Exploring Soil Health Indicators: Yearly Trends in PLFA Biomass and Bacteria-to-Fungi Ratios Across Depths in Regenerative Farming Systems

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Abstract

This study aims to assess how regenerative agriculture affects PLFA biomass and fungal-to-bacteria ratios in soil systems by depth yearly. There is an increased demand for food systems and overwhelming evidence that our current agricultural practices have devastating environmental effects. Results demonstrate that RA significantly enhances microbial biomass compared to conventional methods, with consistently higher biomass across all years. However, both systems experienced declines in biomass in 2023, with RA showing partial recovery in 2024 while conventional systems stagnated. Depth-specific analyses revealed significant increases in F:B ratios at deeper soil layers, though overall trends in F:B ratios fluctuated, with an increase in 2023 followed by a decline in 2024. These patterns suggest that while RA supports soil microbial health, broader environmental or systemic pressures, such as climate variability or soil management practices, may also influence microbial dynamics. These findings align with existing literature highlighting the benefits of RA for soil health and microbial diversity, yet underscore the complexity of soil ecosystems and the need for further research. The work supports the hypothesis that RA enhances microbial biomass over time and offers valuable insights for sustainable agricultural policy and practice while emphasizing the necessity of long-term studies to capture its potential benefits fully.

Keywords: Regenerative Agriculture, Phospholipid Fatty Acid, Fungal-to-Bacterial-Ratios, Soil Health Indicators

1 Introduction

By 2050, the world will need to produce 70% more food to meet the demands of a growing population, but conventional farming methods may not be up to the task (Hannah et al., 2020). Practices like plowing and leaving soil bare degrade soil health, reduce biodiversity, and accelerate climate change, threatening long-term agricultural sustainability (Murphy et al., 2016). Regenerative agriculture offers a promising alternative, combining age-old practices like no-tillage and cover cropping to restore soil health and support sustainable food production (Hermans et al., 2023).

At the heart of regenerative agriculture's benefits lies its impact on soil microbial communities, which are key to ecosystem health. Phospholipid Fatty Acid (PLFA) profiles serve as microbial "fingerprints," providing insights into microbial diversity and biomass (Steer & Harris, 2000; Leckie, 2005). Research shows that intensive farming practices often reduce microbial biomass and skew fungal-to-bacterial (F: B) ratios, favoring bacteria over fungi and diminishing soil's carbon storage capacity (Malik et al., 2016). Regenerative practices, on the other hand, increase organic matter and elevate F: B ratios, fostering healthier soils that can better support crop production (Willers, Jansen van Rensburg, & Claassens, 2015).

These shifts in microbial dynamics highlight the transformative potential of regenerative agriculture.By addressing soil degradation and enhancing microbial ecosystems, it paves the way for a more resilient agricultural future, capable of producing nutritious food while combating environmental challenges.

As researchers explore regenerative agriculture (RA) to improve soil health, challenges persist in securing funding for long-term studies that can fully capture its complexity

(Khangura et al., 2023). This funding gap limits our understanding of key indicators like phospholipid fatty acid (PLFA) profiles, which reveal microbial biomass and structure critical to evaluating farming practices.Evidence supporting RA's effectiveness in enhancing soil health and microbial diversity remains limited, underscoring the need for further research (Khangura et al., 2023).

Our study aims to address this gap by analyzing PLFA biomass data across years and depths over time to compare regenerative and conventional practices. By focusing on microbial structure and fungal-to-bacterial (F: B) ratios, this research will provide insights into how these farming systems impact soil health and microbial communities. Strengthening the evidence base for RA can support its adoption as a sustainable solution to improve soil quality and agricultural resilience.

2 Study Design and Data Collection

This study compared conventional and regenerative farming practices to evaluate the claims supporting regenerative agriculture. Conventional fields utilized practices such as full tillage and bare fallow periods, while regenerative fields incorporated no-tillage, cover cropping (single- and multispecies). Fields were categorized into two treatment types: conventional and regenerative.

