as a response to compost vs. conventional fertilization in a time-dependent manner.

Keywords: Compost amendment, Soil ecology, Soil microbiome, Microbial community, Organic agriculture, Soil fertility

Highlights

- Changes in the structure of soil microbiomes in a dose-dependent manner after manure based compost application
- Compost-amendment causes dynamic short- and long-term changes in soil microbiomes
- Effects of compost amendment are affected by edaphic properties, climate, and soil management
- Compost caused an immediate as well as time-dependent, accumulative effect on soil alpha and beta diversity

1. Introduction

Intensive conventional agricultural practices are largely based on mineral fertilizers of the major elements, NPK, to satisfy crop nutrient requirements and obtain sufficient yields (Savci, 2012). It is widely understood however that the global excessive use of chemical fertilizers leads to extensive environmental pollution (Savci, 2012; Francioli et al., 2016). A sustainable alternative to mineral fertilization is the use of organic, mainly waste based fertilizers such as compost. The use of compost and other organic additives is vital in organic farming and highly recommended to increase soil fertility and health when adopting practices of biodynamic or various regenerative practices (Diacono and Montemurro, 2010).

Application of organic amendments typically increases the basal soil organic matter (SOM) content, microbial biomass carbon (C), enzymatic activities and respiration, and results in high nitrogen (N), potassium (K), and phosphorus (P) availability (Esperschütz et al., 2007; Diacono and Montemurro, 2010; Gomiero et al., 2011; Liang et al., 2012; Cesarano et al., 2017; Liu et al., 2017; van der Bom et al., 2018; Xu et al., 2018; Amadou et al., 2020). As organic matter physically stabilizes the soil, its addition also enhances soil structure, mainly reflected in higher aggregate stability, smaller aggregates size and water retention and lower soil erosion (Abbott and Murphy, 2007; Diacono and Montemurro, 2010; Kirchmann et al., 2013).

The effect and dynamics of long-term organic fertilization are more complex. Repeated amendment with compost or manure enriches soil C and N stocks (Diacono and Montemurro, 2010), as well as total and available P (Lundquist et al., 1999; Esperschütz et al., 2007; Hu et al., 2011; Hartmann et al., 2015; Francioli et al., 2016; Li et al., 2017) compared to mineral fertilization (Zhong et al., 2010). Long-term compost or manure amendment can either increase or decrease pH, depending on the

soil type and its characteristics (Diacono and Montemurro, 2010; Zhang et al., 2017). The slow decomposition of organic fertilizers leads to a prolonged post-application effect on soil biological properties; such as microbial biomass C, basal respiration and more (Diacono and Montemurro, 2010). Taken together, these studies support the understanding that application of organic fertilizers over a long period increases soil organic matter and potentially improves soil quality, fertility and structure (Diacono and Montemurro, 2010; Francioli et al., 2016).

Soil microbiome is very responsive to environmental cues, which makes it a sensitive marker for evaluating agricultural managements (Kennedy and Smith, 1995; Abbott and Murphy, 2007; Kraut-Cohen et al., 2020). Several studies have shown that organic amendment increases soil microbial and fungal biomass (Lundquist et al., 1999; Chu et al., 2007; Esperschütz et al., 2007; Francioli et al., 2016) compared to mineral fertilization. Next-generation sequencing has revealed significant differences between the composition of bacterial and fungal soil communities associated with organic vs. mineral fertilization regime (Francioli et al., 2016; Cesarano et al., 2017; Wang et al., 2017; Zhang et al., 2017; van der Bom et al., 2018). Twenty years of continuous organic (either compost or manure) vs. conventional farming resulted in significant differences between the respective soil microbial communities (Hartmann et al., 2015). Zhang et al., (2017) found a correlation between source soil pH and the effect of fertilization on the soil microbiome. In acidic or near-neutral soils, community composition and Shannon and richness diversity indices were higher with manure vs. mineral fertilization, whereas in alkaline soil, no such effect was observed.

The effect of compost amendment on microbial diversity is complex. According to some studies, bacterial alpha diversity is higher in organically fertilized compared to

mineral-fertilized soil (Hartmann et al., 2015; Francioli et al., 2016; Cesarano et al., 2017; van der Bom et al., 2018).

Beside diversity indices values, compost also changed the taxonomic composition of microbial communities in agricultural soils. Phylotypes of Alphaproteobacteria, Actinobacteria, Pseudomonas and Bacteroidota were found to increase in denaturing gradient gel electrophoresis profiles under organic fertilization, but only for the short term (Stark et al., 2007; Cytryn et al., 2011), which suggests that at least part of the organic fertilization-related taxonomic changes are transient. These phyla are also highly abundant in composts (Neher et al., 2013). In addition, the relative abundances of bacterial taxa known to thrive in nutrient-rich environments, such as Bacillota and Pseudomonadota, were shown to increase in organically fertilized soils (Hartmann et al., 2015; Francioli et al., 2016; Kim et al., 2021). Conversely, under mineral fertilization, increases in the relative abundance of other bacterial groups, such as Actinomycetota and Chloroflexi, have been documented (Liu et al., 2010; Francioli et al., 2016).

Most previous studies focused on one soil type and single sampling time point after compost amendment, under highly conditions while temporal microbiome changes post-compost were not examined in high resolution in field conditions. Our study examined short (week to two months)- and long (annual)-term temporal microbial dynamics in soils of two field sites: Newe Ya'ar (NY, Mediterranean climate winter average rainfall is 570 mm per annum), and Gilat (G, semi-arid climate, winter rainfall is 238 mm per annum), organically managed over four years, using three levels of compost amendment (2, 4 and 6 l/m^2) versus conventional fertilization (zero compost).Soils were sampled at low temporal resolution (yearly intervals between 2014 and 2016) and high temporal resolution (weekly to monthly in 2015–2016) post-

compost amendment. Our results demonstrate that in semi-arid soil (G soil), compost amendment had a long-term dose-dependent effect on microbial community, compared to conventional practice. In NY site, long-term soil microbiome analysis revealed that compost amendment had an accumulative affect, mainly evident in the third year of analysis. Short-term analysis in both sites confirmed that some compost-dependent changes were immediate and visible in the first four weeks post-compost amendment: specifically, Shannon diversity was significantly elevated at both sites and the abundance of specific taxonomic groups and operational taxonomic units (OTUs) was altered under compost vs. conventional fertilization.

2. Materials and methods

2.1. Site description and sampling

The experiments were conducted in two sites: Newe Ya'ar Research Center (NY) in northern Israel (32°70′N, 35°18′E), characterized by a Mediterranean climate where the annual winter rainfall is 570 mm, and soil type is a clay soil (clay- 64%, silt-18%, sand-18%), Vertisol-type, classified as Chromic Haploxerert. Gilat Research Center (G) is located in southern Israel (31º20'N, 34º41'E), and characterized by a semi-arid climate, with annual winter rainfall of 238 mm. Soil type at G is sandy loam (clay-25%, silt-35%, sand-40%), Loess, classified as Calcic Xerosol. Soil parameters for both sites are described in Rotbart et al.,(2017).

Both G and NY sites have been organically managed since 2011, using three levels of compost amendment (20, 40 and 60 m^3/ha, treatments 2, 4, 6 l/m^2 respectively) versus conventional fertilization (0, zero compost). Besides fertilization, all treatments, including the conventional fertilization, were managed under organic practices

regarding plant protection and in NY, green manure incorporation as well. Soils were sampled as described in section 2.2. Briefly, DNA was extracted and 16S rRNA gene amplicons were sequenced for microbiome analysis. The experimental timeline is given in **Fig. 1**.

Section 2.3 and Table S1 summarizes the main properties of the composts applied over the experimental period. The compost was organic cattle manure-based and applied at levels of 2, 4 and 6 l/m^2, equivalent to 20, 40 and 60 m^3/ha, respectively (resulting in 10.9, 21.9, 32.8 t/ha in G and 8.7, 17.4, 26.1 t/ha in NY respectively). The resulting applied quantities of organic carbon were 2.1, 4.1, 6.2 t/ha in G and 2.4, 4.8, 7.3 t/ha in NY respectively. In organic farming, 20 to 40 m^3/ha are the recommended annual treatment doses and 60 m^3/ha per year was selected as two times the median value between 20 and 40. The conventional plots of "no compost" (0 treatment) was fertilized with urea or ammonium sulfate. In both organic and conventional treatments in NY, all plots were amended with harvested vetch (*Vicia sativa*), as commonly used green manure in agriculture. Treatment fields at each site were subdivided into 5 blocks with 4 randomized plots and in each one of the four treatments had 3-5 replicates. All plots have had field crops in rotation since 2009. All plots, including conventional practice plots, were managed without pesticide use. The experiment in the current settings was initiated in 2010 and sampling for the microbiome measurements was begun in 2014 (see sections 2.1.1 and 2.1.2) (Rotbart et al., 2017; Sharma et al., 2017). Rainfall (in mm) records during the experimental period are presented in **Fig. S1**.

2.1.1. Fertilization in G

Compost-treated plots in G were amended with compost in October 2013, February 2015 and December 2015. Urea fertilizer was applied on the zero compost plot in June

2013, December 2013 and February 2014, and ammonium sulfate (21% N) fertilization was applied in March 2015, April 2015 and May 2015.

Shallow soil cultivation was applied in all plots (including conventional practice treatment) using a disc harrow. All field treatments, crop rotations and sampling points are illustrated along an experimental timeline in **Fig. 1**. Compost chemical properties are detailed in **Table S1**.

2.1.2. Fertilization in NY

In April 2013, all NY plots were amended with harvested vetch (*Vicia sativa*) as green manure. Chemical fertilization on the zero compost plot was applied in January 2014 (KCl and 11% P) and with urea (46% N) in July 2014. Because the legume vetch was cultured from November 2015 to April 2016, no additional N fertilization was added in that growing season. Compost treatment plots were amended with compost in June 2014 and November 2015, according to the specified compost levels (**Fig 1**).

