Evaluating Microbial Diversity and Efficacy of Steam Disinfestation Treatments in the Salinas Valley Spinach and Lettuce Fields

By

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ABSTRACT

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As the challenges of climate change intensify, sustainable and environmentally friendly alternatives for pest and pathogen control in agriculture are becoming increasingly essential. Steam disinfestation, once overshadowed by chemical pesticides, is experiencing renewed interest due to rising environmental concerns. This project focuses on evaluating the efficacy of band steaming—a targeted steam disinfestation application along the seed line—in lettuce and spinach fields in the Salinas Valley. Although previous studies have demonstrated the effectiveness of methods like sheet steaming, a critical knowledge gap exists regarding band steaming for targeted pest control. This study takes a comprehensive approach, assessing both pest management efficacy and the impact of band steaming on the soil microbiome. Using a custom-built steam applicator with a low-pressure 1,000 kg/hour steam generator, trials were conducted in Salinas, CA, in 2022 and 2023. Results indicate effective weed suppression and reduced disease pressure from *Pythium spp.* and *Fusarium spp.* Additionally, analysis using 16S amplicon sequencing and enzyme assays reveals that, while band steaming initially disrupts the soil microbiome, significant recovery occurs within 30 days, suggesting that this method can support long-term soil health while controlling soil pests. Notably, all major soil ecological cycles showed resilience, with at least 50% recovery in soil functions and substantial recovery in alpha diversity. This comprehensive study aims to provide valuable insights into the effectiveness and feasibility of band steaming as a sustainable pest control strategy in vegetable crop production and its potential impacts on soil health. By examining the results from these multifaceted trials, we hope to inform the use of band steaming as a viable practice in sustainable agriculture.

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California's agricultural landscape is undergoing significant shifts due to evolving federal, state, and county regulations, alongside persistent labor shortages. Furthermore, the California Department of Pesticide Regulation released the Sustainable Pest Roadmap for California in 2023. This roadmap aims to eliminate high-risk pesticides and adopt sustainable pest management practices by 2025 (California Department of Food and Agriculture, 2023). Central to this roadmap are environmental considerations, emphasizing enhanced buffer zone sensitivity (California Department of Pesticide Regulations, 2021). While the roadmap seeks to support sustainable pest management, including organic agriculture, the sector faces challenges in weed and pathogen control due to the lack of herbicide options. Labor shortages further exacerbate these challenges, with legislation progressively raising the minimum wage to $15.50 in 2023, a 3.3 percent increase since 2022 (Tourte et al., 2023). Weed control significantly contributes to labor expenses, predominantly relying on manual hand-weeding methods that can cost approximately $284 per acre for two hand-weeding operations (Martin, 2019; Tourte et al., 2023).

1.1 Crop Production and Practices in Monterey County

According to the California County Agricultural Commissioners' Reports, Monterey County ranked fourth among the top 10 agricultural counties in California for the years 2021 and 2022. In 2021, Monterey County's total agricultural value was $4.1 billion, and in 2022, the total value increased to $4.6 billion (California Department of Food and Agriculture, 2023b). The leading commodities in Monterey County include lettuce (all types), strawberries, broccoli, and grapes (all types) (California Department of Food and Agriculture, 2023b). The county's fertile land supports a long history of agricultural production, with soils ranging from poorly drained to

well-drained. The best soils for growing leafy greens and strawberries are moderately alkaline clay loam, silty clay loam, and moderately alkaline silt loam. Additionally, the coastal Mediterranean climate allows for a nearly year-round agricultural production, with higher humidity near the coast (PAST Consultants LLC., 2010).

1.1.1 Romaine Lettuce

Lettuce is the second most valuable crop in Monterey County, with a total production value of $1,275,598,000, grown on ~ 40,752 hectares (Monterey County Agricultural Commissioner, 2023). Romaine lettuce (*Lactuca sativa*) is cultivated on about ~ 21,367 hectares, contributing $653,512,000 to the total production value. Lettuce is typically grown in high-density plantings with six rows per 80-inch-wide beds (Tourte et al., 2023). Growing lettuce in the lush Monterey County is favorable due to ideal soil and climate conditions; however, it is crucial to carefully select the land for cultivation because of lettuce's sensitivity to pests such as weeds and pathogens, which can cause severe yield loss (Natwick et al., 2017). Lettuce heavily relies on herbicide applications to prevent weed emergence, as it is a weak competitor against most weeds (Smith et al., 2017). Problematic weeds include groundsel, prickly lettuce, and sow thistle, with limited herbicides available for use in lettuce fields. Commonly used pre-plant herbicides are Paraquat (Gramoxone), pelargonic acid (Scythe), glyphosate (Roundup), and carfentrazone (Shark) (Natwick et al., 2017). Metam sodium, a soil fumigant, is sometimes used in lettuce production to control soilborne diseases, nematodes, and some weeds. Another pre-plant herbicide, benefin (Balan), can be used, but its residual effects can impact subsequent crops like spinach, which are sensitive to benefin (Natwick et al., 2017). During planting, pre-emergence herbicides such as pronamide (Kerb) can be applied through sprinkler irrigation after seeding or via chemigation 3 to 5 days after the first irrigation (Natwick et al., 2017). Despite

these precautions, hand weeding is necessary 10 to 14 days after thinning to remove persistent weeds. Lastly, the lettuce is cultivated twice during its growing season.