Over three years, annual soil sampling was conducted at each field, with 15 randomly selected points per field combined into composite samples. Samples were collected at two depths (0–4 cm and 4–8 cm), resulting in 30 samples per field. Samples were promptly stored in coolers and sent to Regen Ag Lab for phospholipid fatty acids (PLFA) analysis to quantify microbial composition, including fungi and bacteria.

At Regen Ag Lab, PLFA profiles were measured to determine microbial biomass and composition, specifically focusing on total living microbial biomass, fungal-to-bacterial biomass ratio, total fungi, and total bacteria. Data variables also included collection year, research site location, and sampling depth. Results were documented in Excel for further statistical evaluation, providing insights into how regenerative practices influence soil health and microbial diversity.

3 Data Preparation and Statistical Analysis Methods

We used R software to analyze total living microbial biomass, which was log-transformed to meet the requirements for statistical modeling. Other variables, such as sampling depth (0–4 cm and 4–8 cm) and collection year (2022–2024), were treated as categorical variables to account for time and depth differences. Descriptive statistics and visualizations, like violin plots, summarized trends and variations in microbial biomass. A multivariable linear regression model was then used to evaluate how factors like fungal-to-bacterial ratio, total fungi, total bacteria, depth, and year influenced microbial biomass. Model assumptions for linearity, normality, and variance were verified, and significant factors were identified to highlight differences between regenerative and conventional farming.

To explore changes in fungi and bacteria with soil depth, another model was developed using fungal quantity, fungal-to-bacterial ratio, year, and depth. Previously, the RAD Lab used a single depth category ("0–6 cm"), but this was updated to "0–4 cm" and "4–8 cm" for better precision. Older "0–6 cm" data was reclassified into the new categories. The total fungal quantity was divided by 100 to simplify interpretation (measured in nanograms per gram of soil). This model revealed trends in fungal and bacterial dynamics across depths

and years, providing insights into how microbial communities vary in different farming systems.

4   Results

The graph visualizes the distribution of log-transformed microbial biomass across treatment types (regenerative and conventional) for each collection year (2022, 2023, 2024). It uses violin plots to show the density and variability of biomass values within each treatment type, with additional markers for the mean and box plots for detailed comparison. The graph is faceted by year, allowing comparisons of treatment effects within each specific year.
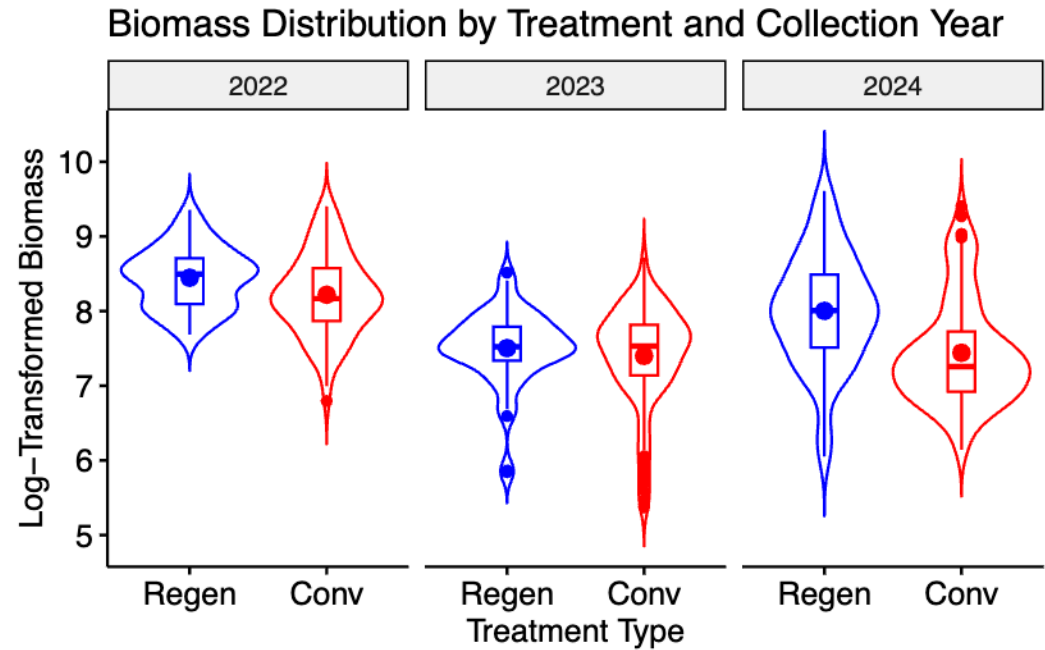