2.2. Sampling

The overall area of each field site was 1 ha, which was subdivided into 20 plots, and the dimension of each plot was 18m*30m = 540 m^2. Soil samples were collected at high frequency (weekly to monthly) for one year and low frequency (annual) for three years. Annual sampling was conducted yearly between 2014 and 2016 in June 2014, November 2015 and August 2016 (G), and in May 2014, November 2015 and October 2016 (NY). High-resolution sampling was conducted in 2015 and 2016 on the following dates: G – pre-compost (November 2015), and 1, 2, 3, 4, 8, 16, 24, 32, 40, 48 weeks post-compost application: NY – pre-compost (November 2015) and 1, 2, 3, 4, 12, 20, 32, 44, 48 weeks post-compost application. At each sampling, a soil composite sample

was taken from 0–15 cm depth. Soil samples were cleaned of stones and plant material, and kept at -80 ^{o}C for DNA extraction. In contrast to lab experiments in controlled settings, field soils, are highly heterogenic. Therefore, care was taken to ensure that sampling will provide as accurate as possible representation of the site soils. Soil sampling was made from five blocks, composed of randomized plots, with each sample composed from three separate soil samples pooled before the final DNA extraction.

2.3. Analysis of compost and soil chemical properties

Commercial compost (Newe Ya'ar – Compost Sade Eliyahu Agricultural Cooperative Society LTD, Gilat – Machluf, Northern Negev manufacturers Hayun Ecology Compost Industry – Cooperative Society LTD) made of cattle manure was applied to each of the organic plots. Organic matter (OM) contents of the compost varied between 24.0 - 53.9%, organic C between 14.1–31.7%, total N between 1.03–1.9% and the C/N ratio between 12.6–16.8. Chemical and physical characteristics of the applied compost during the experiment are described in **Table S1**. Soil for chemical properties analysis (presented in **Table S2)** was sampled from layer 0-15cm, on the following time points: May 2014, May 2015, and November 2016 in NY and June 2014, June 2015 and November 2016 in G. The soil samples were brought to the laboratory, dried in an oven at a 40 °C, ground, and sieved with 2 mm sieves. TOC (total organic carbon) and TN (total nitrogen) were determined using a C/N analyzer (Flash EA 1112, Thermo Fisher Scientific). Soil texture was determined using the hydrometer method based on Stokes' law (analyzed by Dr. G.J. Levy; partly presented by Sharma et al., 2017). Water extracts of 1:5 soil:water ratio (w/v) were obtained, filtered with a slow filter paper (#293; 1-2 µm Sartorius, Thermo Fisher scientific, Finland), and then used to determine EC, pH and water soluble ions. The EC and pH were determined with respective meters. Water-

soluble Na+ and K+ were determined in the extract with a flame photometer (Sherwood Scientific Ltd. Cambridge United Kingdome), and the concentration of Cl- was determined using a Discrete Auto Analyzer (Gallery plus, Thermo Fisher Scientific, Finland). Available NO_3–N and NH_4–N concentrations were determined by soil extraction with 1 mol l−1 KCl, and available P by soil extraction with 0.5 mol l−1 $NaHCO_3$. The extracted NO_3–N, NH_4–N and P were measured calorimetrically using the auto-analyzer described above.

In NY, TOC and TN were measured only on 2014-2015. Na in the soil solution was determined only in Gilat samples.

The main effects of the compost level, blocks, sampling events and their interactions on measured variables were determined statistically using the three-way ANOVA procedure of JMP 16. The significance of comparisons among treatments was tested by the Tukey–Kramer honestly significant difference (HSD) at $p < 0.05$.

2.4. DNA extraction and amplicon sequencing

For analysis of community composition at each site, 5 replicate samples from each treatment were analyzed for years 2015 and 2016, and 3 replicates for 2014. In total, 232 (G) and 212 (NY) soil samples were collected over a 3-year period. Total genomic DNA was extracted from 0.1 g of soil using the Exgene soil DNA isolation kit (GeneAll Biotechnology Co. Ltd., Republic of South Korea), as previously described (Kraut-Cohen et al., 2020). DNA was PCR-amplified according to Earth Microbiome Project recommendations, using primer pair 515F and 806R targeting the variable V4 region of microbial small subunit rRNA genes (Caporaso et al., 2011) using a two-stage protocol, as described previously (Naqib et al., 2018). Amplicons from the first-stage

PCR were used as templates for the second-stage PCR employing Access Array Barcode Library for Illumina Sequencers primers (Fluidigm, San Francisco, CA). Libraries were pooled and sequenced, with a 20% phiX spike-in, on an Illumina MiSeq employing V3 chemistry. Fluidigm sequencing primers targeting the CS1 and CS2 linker regions were used to initiate sequencing. De-multiplexing of reads was performed on-instrument. Library preparation and pooling were performed at the University of Illinois at Chicago Genomics Research Core (GRC).. MiSeq sequencing was performed at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign. Sequences were uploaded to NCBI under Bioproject PRJNA688413.

2.5. Sequence analysis

Samples were sequenced in two batches: 2014 samples, and then all 2015–2016 samples. Sequences were analyzed at yearly resolution as well as at high resolution between 2015 and 2016, as described in section 2.2. In the 2014-2016 annual analysis, we analyzed 897,128 and 886,079 high-quality sequences originating from 50 (NY) or 51 (G) samples, which clustered to 4,128 OTUs. The minimum number of sequences per sample was 11,077 (G) and 11,239 (NY), while the maximum number of sequences per sample was 19,497 (G) and 20,178 (NY), with mean of 18,990 (G) and 17,942 (NY) sequences per sample.

In the high-resolution analysis, 17,605,266 sequences originating from 410 samples were generated and analyzed. These sequences were clustered into 9,373 (NY) and 5,924 (G) OTUs. The minimum number of sequences per sample was 13,037, the maximum number of sequences per sample was 14,525 and the median number of sequences per sample was 13,714. The bioinformatics pipeline was described previously

(Zolti et al., 2019). Briefly, forward and reverse paired-end reads were joined, filtered by quality and trimmed using the MOTHUR software package (Schloss et al., 2009). Clustering sequences into *de novo* OTUs by 97% similarity, and classification and depiction of differentially abundant taxa were performed with QIIME version 1.9 (Caporaso et al., 2010), using Silva 123 as the reference database (Quast et al., 2012). Chimeric sequences were removed using UCHIME (Edgar et al., 2011). Chloroplast and mitochondria sequences were also removed. All generated OTU tables were rarified to the fewest number of sequences per sample before alpha diversity was calculated. All OTUs with fewer than 50 sequences per OTU in all datasets were removed from the dataset. Three samples (two from NY November 2015, 40 m^3/ha compost treatment and one from G August 2016, 20 m^3/ha compost treatment) failed to generate sufficient reads and were removed from the dataset. Three to five biological replicates per treatment were analyzed on each date. The high-resolution OTU BIOM and taxonomy files were exported into QIIME2 (Bolyen et al., 2019) and analyzed using the bioinformatics tools described in sections 2.6–2.9.

2.6. Diversity analysis

The compositional dissimilarity between samples (beta diversity) throughout 2016, was measured using the Bray–Curtis distance metric and visualized using non-metric multidimensional scaling (nMDS) plots (Clarke and Ainsworth, 1993) (annual resolution) or PCoA (high time resolution).

Principal coordinate analysis (PCoA) for the soil treatments (0,2,4,6), was created based on QIIME 2 Bray–Curtis dissimilarity index data, and plotted by ggplot2 package in R (Bolyen et al., 2019) with centroids representing 95% normal data confidence ellipses for each group.

Pairwise comparisons between average Bray–Curtis distance matrices were calculated for compost treatments compared to conventional practice at each time point, and plotted using the ggplot2 package in R. Significance of dissimilarity was estimated with a Kruskal–Wallis non-parametric test (significance threshold of $p < 0.05$); pairwise groups within each category (year, treatment) were compared using ANOSIM test and treatment pairs in each year were compared using PERMANOVA test.

Feature tables was rarified to the minimal feature number per sample (in G 13,000 and in NY 8,500 for 2016, and 11,000 for both NY and G in the three years analysis) before Shannon diversity indices were calculated using QIIME2. Taxonomic differences were calculated by Mann–Whitney test (high-resolution analysis, all time points) or Wilcoxon test (yearly analysis) and considered significant at $p < 0.05$.

2.7. Heat map

For the high-resolution analysis in 2016, all significantly (Kruskal–Wallis test, FDR-corrected $p < 0.05$), enriched or depleted OTUs from each of the compost treatments as compared to the conventional practice treatment were plotted. Significantly different OTUs between treatments 0 and 6 were selected and plotted for all treatments (0, 20, 40, 60 m^3/ha) on the heat map, along the full experimental timeline (either yearly or weekly–monthly resolution), as described in section 2.2). The differentially abundant OTUs were sorted taxonomically at the phylum level, as well as by abundance patterns in each treatment. The heat maps were created using the 'pheatmap' package in R (Kolde, 2019).

2.8. Core microbiome analysis

The core microbiome of all compost treatments together (all compost samples were combined) and the core microbiome (100% prevalence) of the conventional treatment (0) were computed individually for G and NY using the core features plugin in QIIME2 with at least 100% prevalence. Venn diagrams were created at the OTU level to compare different core microbiomes using Venny (Oliveros, J.C. 2015))

2.9. ANCOM and MaAsLin2 analysis

Identification of significantly differentially abundant OTUs between treatments (0, 2, 4, 6) was determined by analysis of composition of microbes (ANCOM) among all treatments using the ANCOM plug-in in QIIME2 (Mandal et al., 2015), with FDR-corrected *p*-values already embedded in the ANCOM test. In addition, centered log ratio-transformed data were used to identify differentially abundant features between the compost and conventional treatments with the Microbiome Multivariable Associations with Linear Models (MaAsLin2) package in R (Mallick et al., 2021). Significant OTUs derived from both methods were compared by Venny (Oliveros, J.C. 2015), and OTUs that were found significant in both were further plotted using the 'pheatmap' package in R (Kolde, 2019).

3. Results

The short- and long-term effects of compost vs. conventional fertilization on microbial community composition were examined over time, at sites G and NY that differ in their soil type (Rotbart et al. 2017), climate, and crop rotation (detailed in section 2.1).