1.1.2 Spinach

Another leafy green of interest grown in Monterey County is spinach (*Spinacia oleracea*), cultivated on ~7,029 hectares with a total production value of $138,963,000 (Monterey County Agricultural Commissioner, 2023). Spinach is typically grown for both the fresh and frozen markets, varying in seasonal days with fresh crops taking 30 to 50 days and frozen market crops taking 70 to 120 days. Like lettuce, spinach can be grown year-round in the Salinas Valley if the climate allows (LeStrange et al. 2012).

Spinach is cultivated in high-density seed lines, typically with 16 to 24 lines per 80-inch bed. The crop is highly sensitive to pests such as weeds, nematodes, and diseases due to its compact planting style and weak competitive ability (LeStrange et al. 2012). Weed control is essential for spinach production, but there are limited herbicide options, with four commonly available: cycloate, phenmedipham, clethodim, and sethoxydim (LeStrange et al. 2012). None of these herbicides control all the weeds that infest in spinach fields (LeStrange et al. 2012). Typically, the best tactic for pest control is pre-plant fumigation with metam sodium or using pre-plant herbicides. Post-emergent herbicides such as phenmedipham (Spin-Aid) or sethoxydim (Poast) can also be used. However, reliance on herbicide applications poses challenges such as poor herbicide application causing discoloration, damage, and deformity of the spinach (LeStrange et al., 2012). Fresh market spinach is challenging to treat with herbicides due to its short growing season, which leaves little time for recovery (Fennimore et al., 2001). Common weeds in spinach fields include burning nettle (*Urtica urens* L.), common chickweed (*Senecio vulgaris* L.), and pigweeds (*Amaranthus)* (Fennimore et al., 2001).

1.1.3 Organic Production of Leafy Greens in Monterey County

Organic production also faces weed control challenges but in a greater urgency than conventional agricultural fields. Without the use of herbicide organic production there are limits in the resources they can use to control for such pest. In lettuce and spinach organic production emphasizes preventative measures such as careful cultivation and extensive hand labor to control for pest such as weeds (LeStrange et al. 2012, De Cauwer et al., 2021). Lettuce is typically grown in 40-inch beds in two rows, but there are cultivars like romaine that are planted in 5-6 seed lines on 80-inch beds. Primarily, the main way to reduce weed pressure is prevention, and that includes field sanitation protocols, crop rotations. Another common technique is preplant germination of weeds before or after final bed shaping to control weeds through shallow cultivation or flaming which can reduce weed pressure up to 50% if done before seeding. Although flaming or the application of organic herbicides can be utilized in lettuce production, these methods are rarely implemented. Lettuce's rapid germination allows insufficient time for effective pre-emergence weed control. Furthermore, these approaches are generally ineffective against grass weed species. Deep plowing, the tillage technique to bury the weed seeds deeply to prevent the seeds capability to emerge is another common tactic. Lastly solarization is another non-pesticidal method to treat pest in organic production. In solarization the soil first needs to be irrigated, then it is covered in clear plastic for 4-6 weeks ideally in hot climatic periods (Elmore et al., 1997). The combination of heat and plastic trapping the sun’s energy in soil can reduce weed seed emergence in the soil and some soil borne pathogens (Natwick et al. 2017). This tactic while promising with its capacity of improving soil structure and increasing nitrogen availability it requires hot climatic temperatures to control soil-borne diseases and weed seeds, thus is less effective in cooler climates such as coastal areas (Samtani et al., 2012; LeStrange et al. 2012).

1.2 Band-Steaming

The growing interest in sustainable practices and the necessity for improved pest management tactics in organic farming have sparked a movement towards alternative pest management strategies. For example, interest in steam disinfestation has increased due to rising labor costs and regulatory pressures. Originating in the 1880s, steam disinfestation effectively controls weeds and eliminates soil-borne pathogens (Baker, 1962). The earliest approach, broadcast sheet steaming, involves applying steam to the entire surface area of a field or plot, but this method consumed excessive energy and had limited efficiency (Gay et al., 2010a). Band-steaming, which injects steam into narrow seed line lanes, offers higher efficacy with reduced resource input (Guerra et al., 2022). This method effectively addresses persistent weed and pathogen control issues (Guerra et al., 2022; Gullino, 2022). The heat inhibits weed seed germination and suppresses pathogens at soil temperatures exceeding 70°C (Baker, 1962; 1970). Furthermore, adjusting the depth and width of band injectors can significantly influence heat retention in the soil (Gay et al., 2010b). The adjustment in the depth of band steam injection can help target weed seeds that germinate from greater depths. Such characteristics are favorable for the refinement of band steaming but also offer room for improvement. Previous research by Gay et al. (2010a) highlighted that heat retention is a critical factor, with deeper soil depths allowing for temperatures to be maintained for longer durations. However, the study by Gay et al. (2010b) was conducted in controlled environments rather than in field settings. Additionally, other band steaming studies have typically focused on shallower depths, averaging around 5 cm. Consequently, further research into the mechanisms of band steaming is crucial to optimize its practical application.