Figure 1. Effect of agricultural treatment type (Regenerative vs. Conventional) on microbial biomass across collection years, adjusted for treatment type, $p < 0.05$.

Regenerative treatments consistently show higher microbial biomass compared to conventional treatments across all years. In 2022, regenerative farming exhibits the highest

biomass with a relatively narrow range, indicating low variability. Both treatments experience a decline in 2023, with conventional farming showing a sharper drop. By 2024, regenerative farming demonstrates a partial recovery, as reflected by an upward shift in biomass, while conventional farming remains stagnant. The variability in biomass increases over time, particularly in regenerative treatments, as seen in the broader distribution in 2024.

Table 1: Mean and Standard Deviation of Biomass by Collection Year and Treatment

collection_year	treatment	mean	SD
2022	Regen	8.446976	0.4044310
2022	Conv	8.218864	0.5485161
2023	Regen	7.504302	0.5064431
2023	Conv	7.399912	0.6799865
2024	Regen	8.002866	0.7585431
2024	Conv	7.441762	0.7111841

Regenerative farming consistently maintained higher microbial biomass than conventional farming across all years. In 2022, the mean log-transformed microbial biomass was 8.4 (SD = 0.4) for regenerative treatments compared to 8.2 (SD = 0.5) for conventional treatments. Both treatments experienced a significant decline in 2023, with regenerative biomass dropping to 7.5 (SD = 0.5), a 10.7% decrease from 2022, and conventional log biomass falling to 7.4 (SD = 0.7), a 9.8% decrease. By 2024, regenerative farming showed resilience, partially recovering to a mean biomass of 8.0 (SD = 0.8), representing a 6.7% increase from 2023. In contrast, conventional farming showed no increase, remaining at 7.4 (SD = 0.7) in both 2023 and 2024. Variability in microbial biomass also increased over time, particularly for

regenerative farming, with the standard deviation rising from 0.4 in 2022 to 0.8 in 2024.
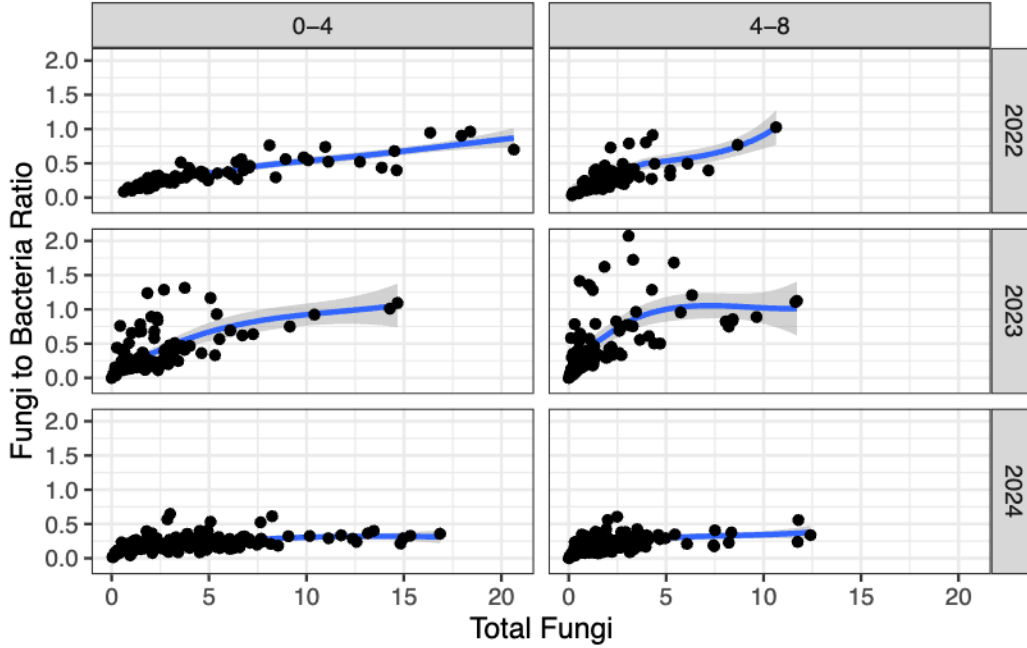