3.1. Effect of yearly compost amendment vs. conventional practice on soil chemistry and community composition

In the semi-arid soil (G), all measured chemical parameters except NH_4, Cl and pH, were significantly elevated in a compost dose dependent manner (Table S2). In the Mediterranean soil (NY), TN, P, K were significantly elevated in compost treatments (and in TN and P in dose dependent manner) while NO_3 exhibited a similar trend. The effect of compost amendment (compost vs. conventional practice) and dose of the amended compost (treatments 2, 4, 6) on microbial community composition was examined yearly. Soils were sampled at site G in June 2014 (Y1), November 2015 (Y2) and August 2016 (Y3) and at site NY in May 2014 (Y1), November 2015 (Y2) and October 2016 (Y3).

The most dominant phyla in the full dataset (all annual sampling times and treatments) were Pseudomonadota (27% in NY, 28% in G), Actinomycetota (22% in NY, 21% in G), Bacillota (15% in NY, 13% in G), Thaumarchaeota (12.8% in NY, 8.1% in G) and Bacteroidota (4% in NY, 8.8% in G).

The effect of compost amendment at annual resolution was initially examined by pairwise comparison based on the Bray–Curtis similarity indices of the three compost levels vs. the conventional treatment. A significant difference for the "year" ($p < 0.0001$ in G and NY) and "compost" (p 0.009, in G) parameters was found by ANOSIM test of all three years (2014–2016). However, the "compost" parameter was not significant in NY samples.

At both sites, all tested years (Y1, Y2, Y3) varied significantly from each other (**Table S3A**, **B**). These differences represent dynamics that may reflect the variation between years in soil management, crop, weather and sequencing batch.

Significant shifts in G soil microbial community structure were observed at the higher rates of compost amendment (4, 6) relative to conventionally treated soil (0) across all three years of the study. Conversely, at the NY site, no significant changes were found between treatments pairs when comparing all three years together (**Table S3 C**, **D**).

The difference in microbial community structure of soils receiving compost and those receiving conventional treatment was visualized using box-plots based on dissimilarity comparison of Bray–Curtis distances for each year (**Fig 2 A and B**). Comparison of compost treatments revealed significant changes. In G, comparison of compost treatments within each year, revealed significant and accumulative changes between treatments: treatments 2 and 6 in Y1, between 2 and 6, 4 and 6 in Y2 and between 2 and 6, 4 and 6 and 2 and 4 in Y3 (**Fig2A, Table S3E**). In NY, significant differences were found between treatments 2 vs. 6, and 4 vs. 6 in Y3 only (**Fig 2B, Table S3F**).

Samples were visualized using nMDS (**Fig. 2C** and **D**), and statistical analyses (PERMANOVA) were conducted to determine whether differences in microbial community structure between treatments was significant (**Table S4**). A cumulative microbiome response to compost levels was demonstrated in G soils as visualized using nMDS (**Figure 2C and D**). In Y1 at the G site, the difference noted between the different fertilization treatment groups was not significant (**Fig. 2C**, **Table S4**). In Y2 and Y3, bacterial communities in soil amended with the lowest compost level (treatment 2) differed significantly from that with the highest compost level (treatment 6) (**Table S4**). Conventionally fertilized soil (treatment 0) differed significantly from the compost treatments (4 and 6 in Y2 and 2, 4 and 6 in Y3) (**Fig. 2C**, **Table S4**).

At NY, nMDS analysis revealed a fertilization and dose-dependent trend that was not significant due to the high variance between replicates in microbial profiles (**Fig. 2D**, **Table S4**). No significant differences were found in the alpha diversity indices between the treatments at either site (not shown).

3.2. Effect of annual compost amendment vs. conventional practice on microbial community structure

Several differentially abundant taxonomic groups were characterized in G soils during the 3 years of observation. In Y2, the relative abundance of OTUs from bacterial orders Gemmatimonadales and Solirubrobacterales was lower in compost-amended soils compared to conventional fertilization (p = 0.0217 and 0.034, respectively, Wilcoxon test. However, none of the 17 tested orders were significant after false-discovery rate correction (*i.e.*, FDR q-value)) (**Table S5)**. In Y3, the relative abundance of Rhizobiales was significantly lower in the compost vs. conventional practice soils. In addition, compost level-dependent patterns were found in the relative abundance of the taxa Cytophagales and Myxococcales, usually associated with high organic matter consumption using Wilcoxon test to compare different doses of compost treatment vs. conventional practice in Y3 (**Table S5**). In G soils, the relative abundance of 134 OTUs differed significantly between conventional fertilization and high compost treatment (0 vs. 6), whereas the relative abundance of only 4 OTUs was significantly different between 0 and 4 soils. No significant difference was found between the other treatments. For the most part, the relative abundance of OTUs in the phyla Bacillota and Bacteroidota was greater in compost-amended soils, whereas OTUs from the Euryarchaeota showed higher relative abundance in the conventional practice soil (**Fig.**

S2). We did not detect any OTUs that differed significantly between treatments at the NY site in the annual analysis.

Overall, unlike in NY, compost amendment affected microbial community composition in the semi-arid G soils. After three years of analysis of an ongoing experiment, we could detect a clear and cumulative influence of compost amendment, suggesting a long-lasting effect.

3.3. High-resolution microbial community temporal dynamics throughout 2016

To further understand the temporal dynamics of bacterial communities in response to compost amendment, we sampled both G and NY soils throughout 2016 at high temporal resolution.

During this high-resolution sampling year, the dynamics of compost amendment were examined at both sites. The dominant phyla in the high-resolution analysis were Pseudomonadota (28% in G and 27% in NY) and Actinomycetota (20% in G and 26% in NY). In G, there was a clear gradient between treatments across all sampling time points, and PERMANOVA analyses demonstrated significant differences between all pairwise treatment comparisons (0, 2, 4, 6) (**Fig. 3A**, **Table S6**). At the NY site, treatment groups were less strongly clustered, but a similar trend was observed, with significant differences between compost level treatments (2, 4, 6) and the conventional treatment (0), and between low (2) and high (6) compost treatments (**Fig. 3B, Table S6**).

At both sites, agricultural management and environmental conditions caused dynamic temporal changes in community structure. To focus on the effects of compost amendment for each sample and time point, we plotted Bray–Curtis differences compared to the site conventional control (0) at the same time point (**Fig. 4A** and **B**).

Compost, at any level of amendment, induced an immediate and sharp shift in microbial community structure, followed by a dynamic change in the community (**Fig. 4C** and **D**). Starting at twelve weeks following compost amendment, there was stabilization in the community dynamics. Still, some residual effects of compost amendment on soil microbial communities lasted 32 or 44 weeks (Y3 in **Fig. 2A** and **B**) and 1 year (**Fig. 4A** and **B**), demonstrating that compost significantly impacted the structure of soil microbial communities on a long-term basis. In G, comparing the distance in community composition at each sampling time, to the equivalent soil community in the conventional practice, a significant dose-dependent response to the compost was observed immediately after compost application. This effect continued throughout the high-resolution sampling period and was still detected one year after compost amendment (**Fig. 4A**; Mann–Whitney pair test p values: 0 vs. 2, $p = 0.00008$; 0 vs. 4, $p = 0.035$; 0 vs. 6, $p = 0.00018$). In NY, moderate differences between treatments were observed, and a significant effect was found only between conventional practice and the high compost treatment in the high-resolution sampling set (Mann–Whitney 0 vs. 6, $p < 0.005$) (**Fig. 2D**, **Table S4** and **Fig. 4B**). At the OTU level, a total of 1,290 (G) and 540 (NY) OTUs differed significantly (Kruskal–Wallis test, $p < 0.05$) in abundance between the high compost treatment and conventional fertilization samples (**Fig. 4C** and **D**, **Fig. S3**). At both sites, an immediate response to compost amendment was observed at all compost levels. Beside the immediate shift in abundance of specific taxa in response to compost amendment, we also noted a dynamic set of changes in communities during the weeks after compost was applied (**Fig. 4C** and **D**, **Fig. S3**). At both G and NY sites, OTUs assigned to the phyla Bacteroidota, Bacillota and Pseudomonadota were tightly and positively associated with compost amendment. Gemmatimonadetes, and Verrucomicrobia showed a mixed response at both sites, with

some OTUs increasing and others decreasing in relative abundance in response to compost amendment (**Fig. 4C** and **D**).

When the mean average of the relative abundance of each phylum of this analysis was plotted over time, we observed that some phyla increased in relative abundance in response to compost amendment: Bacillota, Pseudomonadota and Bacteroidota at both sites (**Fig. S3A** and **B**), and *BRC1*, Planctomyces and Acidobacteria in G **(Fig. S3A)**. The relative abundance of other phyla increased over time in the conventional (0 compost) samples, especially in NY. These phyla included Actinomycetota and Chloroflexi, and for early time points (up to week 20) also included Acidobacteria and Crenarchaeota **(Fig. S3A)**. These observations are likely due to the dynamics of organic matter degradation and composition with time post-amendment (**Fig. S1**). Thus, the relative abundance of many microbial taxa was altered in a dose-dependent fashion in response to compost amendment. A clear gradient between abundance patterns for levels 2, 4 and 6 of compost treatment was observed (**Fig. 4C** and **D**, **Fig. S2**).

3.4. Compost amendment increases soil community diversity

Next, we compared microbial community diversity by comparing Shannon diversity indices for all treatments in 2016 (high temporal resolution) after rarefaction. The initial diversity in G soil was higher than that of NY (Shannon index average was 10.86 in G, 10.22 in NY, for all treatments at a rarefied sequence depth of 13,000 in G and 8500 in NY sequences/sample). Compost amendment increased alpha diversity relative to conventional practice at both sites (**Fig. 5**). At NY, higher compost levels resulted mostly in higher diversity and all compost treatments were significantly different from the conventional practice across the dataset (Kruskal Wallis pair test;

treatments 2, 4, 6 $p < 0.0001$,). Alpha diversity levels were also significantly different between the highest and lowest compost treatments (2 vs. 6; $p < 0.08$) (**Fig. 5B**). At G, alpha diversity (Shannon index) was significantly higher, and similar to one another, in all compost treatment samples relative to conventional practice control samples (Kruskal Wallis pair test; treatments 2, 4, 6 $p < 0.001$; **Fig. 5A**).