1.2.1 Weed Control

Herbicide-resistant weeds present a significant challenge in the agricultural sector (Hanson et al., 2014; Baucom, 2019). Research in the UK has shown a correlation between historical herbicide use and weed resistance, impacting food production and economics (Hicks et al., 2018; Varah et al., 2019). Band steaming offers an alternative, potentially mitigating resistant weeds and reducing environmental toxicity. Current studies have demonstrated the capability of band steaming to reduce weed emergence without the use of herbicides (Fennimore et al., 2014; Guerra et al., 2022)

In a recent study, three different band steamers were used, all successfully reducing weed emergence, leaving only residual weeds, and overall lowering manual labor requirements. Specific weeds such as hairy nightshade, little mallow, shepherd's purse, and burning nettle decreased by 64% or more after steam treatments (Guerra et al., 2023). Similarly, studies conducted in strawberry fields found that steaming effectively controlled weeds such as purslane and nutsedge (Kim et al., 2021). This is consistent with earlier research by Fennimore et al. (2014), which showed that band steaming lowered weed density in strawberry fields in Watsonville and Salinas, specifically reducing weeds such as chickweed, knotweed, mallow, yellow nutsedge, bluegrass, purslane, and burning nettle. In all the studies, band steaming significantly reduced weed emergence, leaving minimal residual weeds, and thereby reducing the need for manual labor in weed control.

1.2.2 Soil-Borne Diseases

An additional advantage of band steaming is its dual benefit: it not only suppresses weed emergence but also controls soil-borne diseases. With climate change driving up temperatures and extending drought periods, the pressure from soil-borne pathogens is expected to increase (Pathak et al., 2017). Pathogens like *Fusarium* and *Pythium* spp. thrive in stressed plants,

particularly under drought and heat conditions. For example, *Fusarium oxysporum* f. sp. *lactucae* is closely associated with warmer climates, additional pressure on lettuce crops (Scott et al., 2009). Historically, chemical pesticides have been the primary means for managing soil-borne pathogens, but they pose significant environmental risks. For instance, methyl bromide, a fumigant that was widely used in strawberry production, has been banned since 2005 due to its harmful impact on the ozone layer (Fennimore et al., 2008). In addition to its toxicity, methyl bromide—along with other fumigants like metam sodium—has been shown to disrupt soil microbial communities, reducing microbial diversity, enzyme activity, and microbial respiration over time (Macalady et al.,1998; Ibekwe et al., 2001). The overuse of pesticides has also led to broader environmental issues, including contamination of soil and water systems (Syafrudin et al., 2021). Runoff from pesticides, for instance, can severely affect aquatic ecosystems and water quality (Syafrudin et al., 2021).

In regions like California's Salinas Valley, where soil-borne pathogens such as *Fusarium* and *Pythium* spp. are prevalent, alternative methods for pest management are increasingly essential (Monterey County Agricultural Commissioner, 2022; Smith et al., 2023). *Fusarium oxysporum* is part of an extensive species complex with over 100 host-specific strains (formae speciales), making it particularly difficult to identify (Gordon, 2017; Burkardt et al., 2019). *Fusarium* spp. is also challenging due to its persistence in soil as dormant chlamydospores, which can be reactivated by root exudates (Gordon, 2017). Once the pathogen reaches the root, the fungus can invade the host vascular system, leading to diseases like *Fusarium* wilt. *Pythium* spp. also displays high resilience through the formation of oospores, which can survive long periods without a host (Martin and Loper, 1999). Among the plant pathogens, *Pythium* spp. displays remarkable complexity, with some species acting as generalists, while others are more

host specific. For instance, *Pythium ultimum*, a major pathogen of lettuce, can infect various plant species. The presence of excessive weeds can exacerbate *P. ultimum* infections, as the pathogen can persist within them (Barboza et al., 2021). Furthermore, *P. ultimum*, rapid sporangia formation allows it to cause significant seedling damage shortly after infection, reducing crop vigor and yield (Martin and Loper, 1999; Schroeder et al., 2013).

Non-chemical management strategies, including crop rotation, resistant varieties, sanitation, and solarization, have been employed but often offer limited and inconsistent pathogen control, and can be less economically viable (Martin and Loper, 1999; Gordon and Koike, 2015). For instance, although genetic resistance has been used against *Fusarium oxysporum* f. sp. *lycopersici* in tomatoes, successive pathogen races have repeatedly overcome this resistance, leading to widespread crop losses (Swett et al., 2023). Solarization, another pest control alternative, has shown variable effectiveness in coastal California due to its reliance on consistently high temperatures for optimal results. For instance, Samtani et al. (2012) found that solarization alone did not effectively reduce *Verticillium dahliae* populations. In the same study they found that solarization generally was less effective than steam treatments for managing pests like weeds and pathogens, with steam significantly lowering pathogen pressure.

Given these challenges, band steaming has emerged as a promising, eco-friendly alternative for managing soil-borne pathogens. Studies have demonstrated its effectiveness in reducing *Fusarium* and *Pythium* populations in the soil (Triolo et al., 2004; Fennimore, 2014; Kim, 2021; Guerra et al., 2022). For instance, recent work by Guerra et al. (2023) showed that *Pythium* spp. pressure was reduced by 50–100% in steamed soil compared to untreated plots. These findings align with Kim et al. (2022), who reported that steam treatment was similarly effective to methyl bromide in controlling *Pythium ultimum*. In a long-term study, Triolo et al.

(2004) tested steam in open-field conditions for its efficacy against *Fusarium oxysporum* f. sp. *basilici* in basil and achieved pathogen reductions of 74.9–76.8%. Such results highlight steam's potential. As climate change intensifies disease pressures, sustainable strategies like band steaming could play a critical role in resilient agriculture, offering an effective solution for managing resilient soil-borne pathogens.