Figure 2. Effect of total fungi on the Fungi-to-Bacteria Ratio across soil depths (0-4 cm vs. 4-8 cm) and collection years (2022, 2023, 2024), p < 0.001.

This facet graph shows how the Fungi-to-Bacteria Ratio changes with Total Fungi at two soil depths (0-4 cm and 4-8 cm) over three years: 2022, 2023, and 2024. In the 0-4 cm depth, the ratio of fungi to bacteria increases as total fungi increases in 2022 and 2023, but by 2024, the trend flattens, showing little change regardless of fungi levels. At the deeper 4-8 cm depth, a similar increasing trend appears in 2022 and 2023, but the data is more scattered, as shown by the wider gray bands (confidence intervals). By 2024, the trend stabilizes, with the fungi-to-bacteria ratio remaining relatively flat.

Characteristic	Beta	95% CI[1]	p-value
(Intercept)	0.13	0.08, 0.17	<0.001
total_fungiper100	0.04	0.04, 0.05	<0.001
depth			

0-4	—	—	
4-8	0.07	0.04, 0.10	<0.001
collection_year			
2022	—	—	
2023	0.12	0.08, 0.17	<0.001
2024	-0.09	-0.12, -0.05	<0.001
Adjusted R²	0.340		
No. Obs.	796		

[1]CI = Confidence Interval

Table 2. Impact of total fungi, soil depth, and collection year on Fungi-to-Bacteria Ratios, adjusted for all variables, p < 0.001.

The regression analysis examining how Fungi-to-Bacteria Ratios are influenced by total fungi, soil depth, and collection year. The intercept value is 0.13, showing the baseline fungi-to-bacteria ratio when other variables are held constant. Total fungi (per 100) has a small but statistically significant positive effect (95% CI: 0.04–0.05, p < 0.001), meaning as total fungi increases, the fungi-to-bacteria ratio slightly increases. For soil depth, the 4-8 cm depth shows a positive effect (95% CI: 0.04–0.10, p < 0.001) compared to the 0-4 cm depth, indicating slightly higher ratios at deeper depths. Collection year also significantly impacts the ratio: compared to 2022, the ratio increased in 2023 (95% CI: 0.08–0.17, p < 0.001), but decreased in 2024 (95% CI: -0.12 to -0.05, p < 0.001). The adjusted R² value of 0.34 indicates that about 34% of the variation in the fungi-to-bacteria ratio is explained by this model, based on 796 observations.

5 Conclusion

The results of this study highlight that regenerative agriculture significantly enhances microbial biomass compared to conventional practices, with biomass consistently higher in regenerative systems over time. However, a decline in total microbial biomass was observed across the 2023 and 2024 collection years compared to 2022, indicating that other environmental or systemic factors may be influencing microbial communities beyond the practices themselves. These findings suggest that regenerative practices alone may not be sufficient to fully counteract broader pressures, such as climate variability or soil management practices, that could be impacting microbial populations.