3.5. Compost-amended soils have unique microbial populations

We identified 332 core OTUs in conventionally fertilized soils vs. 205 OTUs in compost-amended soils at the G site. Similarly, we identified 268 core OTUs in conventionally fertilized soils vs. 174 OTUs in compost-amended soils at the NY site. At each site, the core microbiome was determined based on OTUs represented in all compost-amended soil samples from all sampling time points (treatments 2, 4, 6). This site-specific core microbiome was compared to that of the conventional treatment. In G, 30 core OTUs were unique to the compost-amended soils, and 157 were unique to the conventionally fertilized soils (**Fig. S4A**). In NY, 16 OTUs were unique to compost-amended soils and 110 OTUs were unique to the conventional treatment (**Fig. S4B**). In G, most compost core OTUs were annotated to the Bacillota (9) and Pseudomonadota (7), while in NY, most compost core OTUs were annotated to Actinomycetota (5) and Pseudomonadota (4). Most conventional-treatment core OTUs at both sites were annotated as Pseudomonadota (37 in G and 31 in NY) and Actinomycetota (32 in G and 28 in NY). Alphaproteobacteria (26 in G and 20 in NY) and Actinobacteria (16 in G) and Thermoleophilia (13 in NY) were the most abundant class level annotations in conventional-treatment core OTUs. Bacilli (9) in G, and Gammaproteobacteria at both site (G-4, NY-3) were the most abundant class level annotations in the compost core OTUs. When the core communities from both experimental sites were compared, we

found 2 OTUs that were shared by the compost-amended soils of G and NY; 12 OTUs were shared by the core communities of the conventionally fertilized soils at both sites (**Fig. 6A; Table S7**).

To further characterize compost indicator OTUs, we analyzed the OTUs that were differentially abundant between compost and conventional soils by two methods that rely on different hypothesis tests (Nearing et al., 2022): (i) we compared all compost treatments (2, 4, 6) to conventional soil treatments (0) using MaAsLin2, and identified 1173 unique OTUs in G and 153 unique OTUs in NY; (ii) significantly differentially abundant OTUs in any treatment (0, 2, 4, 6) were computed using ANCOM, identifying 136 OTUs in G and 36 in NY (**Table S8A and B** respectively). Finally, the lists of significant OTUs resulting from both MaAsLin2 and ANCOM analyses were compared and 111 class level-annotated OTUs in G and 25 in NY were identified by both analyses, most of which increased as a result of compost amendment. At both sites, most of these OTUs belonged to the Bacilli or Cytophagia, as well as Planctomycetia, Deltaproteobacteria and Actinomycetota. Some of the annotated OTUs were site-specific: Pedosphaerae (higher relative abundance in the conventional treatment) in NY (**Fig. 6C**) and Acidimicrobiia, Saprospirae, Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria (most of which increased in relative abundance in compost treatments) in G (**Fig. 6B**). These OTUs thus represent the effect of compost on the microbiome of amended soils.

4. Summary

Intensive agricultural practices require external inputs to meet crop nutrient demands. Compost is one of the most common soil amendments, and is widely used on a global scale as an alternative to mineral fertilization. Numerous studies have described

the beneficial effects of organic fertilization on soil- and microbe-related parameters such as increases in microbial biomass and total organic carbon. Nevertheless, detailed information on the immediate and prolonged effects of compost vs. conventional fertilization on the dynamics and ecology of soil microbial communities is still lacking, especially under field conditions. In this study, performed at two agricultural sites with different climates, soil types and agricultural practices, after application of two different composts in each site, we characterized both the immediate and prolonged effects of conventional, chemical fertilization and three levels of compost amendment on soil chemical parameters and microbial community structure.

4.1. Single annual compost amendments significantly alter soil microbial communities relative to conventionally fertilized soil communities

Annual sampling between 2014 and 2016 showed that even a single compost amendment can affect microbial community structure. Similarly, previous studies showed that single organic soil fertilization, alone or combined with mineral fertilization, may affect microbial soil community composition (Inbar et al., 2005; Jechalke et al., 2014; Liu et al., 2017). In our study, G (extremely low organic content soil), compost amendment significantly altered the microbial community structure of agricultural soils in the first, second and third years of the experiment, in a dose-dependent manner (**Fig. 2A**, **Table S3E**). In NY (low organic content soil), a significant effect was observed only in the third year of treatment, and only between the lower (treatment 2 or 4) and highest (treatment 6) compost-amended soils (**Fig. 2B**, **Table S3F**). In 2013, harvested vetch (*Vicia sativa*) was added to NY fields as green manure, which may have reduced the impacts of the compost amendment in 2014. The compost-dependent shift in microbial community was evident in G and NY, even 8 and 10

months (respectively) after compost application (Y3 in **Fig. 2A** and **B**). Such a prolonged effect on microbial community structure (vs. mineral fertilization or untreated soil) was previously found after single (Saison et al., 2006) or repetitive (Zhong et al., 2010; Hartmann et al., 2015; Francioli et al., 2016; Chowdhury et al., 2019) compost application.

Shifts in microbial community structure associated with compost amendment in G were examined closely, and in compost-amended soils, taxa known to prefer high organic matter (e.g., Cytophagales and Myxococcales), and those described as copiotrophs and organic matter-decomposing bacteria (Bacteroidota and Bacillota) increased in relative abundance compared to the conventionally fertilized plots. The relative abundance of Bacteroidota and Bacillota has been shown to be high during the composting processes (Neher et al., 2013), and this may explain their elevated levels in the compost amended soils. Conversely, OTUs annotated as Rhizobiales were more abundant in conventional treatment soils (**Fig. S2, Table S5**). G and NY soils have low organic content and originate from sites of Mediterranean semi-arid weather conditions, with high temperatures. Interestingly, both sites have high relative abundance of Thaumarchaeota (12.8% in NY, 8.1% in G). This phylum, which contains some ammonia-oxidizing archaea, is usually more abundant in low fertility soils and lower in soils with higher availability of inorganic N (Bates et al., 2011; Pester et al., 2011; Li et al., 2020).

4.2. Compost effects on soil chemistry and microbial community are dose-dependent and accumulate over time

Compost elevated many of the measure chemical parameters, mainly in G but also in NY soil. This is in agreement with previous studies that showed that organic fertilization increased TN, available P, TOC and pH compared to non-fertilized or

mineral fertilized soil (Luo et al., 2015; Francioli et al., 2016; Amadou et al., 2020; Yang and Zhang, 2022). This compost effect on soil chemical parameters was in a dose-dependent manner, and this phenomenon was most strongly observable in soils from G. For example, a significant effect of compost on the microbial community structure of G soils was visible at our first sampling point (Y1). However, this effect was stronger in 2015–2016 (Y2 and Y3 in G) or 2016 (Y3 in NY), as assessed by Bray Curtis distances (**Fig 2**). This enhanced compost effect in Y2 and Y3 in G and Y3 in NY, compared to Y1 of sampling, likely reflects a cumulative effect after repeated compost amendment over several years. Alternatively, it may also be affected by environmental factors (such as weather differences between Y1, Y2 and Y3; **Fig. S1**), or technical variability with different sequencing batches. When environmental and technical factors were eliminated by comparing each compost treatment to the respective conventional control (with the same environmental and technical settings) significant difference in Y1 in G site between treatment 2 and 6 was evident as well. Despite differences in response to amendment between the years, we observed a compost-related gradient in community composition in each of the sampling years at both sites. Such dose-dependent change was previously observed in controlled lab experiments, as higher levels of compost amendment significantly affected microbial community composition (based on PLFA or DGGE profiles) while lower dose did not (Inbar et al., 2005; Saison et al., 2006). Repetitive compost application was found to affect the community structure more than a single one (Diacono and Montemurro, 2010; Cesarano et al., 2017). Our study demonstrates that this accumulative, dose-dependent change is also dynamic, as the community shifts over time.

4.3. Amendment with compost has an immediate and time-dependent effect on the soil microbiome

To follow the temporal dynamics and understand the immediate and delayed responses of the soil microbiome to compost amendment, we analyzed the microbiome dynamics at high (weekly to monthly) temporal resolution from November 2015 through 2016. This high-resolution analysis revealed significant differences in microbiome composition between conventionally fertilized (treatment 0) and compost-amended (treatments 2, 4, 6) soils, as well as between high (6) and low (2) compost doses, at both sites (**Fig. 3A** and **B**). The observed community dissimilarity after compost application was in accordance with a previous study that found that organic and mineral fertilization explained, respectively, 15.3 and 14.4% of the dissimilarities in community composition (Francioli et al., 2016). Additionally, a study by (Saison et al., 2006) showed that compost can affect the microbial composition both in the immediate and later (6 months) time points, in a dose dependent manner (Saison et al., 2006).

In our study, compost application immediately shifted the soil microbial community composition compared to conventionally fertilized soils at both examined sites. This shift was reduced 4 weeks post-compost amendment and onward. In G (but not in NY), a compost dose-dependent change was also observed compared to the microbial community of conventionally fertilized soil (**Fig. 4A** and **B**).

Compost had a different impact in each of the sites. The effect of compost dose on community composition in NY soils was milder than that in G soils, probably due to environmental parameters such as soil type and chemical properties (**Table S2**), heavy rain events (**Fig. S1**), differences between compost composition in each site and field management, including tillage practices, which are known to greatly impact the

soil microbiome (Cohen et al., 2019, **Fig. 1, Fig. S1**). One of these differentiating parameters is the organic carbon content, which was higher in NY soils than G soils (**Table S2**).

Our results suggest that dry, low OM sandy soils, under semi-arid climate (as in the G field), are more susceptible to microbiome shifts resulting from agricultural management, but this finding requires further validation. Furthermore, our results suggest that frequent sampling is necessary to reliably describe the microbiome response to a disturbance such as compost amendment, as the effects of such amendments can be influenced by soil type and climate. In our case, weekly resolution was adequate for tracking immediate changes in the first month after compost amendment. To examine the delayed effect of compost, sampling between weeks 15-20 following compost amendment was sufficient, as the community started to stabilize in at this stage.