1.3 Soil Health: Soil Microbial Community

As we continue to evaluate the efficiency of band steaming in pest control, it is crucial to consider its potential impacts on soil health, much like other agricultural methods. California has recently emphasized the preservation of nutrient cycling and microbial biodiversity, which are essential for the long-term sustainability of agricultural fields (California Department of Food and Agriculture, 2023b). To illustrate the potential impacts on soil health, a study conducted by Roux-Michollet et al. (2010) applied steam at 120°C to the topsoil layer. The soil temperature rose from 17°C to 100°C in the 0–5 cm layer and reached approximately 55°C at an 8 cm depth. After steaming, 24 treated and 12 untreated soil samples were incubated at 21°C for up to 10 days and analyzed for organic matter, soil respiration, and bacterial populations (Roux-Michollet et al., 2010) The results showed an initial decline in bacterial population, followed by a tenfold increase, a surge in carbon mineralization, and changes in genetic structure of the microbial community. These effects were strong but quickly reversible, highlighting the short-term impact of steam treatment on soil health and microbial activity. Roux-Michollet et al. (2010) concluded that steaming likely breaks down organic materials, including lignin-like substances and carbohydrates, disrupting soil structures. This supports previous findings from Roux-Michollet et al. (2008) regarding immediate decreases in community activity after steam treatments, particularly affecting nitrogen cycling—increased respiration and denitrification but lower

nitrification levels after 62 days. Thus, nitrifiers were more affected than denitrifiers and heterotrophic bacteria.

Band steaming may have less drastic results compared to broadcast steaming as suggested by Elsgaard et al. (2010), who investigated band steaming at 80-90°C. They found minimal impact on soil pH and water content, with soil respiration mostly unaffected. However, enzyme activities were significantly inhibited with weak recovery over 90 days. Bacterial colonies increased, while fungal propagules were reduced by 50%, persisting at a 38% reduction after 90 days. Although short-term effects were ecologically tolerable, potential long-term impacts exist due to weaker recovery. However, since band-steaming affects only a small portion of the plow layer it could be mitigated by annual tillage. Therefore, there is a need to fully investigate band-steaming effects, considering its refinement in treatment that can decrease the impact on soil microflora.

Band steaming, which targets specific areas, may offer a refined approach with potentially less microbial disruption. Therefore, this study includes microbial profiling via 16S rRNA gene sequencing and enzymatic activity assays to evaluate the impacts of band steam treatments. Recent advances in 16S rRNA gene sequencing have made it an accessible tool for characterizing microbial communities at a detailed taxonomic level, allowing for a comprehensive assessment that was previously challenging (Fadrosh et al., 2014). This study also incorporates enzyme assays to provide complementary data alongside microbial DNA analysis, offering a more comprehensive view of soil health. Enzymes play a crucial role in decomposing organic matter and facilitating nutrient availability in soil ecosystems. The enzyme assays included in this study target key nutrient cycles, focusing on β-glucosidase (BG), cellobiohydrolase (CB), β-xylosidase (XYL), α-glucosidase (AG), and N-acetyl-β-D-

glucosaminidase (NAG) for carbon cycling; phosphatase (PHOS) for phosphorus cycling; and leucine aminopeptidase (LAP) for nitrogen cycling, following protocols established by Bell et al. (2013). However, it is important to note that 16S rRNA gene sequencing at the species level is still evolving, with many taxa yet to be identified. While this technique offers significant advancements over previous microbial community studies, it still has limitations in fully characterizing microbial diversity. Similarly, enzyme activity assays are constrained by the range of enzymes that can be feasibly tested, which may not capture the full complexity of nutrient cycling processes in soil.

1.4 Conclusion

This study highlights the potential of band steaming as a viable pest management solution, focusing on both weed and pathogen control while preserving soil health. Furthermore, our research evaluates various configurations of the steam applicator to optimize its efficacy. Additionally, understanding the impact of band steaming on the soil microbiome is essential for assessing its long-term implications on soil health and sustainability. By thoroughly examining the effectiveness of band steaming for pest control and its influence on soil microbial communities, this project offers valuable insights into its viability as an alternative pest management strategy. Its successful application in Monterey County demonstrates band steaming's promise as a sustainable agricultural practice, balancing effective pest control with minimal impact on soil health

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CHAPTER 2: Band Steaming Efficacy for Pest Control in Monterey County lettuce production

Monterey County is a key player in California's agricultural production, especially in the cultivation of leafy greens like romaine lettuce, covering approximately 40,752 hectares in 2023 (California Department of Food and Agriculture, 2023). Like much of the region, romaine lettuce farming heavily relies on pesticides and herbicides due to the crop's limited ability to compete with weeds (Smith et al., 2017; Natwick et al., 2017). However, the intensive use of pesticides has led to significant issues, including pesticide-resistant weeds and water contamination (Hicks et al., 2018; Varah et al., 2020; Syafrudin, 2021). Additionally, rising labor costs and ongoing labor shortages have made traditional, labor-intensive weed control methods less viable (Martin, 2019; Tourte et al., 2023). Weed control significantly contributes to labor expenses, predominantly relying on manual hand-weeding methods that can cost approximately $284 per acre for two hand-weeding operations for conventional production (Martin, 2019; Tourte et al., 2023). These challenges are even more pronounced in organic agriculture, where the absence of herbicides results in higher weed densities (Guerra et al., 2022).