This supports the research hypothesis that regenerative agriculture increases microbial biomass over time, though the observed decline suggests additional factors may need to be considered. Outliers in the data point to the complexity of microbial biomass and highlight the importance of considering other contributing factors when evaluating the effectiveness of regenerative practices. This trend is consistent with studies such as Fenster et al. (2021), which found that regenerative practices in almond production enhanced microbial biomass and soil health, and Khangura et al. (2023), which suggested that practices like crop rotation and reduced chemical use promote microbial diversity and soil health.

Soil depth plays a significant role in shaping the Fungi-to-Bacteria Ratio, with deeper soils (4-8 cm) showing a higher ratio compared to shallower soils (0-4 cm). However, the trend begins to level off by 2024, suggesting that other factors may be influencing microbial communities over time. The presence of outliers further emphasizes the complexity of microbial dynamics, highlighting the need for additional research into the factors driving these changes. These observations are consistent with studies like Fenster et al. (2021),

which found that soil depth impacts microbial biomass and diversity, and Khangura et al. (2023), which highlighted that soil management practices significantly affect microbial communities.

The overall results align with broader literature indicating that regenerative agriculture can improve soil health, microbial diversity, and ecosystem functioning. However, as the research does not establish definitive causality, further studies are needed to investigate the specific factors, such as soil type, climate, and crop rotations, that could influence these results. Future research should also explore the role of additional soil factors, such as organic matter, pH, moisture, and temperature, which directly impact microbial communities. These findings have practical implications for policy development, offering evidence for the benefits of regenerative practices in sustainable agriculture, and could help guide further research into their long-term effects across different environments and farming systems.

6 References

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7 Appendix

```
load(here::here("data/PLFA_clean.Rdata"))

load(here::here("CRARS_clean.Rdata"))


# fig 1

linda_clean <- linda_clean %>%

  mutate(logbiomass = log(total_living_microbial_biomass))
```

```r
treat_biomass <- linda_clean %>%

  select(treatment, logbiomass, collection_year) %>%

  na.omit()


treat_biomass %>%

  ggviolin(x = "treatment",

           y = "logbiomass",

           color = "treatment",

           add = c("mean", "boxplot")) +

  facet_wrap(~collection_year) +

  scale_color_manual(values = c("blue", "red")) +

  xlab("Treatment Type") +

  ylab("Log-Transformed Biomass") +   # Change the y-axis title here

  ggtitle("Biomass Distribution by Treatment and Collection Year")


# fig 2

treat_biomass %>%

  group_by(collection_year, treatment) %>%

  summarise(

    mean = mean(logbiomass, na.rm = TRUE),

    SD = sd(logbiomass, na.rm = TRUE)

  ) %>%

  kable(caption = "Mean and Standard Deviation of Biomass by Collection Year and Treatm
```

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2024	Regen	8.002866	0.7585431
2024	Conv	7.441762	0.7111841

```
# fig 3

bidepth %>%

  select(total_fungiper100, fungi_bacteria, depth, collection_year) %>%

  na.omit() %>%

  ggplot(aes(x = total_fungiper100, y = fungi_bacteria)) +

  geom_smooth() +

  geom_point() +

  theme_bw() +

  facet_grid(collection_year ~ depth) +

  labs(x = "Total Fungi", y = "Fungi to Bacteria Ratio")


# fig 4

model2 <- lm(fungi_bacteria ~ total_fungiper100 + depth + collection_year, data=bidepth)

model2 |>
```

```
tbl_regression(intercept=TRUE) |>

add_glance_table(include = c(adj.r.squared, nobs))
```

Characteristic	Beta	95% CI[1]	p-value
(Intercept)	0.13	0.08, 0.17	<0.001
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