4.4. Higher abundance of taxa putatively involved in organic decomposition in compost-amended soil

Organic fertilization increases soil metabolic activity and microbial functional diversity (Zak et al., 1994; Zhong et al., 2010), and similar changes are likely to result from compost-mediated shifts in community structure. Our results showed that indeed, compost amendment induces taxonomic changes from the phylum to the OTU levels. At both sites, most of the OTUs that were more abundant in the compost-amended soils belonged to the phyla Bacteroidota, Pseudomonadota and Bacillota (**Fig. 4**, **Fig. S3**). Members of these bacterial groups are often involved in the degradation of complex organic compounds (Fierer et al., 2007; McBride et al., 2014; Cesarano et al., 2017; van der Bom et al., 2018) and are highly abundant during composting (Neher et al., 2013).

Pseudomonadota, Bacteroidota and Bacillota are known as hydrolytic, fermentative bacteria (Shanks et al., 2011; Rath et al., 2022), and have been found to be relatively more abundant in manure-fertilized soils (Hartmann et al., 2015; Francioli et al., 2016; Hartman et al., 2018; van der Bom et al., 2018; Yang and Zhang, 2022). Some of these taxonomic groups have high relative abundance in nutrient-rich environments, and Bacteroidota and Pseudomonadota have also been generally described as copiotrophic (Fierer et al., 2007; Cesarano et al., 2017).

In NY, OTUs belonging to the phyla Actinomycetota, Chloroflexi and Crenarchaeota had higher relative abundance in conventionally fertilized soils (**Fig. S3**, **Fig. 4D**). These results are in accordance with previous studies showing a significantly higher proportion of Actinomycetota and Chloroflexi in mineral- compared to organic-fertilized soils (Hartmann et al., 2015; Francioli et al., 2016; van der Bom et al., 2018).

4.5. Compost amendment indicator OTUs

Our heat map analysis showed differences in OTU composition between treatments immediately after compost amendment. Nevertheless, the relative abundance of some OTUs shifted significantly only a few weeks after compost application, thus demonstrating that compost has an additional, prolonged effect on soil microbiome, with some OTUs belonging to late responders. This dynamic likely reflects the balance between the fast-growing copiotrophic bacteria, which proliferate in response to the supply of readily available substrates, and slower growing bacteria that replace them later and utilize more recalcitrant compounds.

For the detection of compost amendment indicator species, we identified OTUs that differ significantly in relative abundance between the compost-amended and conventionally fertilized soils. Since many tools used to detect differential abundance

show variable performance between datasets, we used both MaAsLin2 and the compositionally-aware method ANCOM (Nearing et al., 2022). Because ANCOM is a more conservative approach, it identifies fewer features than MaAsLin2. Most of the significantly altered OTUs were more abundant in the compost-amended soils, especially in soils amended with the highest compost levels. At both sites, most of these OTUs belonged to the class Bacilli as well as the Actinobacteria, which are known to be associated with composting processes or enriched in organically fertilized soils (Paul Chowdhury et al., 2019; Rath et al., 2022), and the class Cytophagia. In contrast, the relative abundance of Pedosphaerae decreased in the compost-amended soil in NY. This class was previously demonstrated to be reduced in soil following high-concentration nitrogen- based fertilization (Zhou et al., 2015).

Core microbiome analysis identified a larger core microbiome for conventionally fertilized vs. compost-amended soils (157 vs. 30 OTUs in G, and 110 vs. 16 OTUs in NY). The smaller microbiome core size is likely the result of a more dynamic microbiome in soils with compost amendment, as also reflected in **Fig. 4C** and **D**.

Two OTUs from the compost core were shared between the G and NY sites. Both were annotated as members of the Sinobacteraceae. Originally, this bacterial family was identified upon isolation of a representative member from a polluted farmland sample, and was described as chemo-organotroph (Zhou et al., 2008). One of the two OTUs was further classified as a member of the genus *Steroidobacter*, reported to be among the most abundant genera in composting processes of dairy manure with plant additives, textile or distilled grain waste (Biyada et al., 2021; Rath et al., 2022; Wang et al., 2022).

4.6. Effect of compost amendment on alpha diversity of microbial communities

Some studies on long-term (21–33 years) compost application or organic fertilization have reported an increase in microbial diversity in bulk soil (Zhong et al., 2010; Luo et al., 2015; Lupatini et al., 2017) and the rhizosphere (Schmidt et al., 2020), whereas others did not find fertilization-related differences in diversity (Paul Chowdhury et al., 2019). Previous reports of long- and short-term compost-amendment effects on soil microbial diversity have seemed to be inconclusive. In some studies, bacterial diversity and richness indices were significantly higher in soils amended with organic fertilizer compared to mineral NPK-fertilized soils (Zhong et al., 2010; Li et al., 2012; Luo et al., 2015; Hartmann et al., 2015; Francioli et al., 2016, Lupatini et al., 2017), whereas in others, the opposite trend was reported (Amadou et al., 2020). We could not detect a significant increase in microbial diversity in the annual sampling over 3 years (not shown), either because the sampling was done at least 8–10 months after the compost application, or due to different environments and resolution of the methodology.

However, sampling at higher temporal resolution in a single year (2016, Y3) revealed a significant compost-related effect on diversity, despite major differences in compost and soil characteristics, as well as crops and weather events at both sites (**Table S1** and **S2**, **Fig S1**). The Shannon diversity index of compost-amended soils was higher throughout 2016 relative to that of conventionally fertilized soils at both sites. In NY, there was a clear effect of compost dose on the diversity, with higher compost levels leading to higher diversity (**Fig. 5B**), whereas in G soil, diversity became similar and high at the three compost levels compared with the non-compost control (**Fig. 5A**). NY soils were highly affected by the compost amendment dose compared to G soils, possibly due to the lower basal Shannon diversity in the former.

Compost amendment may increase community diversity through the addition of external microorganisms originating from the compost, or through promotion of the decomposition of complex organic matter by existing copiotrophic bacteria, the relative abundance of which is higher immediately after compost amendment. The significant difference between treatment 0 and treatments 2, 4 and 6 reflects the immediate (in the first month) fluctuations following compost amendment, as well as milder fluctuations later on, when more recalcitrant compounds are being degraded by different groups of organisms.

4.7. Compost-mediated site-specific differences in microbiome

We examined microbial dynamics at two environmentally different agricultural fields that differ not only in their environmental settings (soil type, weather, rainfalls) but were also amended with different commercial compost on each site and differed in their field management (various crop rotation at G and NY and green manure applied in NY, but not in G) . At all examined taxonomic levels, compost-related changes in soil microbiome composition were more significant in G than in NY. In NY, fewer OTUs were significantly enriched or depleted as a result of compost amendment. G soil had an initial lower TOC, TN content and NO_3, NH_4, Cl and EC levels (**Table S2**) relative to soils of the NY site. The poor sandy soil, along with the semi-arid climate in G, likely renders this agricultural soil more susceptible to external perturbation. However, the Mediterranean site of NY, which has higher TOC and TN levels to begin with, might require a higher dose of compost to disturb its soil microbiome. NY fields were amended with harvested vetch (*Vicia sativa*) as green manure on 2013, which might minimized the effects of the compost amendment on 2014. Earlier studies similarly demonstrated dose-dependent effect of compost amendment on rhizosphere

and root microbial community structure and on root colonization (Inbar et al., 2005). Other studies have shown that site as variable can have a stronger influence on the microbiome than agricultural management (Schmidt et al., 2020). Local environmental conditions may also affect the soil microbiome's stability and responses to perturbations. For example, in our study, heavy rains in the winters of 2015 and 2016 (**Fig. S1**) may also have contributed to the reduction of compost-related effects on NY's microbial community.

A single application of mineral fertilizer does not necessarily induce long-term changes in microbial communities (Stark et al., 2007; Geisseler and Scow, 2014). On the other hand, continuous fertilization practice, organic or mineral, has been shown to alter soil properties and microbial community composition (Lentendu et al., 2014; Hartmann et al., 2015; Eo and Park, 2016). Continuous fertilization, more than a single fertilization event, markedly disturbs the ecological system, and may result in a new ecological steady state for the soil microbiome (Eo and Park, 2016; Francioli et al., 2016). The differences between the examined sites in our study demonstrate that these long-lasting effects are site-dependent, but agricultural management and environmental variables contribute as well.

4.8. Summary

Results indicated that compost significantly affects soil microbiome structure immediately after amendment. This shift, which correlated to the initial applied compost dose, decayed over time. Still, some changes in the microbial community structure and ecological parameters (i.e. alpha- and beta diversity) persisted for months following compost application. These changes in soil microbiome occurred in parallel

to changes in soil chemical parameters. Fertilization practices were found to be more influential in shaping the soil microbiome than other site-specific or environmental factors, promoting the enrichment of microorganisms specific to compost amendment, including copiotrophic and organic matter degrading bacteria.

Supplementary material

Supplementary material accompanies the paper.

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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.

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Figure legends

Fig. 1. Experimental timeline in Gilat (G) and Newe Ya'ar (NY) sites. The experiment was conducted between 2013 and 2016. Each field site (G, NY) had four treatments: 0 (conventional fertilization) and 2, 4, 6 (20, 40 and 60 m^3/ha of compost amendment, respectively). The crops grown at each site are indicated at the top. All sampling events are marked with "S": a red "S" indicates that the sampling event was part of the annual analysis from 2014 to 2016. Lightening symbols represent soil cultivation (tillage/loosening).

Fig. 2. Compost amendment alters soil community composition. Bray–Curtis dissimilarities between microbiomes of compost-amended soils (2, 4, 6) and conventionally fertilized soil for the period 2014–2016 at yearly resolution. Pairwise comparisons are indicated for soils from G (A) and NY (B). Asterisks represent significant differences between groups ($p \leq 0.05$, Wilxcon test). The 2014 (Y1) samples are marked in red, 2015 (Y2) in blue and 2016 (Y3) in green. nMDS ordination was plotted for the Bray–Curtis dissimilarities in soil microbial community composition (OTU level) at G (C) and NY (D) sites between 2014 and 2016. Conventionally

fertilized (0) samples are marked in violet, and compost-amendment treatments are marked in light blue (2), blue (4), and dark blue (6). Stress values were <0.1 for all analyses.