In response to these challenges, steam pasteurization has emerged as a sustainable alternative, using heat to control both weeds and pathogens. Steam pasteurization has a longstanding history in agriculture, tracing its use back to the 1880s (Baker 1962). Distinct from traditional pesticides, steam pasteurization uses heat to kill weed seeds before their emergence and to pasteurize the soil-borne pathogens. Temperatures above 60-70°C are applied to the top 0-15 cm layer of soil for vegetables, effectively suppress soil pathogens (Lu 2009; Kim et al., 2021). Traditionally, steam was applied via a broadcast sheet, a method involving steaming under a sheet to treat the entire surface area of a field or plot, though it proved to be fuel intensive (Gay et al 2010a). In contrast, mobile band steaming has emerged as a less costly and

more efficient alternative (Guerra et al. 2022). This technique applies steam in narrow strips along the intended seed lines before planting and has been shown to be effective in controlling pests such as weeds and pathogens (Pinel et al. 1999; Guerra et al. 2022).

Steam pasteurization's ability to target both weeds and soil-borne pathogens offers a significant advantage for agricultural management. In a recent study by Guerra et al. (2022), band steaming significantly reduced weed emergence, resulting in minimal residual weed emergence after treatment and thereby decreasing the need for manual weed control. This aligns with earlier findings by Fennimore et al. (2014), who demonstrated that steaming reduced weed density in strawberry fields in Watsonville and Salinas. Similarly, Kim et al. (2021) found that steam effectively controlled weed emergence in strawberry fields. Furthermore, Guerra et al. (2022) showed that band steaming also effectively reduces pathogens, such as *Pythium* spp. and *Sclerotinia minor*. Additionally, Lu et al. (2009) reported a 90% reduction in *Fusarium oxysporum* with steam treatments at 70°C or higher. In regions like Monterey County, where *Fusarium oxysporum* f. sp. *lactucae* severely affects lettuce production and *Pythium ultimum* threatens leafy greens, addressing these diseases is critical (Martin and Loper, 1999; LeStrange et al., 2012; Natwick et al., 2017). Traditional management practices include avoiding overwatering or applying chemical inputs (Martin and Loper, 1999; Koike et al., 2020). However, chemical applications can negatively impact the environment and pollinators, like bees, if misapplied (Natwick et al., 2017). While non-chemical strategies such as crop rotation, resistant varieties, sanitation, and solarization provide some benefits, they often face economic challenges and inconsistent results (Martin and Loper, 1999; Gordon and Koike, 2015; Natwick et al., 2017). For instance, Samtani et al. (2012) found that solarization alone did not effectively reduce pathogen pressure, since it was highly reliant on consistently high temperatures for

optimal results. Thus, band steaming emerges as an environmentally friendly and effective alternative for simultaneously targeting two types of pests in agricultural fields.

Nevertheless, the application of steam pasteurization, through band steaming, requires further research to fully refine its effectiveness much like other pest management techniques. Steam injection introduces heat flux, facilitating an exchange among the soil's solid, liquid, and vapor phases, with steam naturally rising to the surface, leading to heat loss (Gay 2010a). The increase in soil temperature is significantly influenced by the configuration of treatment injectors, including their depth and width. Research by Gay et al. (2010b) examined the temporal and spatial distribution of temperature following steam injection, finding that at an injector depth of 15 cm, temperatures initially exceeded 90°C but decreased as heat moved towards the surface, averaging 60°C at a 9 cm depth. This variability in optimal band dimension underscores the need for tailored approaches to optimize injector configurations to maximize steam efficiency.

Furthermore, this research continues to explore and optimize band steaming by manipulating the dimensions of steam injectors. Specifically, it calls for an examination of how varying injector depths and widths affect soil temperature while simultaneously evaluating pathogen and weed control in vegetable crop production. This study specifically evaluated greater injector depths and widths than those used in previous studies, where injectors were typically placed at 5 cm depths in experimental fields (Hansson and Svensson, 2007; Elsgaard et al., 2010).

This study evaluates band steaming as a sustainable alternative for weed and disease This study evaluates band steaming as a sustainable alternative for pest management in both experimental and commercial fields. It is divided into two parts: the first focuses on optimizing steam injector width and depth, while the second assesses its effectiveness in commercial lettuce

fields. The results highlight band steaming's potential as an eco-friendly method for reducing soil-borne diseases and weeds, with promising applications in vegetable production in Monterey County. As environmental concerns and regulations intensify, band steaming presents an innovative, sustainable solution for pest control in agriculture.