Fig. 3. High-resolution analysis of compost-mediated changes in soil microbiomes. Ordination of soil microbiomes was conducted using PCoA based on a Bray–Curtis dissimilarity matrix of all high-resolution-sampled time points in 2016, in G (A) and NY (B) soils. Conventionally fertilized (0) samples are marked in violet, and compost-amendment treatments are marked in light blue (2), blue (4), and dark blue (6).

Fig. 4. High-resolution temporal dynamics of compost-mediated shifts in microbial community structure during 2016. Bray–Curtis dissimilarities between microbiomes of the compost-amended soils—treatments 2, 4, 6 (marked with light blue, blue and dark blue, respectively)—and that of conventionally fertilized samples (treatment 0) at high temporal resolution over 2016 were visualized for G (A) and NY (B) soils. Heat maps of treatment-enriched OTUs at multiple sampling points in 2016 are shown for G (C) and NY (D) sites. All visualized OTUs were significantly (Kruskal–Wallis test, FDR-corrected $p < 0.05$) enriched or depleted in treatment 0 compared to any of the compost treatments (2, 4 or 6). The heat map presents average OTU abundance (at all corresponding replicated treatments) for each sampling point, normalized per row (Z-score). The colored annotation bars represent the classification of each OTU at the taxonomic level of phylum.

Fig. 5. Temporal dynamics of alpha diversity in the microbiomes of compost-amended and conventionally fertilized soils during 2016. Alpha diversity, as measured by Shannon diversity index, in conventional practice (0) samples is marked in violet and compost-amendment treatments in light blue (2), blue (4), and dark blue (6), over high-resolution 2016 sampling in G (A), and NY (B).

Fig. 6. Core and indicator OTUs for compost-amended soils. (A) Venn diagram illustrating core microbiome (OTU level) of compost-amended and conventionally fertilized soils at G and NY sites. Heat map for the indicator species (significantly enriched at $p < 0.05$, OTU level) detected and shared by MaAsLin2 analysis and ANCOM (the OTU list is presented in **Table S8**) in G (B) and NY (C) soils. The heat map presents average OTU abundance at all-time points for the four treatments (0, 2, 4, 6), normalized per row (Z-score). The color bars represent class-level annotation of the indicator OTUs.

Supplementary material

Fig. S1. Precipitation at the sampling sites during 2014–2016. Rainfall measurements (mm) in G (A) and NY (B) during the experimental time period.

Fig. S2. Compost-mediated changes in relative abundance of OTU-level taxa in yearly resolution sampling. Heat map of treatment-specific OTUs calculated throughout the 3 years of sampling. All presented OTUs were significantly (Kruskal–Wallis test, FDR corrected $p < 0.05$) enriched or depleted at 0 vs. any compost treatment (2, 4 or 6). The heat map presents an average of OTU abundance on each sampling date of the 3 years of study, normalized per row. The color bars correspond to phylum-level classification of the different OTUs.

Fig. S3. Temporal dynamics of dominant phyla representing significantly enriched or depleted OTUs in 2016, in response to compost dose. The average relative abundance of phylum-level taxa that represent all OTUs which were significantly (Kruskal–Wallis test, FDR corrected $p < 0.05$) enriched or depleted in 0 vs. compost treatments (and plotted in a heat map in Fig. 4C and D) in NY (A) and G (B) is presented. Conventional

practice (0) samples are marked in violet, and compost-amendment treatments are marked in light blue (2), blue (4), and dark blue (6).

Fig. S4. Comparison of core microbiomes in compost-amended vs. conventionally fertilized soils. The Venn diagram depicts the unique and shared OTUs among the core microbiomes of compost amended vs. conventionally fertilized samples in G (A) and NY (B).

Table S1. Chemical and physical characteristics of compost applied during the study. Various chemical and physical properties of the compost applied in NY (left) and G (right), as measured in 2013, and in January and December 2015.

Table S2. The effects of compost load on Gilat and Newe Ya'ar soil properties during 2014-2016. Chemical soil parameters were measured between 2014-2016 and analyzed by Tukey HSD. Letters indicate statistical difference between compost treatments. Asterisks indicate measurements done in 2014-2015.

Table S3. Pairwise comparison between p-values based on year or compost parameters in the microbial community analysis during 2014-2016. Comparison of the variation between years (2014, 2015, 2016) in G (A) and NY (B) analyzed by ANOSIM. Comparison of compost treatments in all samples during 2014-2016 in G (C) and NY (D) was analyzed by ANOSIM. Dissimilarity distance comparison between treatment/year pairs in Gilat (E) and NY (F) in each year, analyzed by PERMANOVA. Significant p-values are marked in red.

Table S4. PERMANOVA results comparing microbial communities (nMDS ordination based distances) between all treatments in each year. Pairwise comparison using PERMANOVA to all treatment pairs (0, 2, 4, 6 indicates for 20, 40 and 60 m^3ha^{-1} compost amendment soils) in 2014 (Y1), 2015 (Y2) and 2016 (Y3) are presented after

999 permutations as presented in the nMDS in Fig. 2C and D. Significant p-values are marked in red.

Table S5. Relative abundance comparison for microbial orders between different doses of compost amendment vs. conventional practice. The relative abundance of major orders was compared between treatments in each year (2014-2016). Significant (Wilcoxon test) differential abundance between treatments are marked in red.

Table S6. PERMANOVA results for all treatments in G and NY during 2016. Pairwise comparison using PERMANOVA of treatment pairs (0, 2, 4, 6 indicates 0, 20, 40 and 60 m^3/ha compost amendment) throughout all 2016 sampling time points together. Significant p-values are marked in red.

Table S7. Compost-amended and conventionally fertilized soil core microbiome features in NY and G. Shown is a list of the identified core OTUs for compost treatments in G, NY or both, as well as core OTUs that are shared between conventional treatments in NY and G.

Table S8. Significant OTUs identified by MaAsLin2 and ANCOM. List of significant OTUs that were identified by ANCOM, MaAsLin2, or shared by both methods, are presented for G (A) and NY (B) sites.

Figures - Cohen et al

Short- and long-term effects of continuous compost amendment on soil microbiome community

Figure 1

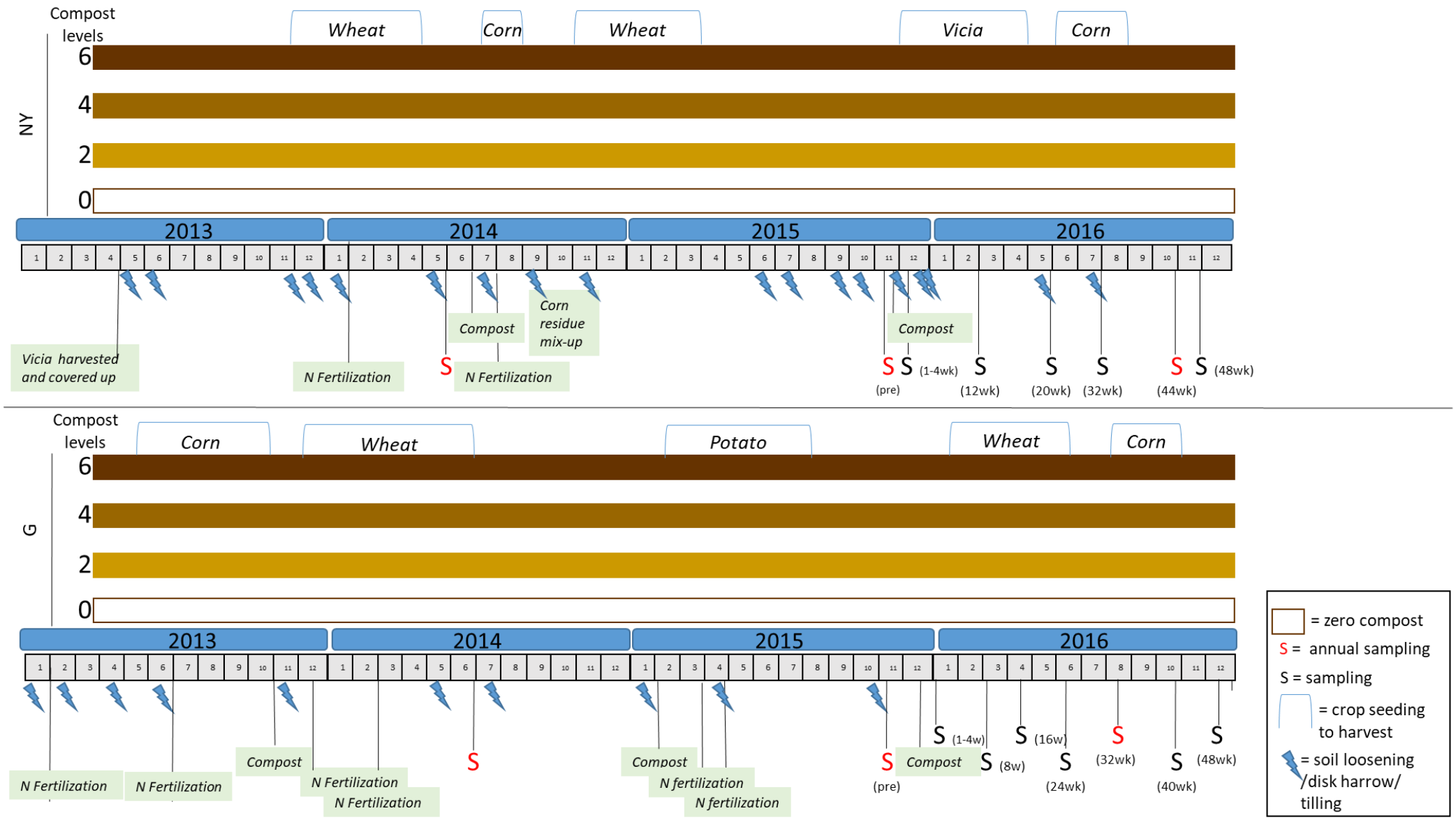

Compost levels
NY
6
4
2
0
Wheat
Corn
Wheat
Vicia
Corn
2013
2014
2015
2016
Vicia harvested and covered up
N Fertilization
S
Compost
N Fertilization
Corn residue mix-up
Compost
S (pre)
S (1-4wk)
S (12wk)
S (20wk)
S (32wk)
S (44wk)
S (48wk)
G
Compost levels
6
4
2
0
Corn
Wheat
Potato
Wheat
Corn
2013
2014
2015
2016
N Fertilization
N Fertilization
Compost
N Fertilization
N Fertilization
S
Compost
N fertilization
N fertilization
S (pre)
Compost
S (1-4w)
S (8w)
S (16w)
S (24wk)
S (32wk)
S (40wk)
S (48wk)
= zero compost
S = annual sampling
S = sampling
= crop seeding to harvest
= soil loosening /disk harrow/ tilling

Figure 2

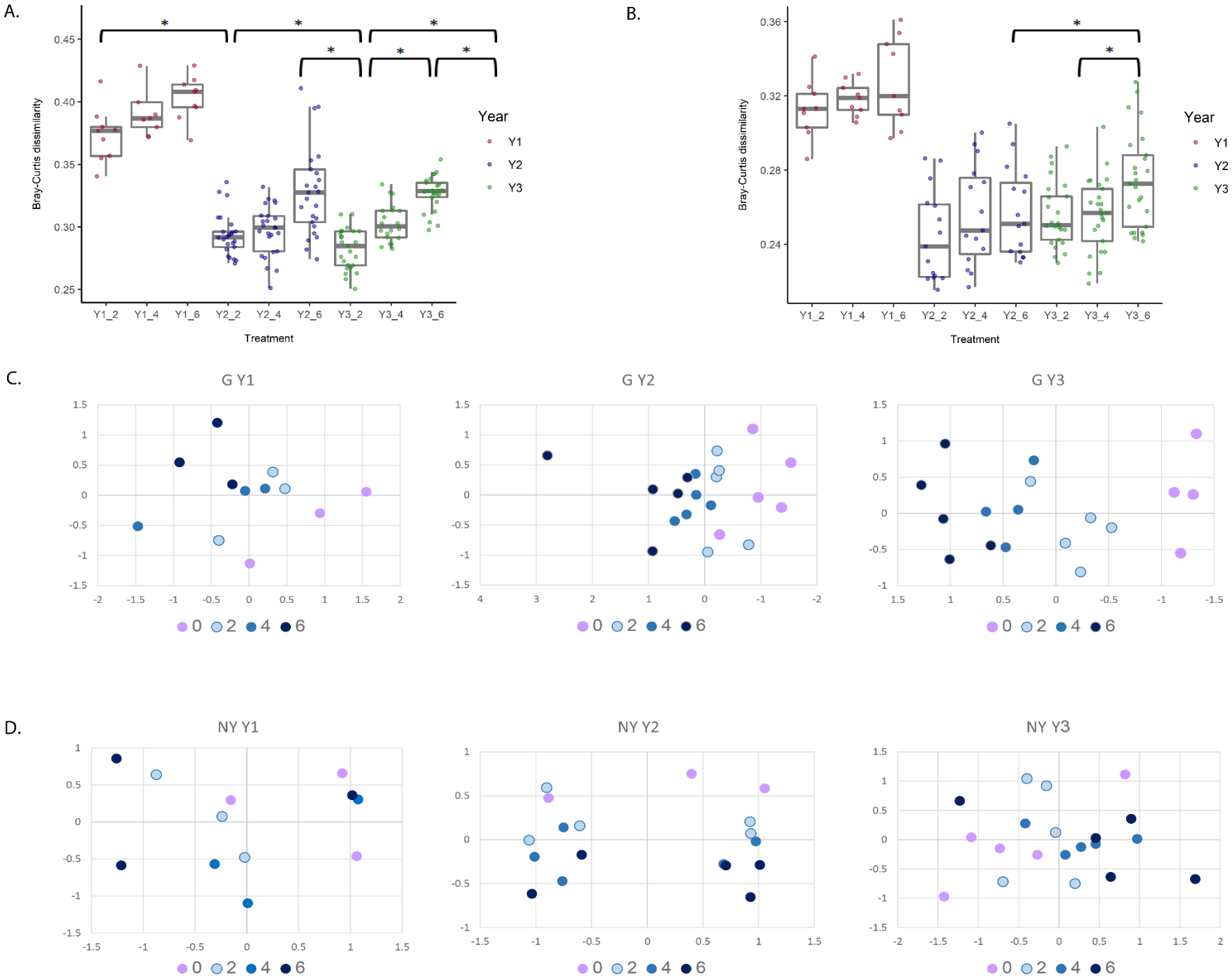

A.
Bray-Curtis dissimilarity
Treatment
Y1_2
Y1_4
Y1_6
Y2_2
Y2_4
Y2_6
Y3_2
Y3_4
Y3_6
Year
Y1
Y2
Y3
B.
C.
G Y1
G Y2
G Y3
D.
NY Y1
NY Y2
NY Y3
0
2
4
6