MATERIALS AND METHODS

During 2022-2023, five steam trials were conducted on lettuce fields. Each trial employed a band steam applicator built (Fig. 1) at the Keithly Williams shop at Yuma, AZ set to maintain soil temperatures between 60 and 70°C for 20 minutes, aiming to suppress soil pathogens and weeds. This design builds upon the previous work by Guerra et al. (2021) and incorporates a Simox Agrivap 2008 model steam generator. The steam generator, with a capacity of 65 BHP (boiler horsepower), will be mounted on a bed shaper sled (Simox, La Forêt, France). The steam implement was towed by a 5520 John Deere tractor with the engine set at a 1500 to 1700 RPM, moving 2.4 to 3.7 meters per minute while steaming is being injected via shank injectors. Trials 1 through 2 assessed the efficacy of different injector dimensions on lettuce fields. Trials 3 through 5, were conducted in Soledad, California, focused on the efficacy of steam treatment for weed and pathogen control in commercial lettuce fields. A randomized complete block design was implemented, with Hartnell trials assigning individual beds to treatments. In the Soledad trials, each trial featured two blocks, with each block containing a single lettuce bed partitioned into three steam-treated plots and two untreated control plots. Temperature data were recorded at a depth of 10 cm using HOBO T-Type thermocouples (U12 Outdoor, Onset Computer Corp., Pocasset, MA) during steaming and for 24 hours post-steaming in trials 1-5. The highest soil temperatures from two points within the 10 cm depth range were averaged to determine the peak temperature reached during the first hour of steaming.

Study location and environmental conditions.

Field trials 1 and 2 were conducted in Hartnell College Research fields near the USDA Salinas Center during the summer of 2023, using a custom-built steam applicator (36°40′ 10.0399 N; 121°36′ 19.9784 W). The soil type is a loam with 53% sand, 32% silt, 15% clay and with 2.09% organic matter. The electric conductivity of the soil is 1.65 dS/m with a pH of 7.03. Trials 3 to 5 were conducted in commercial fields in the Soledad region, characterized by fine loamy soil with a composition of 35% sand, 31.5% silt, 31% clay, and 2.5% organic matter. The soil's electrical conductivity is 1 dS/m, with a pH of 7. All trials were conducted on romaine lettuce (*Lactuca sativa var. longifolia*).

Steam application

In 2022, trial 1 was initiated in August, at the Hartnell College Research fields near the USDA Salinas Center to investigate the effects of steam treatment band width and depth on lettuce. Trial 1 tested two band widths— 5 and 10 cm—and three depths—5, 10, and 15 cm. Furthermore, the trial comprised six different band configurations (treatments) plus a non-treated, with four replicates for each treatment. The steam treatments specific configurations for trial 1 were as follows: 5-cm width by 5 cm depth (treatment 1), 5-cm width by 10 cm depth (treatment 2), 5-cm width by 15 cm wide depth (treatment 3), 10 cm width by 5cm depth (treatment 4), 10 cm width by 10cm depth (treatment 5), and 10 cm width by 15 cm depth (treatment 6). Each replicate consisted of a single bed measuring 1.02 m wide by 25.9 m long.

In 2023, a similar layout was conducted in trial 2 and included six treatments in total, each replicated four times within a single bed measuring 1.02 m wide by 36.6 m long. On August 29 and 30, 2023, steam was applied as a band along the seed lines in raised beds. This trial explored four different band configurations (treatments), a pronamide treatment (Kerb SC3.5 p.t/A) and non-treated plots. The steam treatments specific band configurations are as followed:

10 cm width by 7.6 cm depth (treatment 7), 10 cm width by 12.7 cm depths (treatment 8), 12.7 cm width by 7.6cm depth (treatment 9) and 12.7 cm width by 12.7 cm depth (treatment 10). Romaine lettuce (*Lactuca sativa var. longifolia*) was direct seeded into two plant lines with 30.5 cm of inter-row spacing using a Stanhay precision planter on August 31, 2023. On September 1, pronamide (Kerb SC) was applied at 3.5 pt/A as a broadcast spray over 40 gallons per acre.

Trials 3 to 5 were conducted in commercial fields in Soledad, California. Each of these trials was carried out in two beds, each 2 m wide by 366 m long, within a specific section of the field. Within each bed, two sections measuring approximately 2 m wide by 9 m were designated for non-treated control plots, effectively dividing the rest of the bed into three distinct sections, with the remaining areas receiving the steamed treatment. Subsequently, romaine lettuce was direct seeded across six plant lines post-treatment.

Recording weed densities and hand weeding

Weed density and hand weeding time were measured in the treated area on August 18, 2022 for Trial 1. Weed counts by species were recorded in two sample zones, each 0.1 m wide by 0.9 m long. On September 19, 2023, Trial 2 employed a similar method, counting weed density by species within two .10 m wide bands extending .90 m in bed length, prior to thinning. Trials 3 to 5 followed a different protocol for weed assessment: weed counts were conducted at five weeks post-steam for Trials 3 and 5, and at three weeks post-steam for Trial 4, all relative to three weeks after planting. Total weed counts were collected across the entire lettuce beds, with counts allocated according to their respective sections and treatments. Hand weeding times were only recorded for treated plots in trials 1 and 2. Specifically, the time to hand weed a 9.1 m section for trial 1 was noted for each replicate. For trial 2 on September 21, 2023, the time spent weeding and thinning was documented over two plant lines within a 4.5 m bed segment. Hand

weeding times for Trials 3 through 5 were not recorded due to limited control over experimental conditions in commercial field settings.