Figure 3

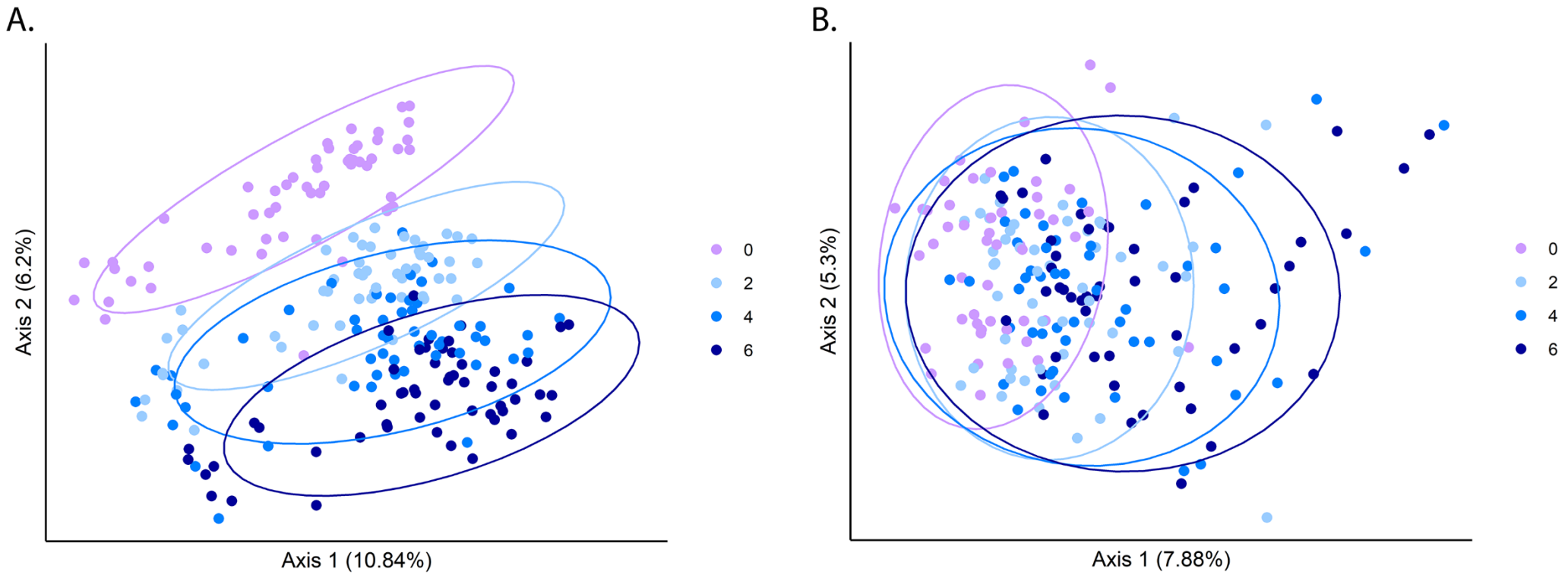


Figure 4

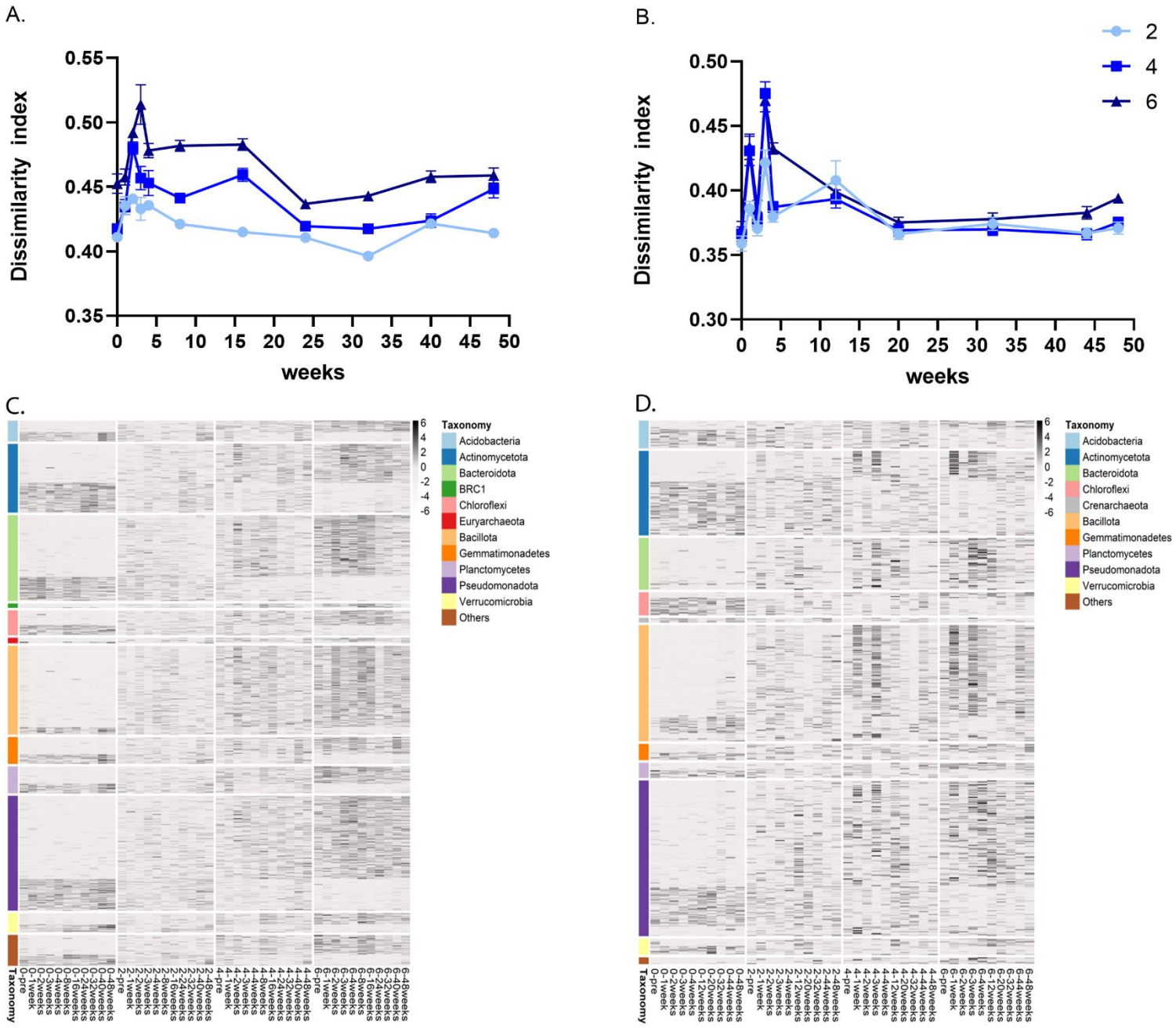
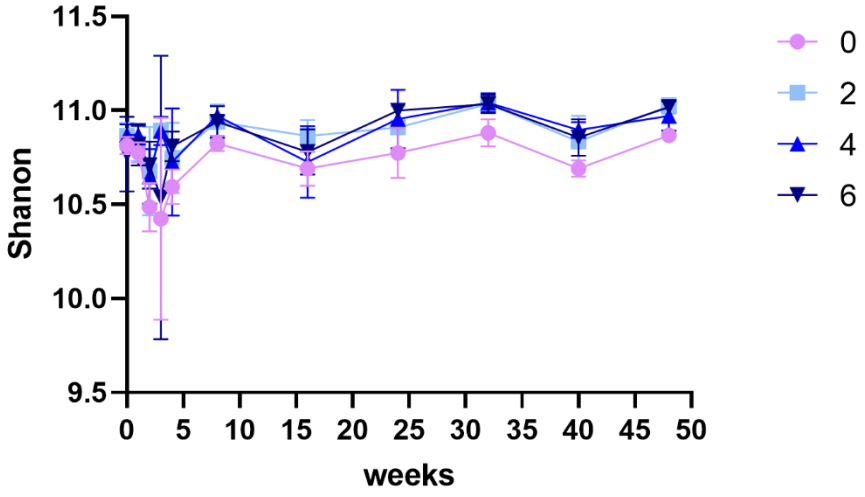

A.
Dissimilarity index
0.55
0.50
0.45
0.40
0.35
0 5 10 15 20 25 30 35 40 45 50
weeks
B.
2
4
6
Dissimilarity index
0.50
0.45
0.40
0.35
0.30
0 5 10 15 20 25 30 35 40 45 50
weeks
C.
Taxonomy
Acidobacteria
Actinomycetota
Bacteroidota
BRC1
Chloroflexi
Euryarchaeota
Bacillota
Gemmatimonadetes
Planctomycetes
Pseudomonadota
Verrucomicrobia
Others
D.
Taxonomy
Acidobacteria
Actinomycetota
Bacteroidota
Chloroflexi
Crenarchaeota
Bacillota
Gemmatimonadetes
Planctomycetes
Pseudomonadota
Verrucomicrobia
Others

Figure 5

A.


11.5
11.0
10.5
10.0
9.5
Shanon
0
5
10
15
20
25
30
35
40
45
50
weeks
0
2
4
6

B.

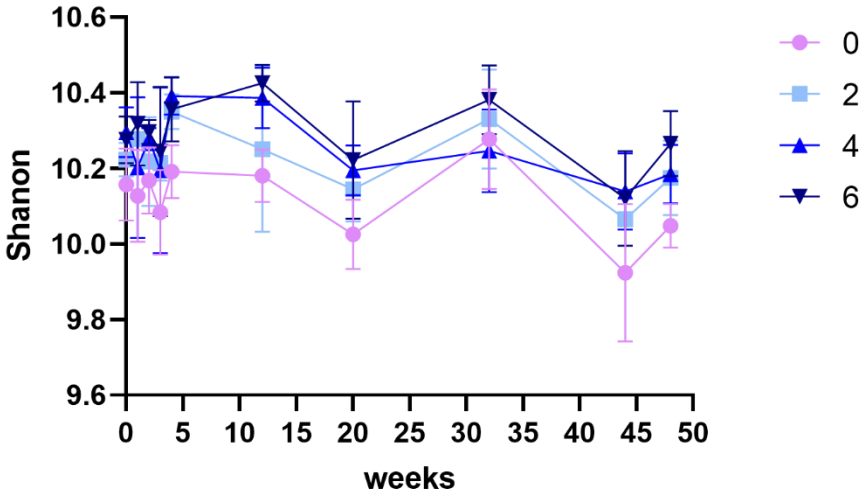
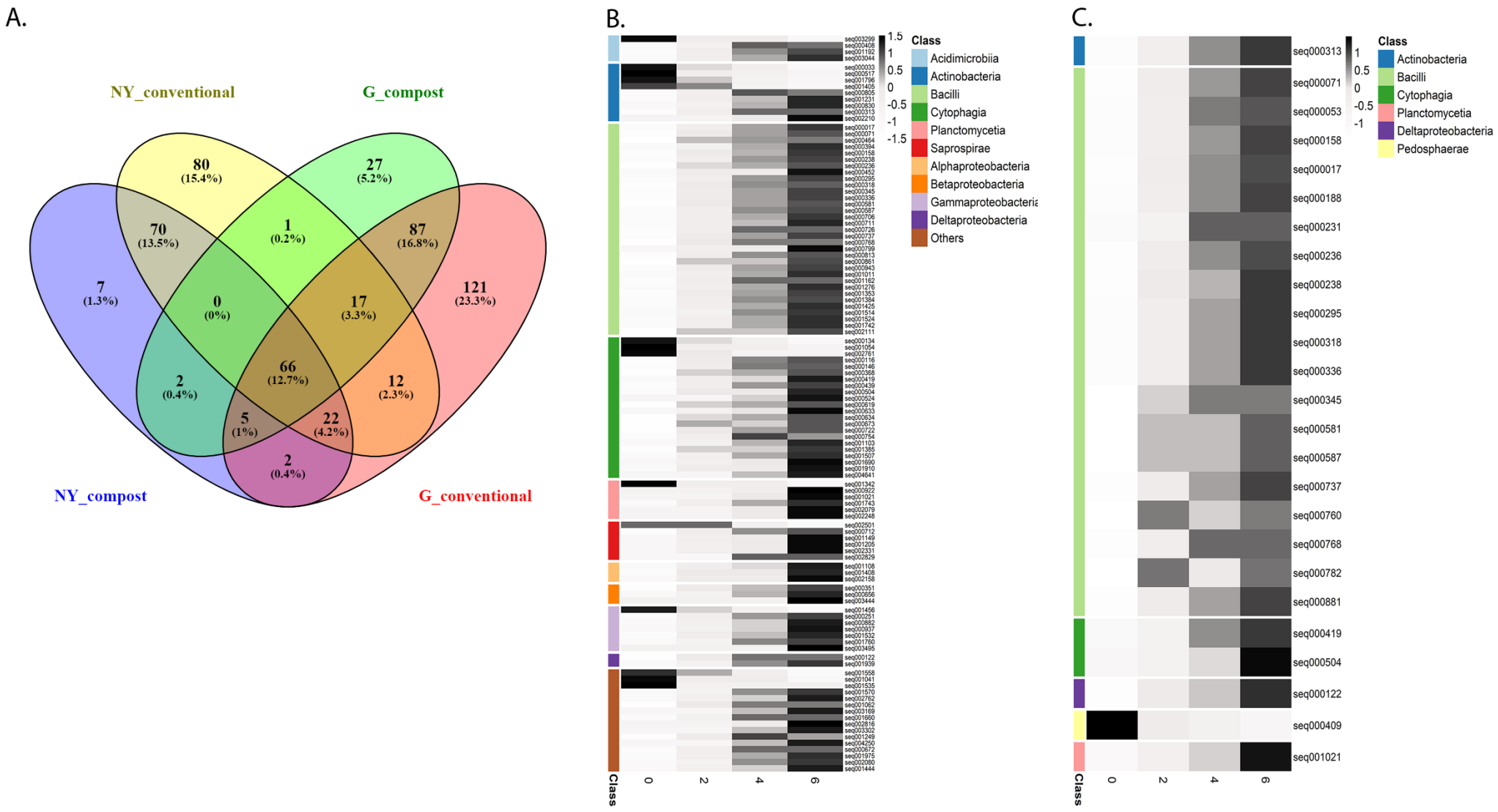

10.6
10.4
10.2
10.0
9.8
9.6
Shanon
0
5
10
15
20
25
30
35
40
45
50
weeks
0
2
4
6

Figure 6


A.
NY_conventional
G_compost
NY_compost
G_conventional
80 (15.4%)
27 (5.2%)
70 (13.5%)
1 (0.2%)
87 (16.8%)
7 (1.3%)
0 (0%)
17 (3.3%)
121 (23.3%)
66 (12.7%)
2 (0.4%)
12 (2.3%)
5 (1%)
22 (4.2%)
2 (0.4%)
B.
Class
Acidimicrobiia
Actinobacteria
Bacilli
Cytophagia
Planctomycetia
Saprospirae
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
Others
1.5
1
0.5
0
-0.5
-1
-1.5
Class
0
2
4
6
C.
Class
Actinobacteria
Bacilli
Cytophagia
Planctomycetia
Deltaproteobacteria
Pedosphaerae
1
0.5
0
-0.5
-1
seq000313
seq000071
seq000053
seq000158
seq000017
seq000188
seq000231
seq000236
seq000238
seq000295
seq000318
seq000336
seq000345
seq000581
seq000587
seq000737
seq000760
seq000768
seq000782
seq000881
seq000419
seq000504
seq000122
seq000409
seq001021
Class
0
2
4
6