Soil sampling

For trials 1-2, soil samples weighing 500g were gathered from each treatment zone pre- and post-steaming. In trial 1, a single sample was collected from approximately 15 – 18 m from the plots in each replicated bed. In contrast, trial 2 involved taking samples from two points, 12 and 24 m the plot's perimeter, which were then combined into a single paper bag. Trials 3-5 consisted of obtaining six random 500g soil samples from every treated block, subsequently consolidated into one bag for collective analysis. Sampling depth was consistent at roughly 8-12 cm for trials 1-2, while a shallower depth of ~7.62 cm was gathered for trials 3-5 to minimize disturbance to the commercial beds

Pathogen assays through plating

Soil samples from trials 1-5 were air-dried for a week, and finely ground. Trials 1-2 were analyzed for *Pythium* spp. detection using a soil plating method on Difco Corn Meal Agar that consisted of 0.1% Tween 20, pimaricin, penicillin, ampicillin, rifampicin, rose bengal, and Benomyl 50WP ((Klose et al. 2008; Martin 1992). Each sample, comprised of 1 gram of soil in 15 mL of distilled water, 0.5ml of the solution was plated onto five petri dishes with the medium. The dishes were incubated in the dark at 21 to 24°C and colonies were counted at 24 and 48 hours (Klose et al. 2008; Martin 1992).

In trials 3-5, soil samples were analyzed for *Fusarium oxysporum* prior and post-steam treatments using Komada's medium, conducive to *F. oxysporum* detection. For each soil sample, three plates were prepared. On two separate occasions—December 17 and January 14—assays were performed, yielding six plates per sample. A 10-gram soil sample was blended with 200

mL of sodium hexametaphosphate (NaHMP) solution (Komada, 1975), stirred for 5 minutes, then 10 mL of this slurry was diluted with 90 mL of 0.1% water agar and stirred again for 5 minutes. A 0.250 mL sample of this dilution was applied to the plates, which were then placed under full-spectrum LED lights for 5-7 days, reversing the plates after 1-2 days.

TaqMan qPCR assay for Fusarium oxysporum f. sp. *Lactucae*

Large-scale DNA extractions were performed from soil samples (Matson et al., 2024), incorporating an internal control (IC) to check for PCR inhibition (Bilodeau et al., 2012). A TaqMan qPCR assay was developed following previous protocols (Bilodeau et al., 2012; Burkhardt et al., 2018; Matson et al., 2024) to quantify *Fusarium oxysporum* f. sp. *lactucae* in soil samples. This study utilized an early development version of the TaqMan qPCR assay by Li et al. (2024) to specifically detect and quantify *F. oxysporum* f. sp. *lactucae* race 1 using 1 µl of soil DNA. Each 25 µl reaction contained the standard curve samples (JCP024; ranging from 200 fg to 2 ng), 1 µl of the exogenous IC template, 400 nM Folac_TaqMan_F and Folac_TaqMan_R primers, 200 nM Folac_TaqMan_probe, 400 nM Vd-F929-947, Vd-R1076-1094 primers, 40 nM PPF_Probe_543 probe, and 1× Perfecta Multiplex qPCR ToughMix, (Quantabio, MA). The negative control included everything except DNA of JCP024 (Li et al., 2024). One set of the standard curve samples without soil DNA was used as positive controls. Three technical replicates were tested per sample. The IC primers used were F: 5′-CCACATAATAGACAGTGAAC -3′ and R: 5′-CAATGAGATGGGAGATTT -3′, with a probe sequence of 5′ -[FAM]CGCTGTCTGTAACCTTCTTCCGCA[BHQ1] -3′. Assays were run with and without IC to assess its effect on amplification efficiency. Pathogen load was quantified by plotting colony-forming units (CFU) against cycle threshold (Ct) values, and standard curves were generated for each run. The qPCR assay was conducted on a CFX96 Real-Time PCR

Detection System (Bio-Rad, CA) with the following conditions: 95°C for 3 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds. For soil DNA samples with a Ct value of 32 or higher, a second qPCR assay without IC was conducted to accurately quantify the pathogen. A Ct value of 36 was set as the upper limit for valid quantification. Ct values were recorded after the second amplification step.

Statistics

Data from trial 1 and 2 were subjected to statistical analysis using with R (R Core Team, 2022) in RStudio. An independent sample t-test was employed to assess differences in means across treatments. For trials 3 through 5, analysis of variance (ANOVA) was conducted, along with post-hoc tests for mean separation using the least significant difference (LSD) method, and Tukey's Honest Significant Difference (HSD), utilizing tools from Agricultural Research Manager (Gyllings Data Management, Brookings, SD) and R Studio (R Studio, Boston, MA). These analyses determined statistical significance at the $P = 0.05$ level. When analyzing the influence depth and width had on the total weed densities in trial 1-2 there only the comparison of the data with each other was considered without the control treatments, to ensure a factorial statistical analysis.

Figure 2.1 Band steam applicator used in trials 1 to 5.

RESULTS

Soil temperatures varied depending on the configuration of the band steamers. In Trial 1, the average maximum temperatures for the 5 cm wide treatments were as follows: 60°C at 5cm deep, 85°C at 10 cm deep, and 80°C at 15 cm deep. Similarly in trial 1, for the 10 cm wide treatments, the average maximum temperatures were 58°C at 5 cm deep, 82°C at 10 cm deep, and 70°C at 15 cm deep. In Trial 2, the average maximum temperatures for the 10 cm inch wide treatments were 60°C at 7.6 cm depth and 80°C at 12.7 cm depth. For the 12.7 cm wide treatments in Trial 2, the temperatures were 62°C at 7.6 cm depth and 80°C at 12.7 cm depth.

Steam treatments reduced weed pressure across trials, with weed density influenced by band width only in Trial 1. In Trial 1, steam treatments led to a reduction in weed pressure

compared to the non-treated plots. The steam treatments in Trial 1, reduced weed density for the four most prevalent weeds: purslane (*Portulaca oleracea*), shepherd's purse (*Capsella bursa-pastoris*), goosefoot (*Chenopodium*), and burning nettle (*Urtica dioica*), with reductions ranging from 80-98% compared to the untreated control (Table 2). Total weed emergence decreased by at least 90% across all treatments in Trial 1. Hand weeding time decreased by 74% in comparison to non-treated treatments. The treatment with the lowest total weed emergence and hand weeding time was the 5 cm wide x 10 cm deep treatment, followed by the 5 cm wide x 15 cm deep treatment. In Trial 2, steam treatments also resulted in reduced weed pressure compared to the non-treated plots. Steam treatments also reduced overall weed density and hand weeding times compared to Kerb and control treatments (Table 3). The reductions in weed density for the four most prevalent weeds: purslane (*Portulaca oleracea*), shepherd's purse (*Capsella bursa-pastoris*), chickweed (*Stellaria media*), and burning nettle (*Urtica dioica*), ranged from 82-100% reduction compared to the control. Total weed emergence decreased by at least 91% in all treatments. Hand weeding time decreased by at least 79% compared to control treatments. The treatment with the lowest total weed density and hand weeding time was the 12.7 wide by 12.7 deep cm steam band, followed by the 10 cm by 12.7 cm deep steam band. When analyzing the influence of depth and width on total weed densities, in Trial 1, width had a significant impact on total weed density, whereas in Trial 2, neither width nor depth had a significant effect on weed densities (Table 4). Weed density in commercial fields (Trials 3-5) also showed significantly lower weed densities compared to control treatments (Table 5).

Steam treatments also reduced pathogen pressure across all trials, effectively suppressing *Pythium* spp. and *Fusarium oxysporum* spp. densities. In Trial 1, all steam treatments resulted in lower *Pythium* spp. densities, with treatments 3, 5, and 6 achieving a 99% reduction. In Trial 2,

all steam treatments experienced a reduction in *Pythium* spp. density, with treatments 8 and 10 achieving a 100% reduction (Table 7). Neither depth nor width was significant in influencing the differences between control and steam treatments in Trial 2 for *Pythium* spp. density (Table 8). In Trial 1, width was statistically relevant in influencing the effects between steam and control treatments. Lastly, in the commercial fields (Trial 3-5), there was a reduction in *Fusarium oxysporum* spp., with all steam treatments showing a reduction ranging from 88% to 95% lower CFU compared to post samples (Table 10). Moreover, the molecular characterization in the commercial fields of *Fusarium oxysporum* f. sp. *lactucae* through target amplification using the TaqMan assay was unsuccessful. This was due to the insufficient detection of the pathogen in the soil DNA extractions, which contained low pathogen concentrations. As a result, the TaqMan assay data were excluded from further analysis because the pathogen could not be reliably detected at these low levels.

DISCUSSION

As the agricultural industry evolves to incorporate sustainable practices, there is a pressing need to explore alternative pest management methods like band steaming. This study evaluated the efficacy of steam treatments for controlling weeds and pathogens, demonstrating reductions in weed density, hand weeding times, and pathogen pressure, particularly for *Pythium* spp. and *Fusarium oxysporum*. These results align with previous research on the effectiveness of thermal treatments in weed and pathogen control (Guerra et al. 2022).

The optimal band dimensions varied across trials, suggesting that depth and width influenced the effectiveness of steam treatments differently based on the trial. In Trial 1, the width of the steam bands had a significant impact on weed emergence, whereas depth did not. Conversely, in Trial 2, neither width nor depth was a critical factor for weed control. This

variability could be attributed to the different depths and widths used, with Trial 2 employing a 12.7 cm depth compared to the 15 cm depth in Trial 1. Additionally, Trial 1 had a larger variation in the width and depth values than Trial 2. Notably, the lowest weed densities were observed from the 10 cm width and 10 cm depth treatments in Trial 1 and the 12.7 cm width and 12.7 cm depth treatment in Trial 2.

In Trial 2, increasing depths improved weed control, but since all treatments were highly effective, the differences compared to the control treatments were less relevant. Changing the width setting in Trial 1 was pivotal for weed control effectiveness, with narrowest width being less effective than the larger widths. Previous studies (Gay et al. 2010b) have indicated that injector dimensions impact soil heat retention, with deeper injections retaining heat longer before aerating to the surface. However, the direction of steam release remains difficult to control. As a result, Trial 1 showed decreased effectiveness at the deepest injector setting, possibly due to inadequate soil aeration at greater depths. In contrast, Trial 2 showed no decrease in weed control at the deepest injection.

Pathogen control results for *Pythium* spp. generally mirrored those observed for weed control with width influencing the effectiveness of steam treatments in Trial 1. In Trial 2, the deepest injector achieved the highest level of *Pythium* spp. control, reducing pathogen presence to nearly undetectable levels. Furthermore, in Trial 1, the highest temperature and pathogen control were observed at the second-deepest injection depth, while the deepest injector did not provide the highest temperature or control for *Pythium* spp. This suggests there may be a limit to the effectiveness gained by increasing injector depth, as the deepest setting in Trial 1 (15 cm) did not perform as well as the slightly shallower depth (12.7 cm) used in Trial 2. Despite these differences across trials, all steam treatments effectively reduced pathogen pressure relative to

their pretreatment state thus differences in depths were negligible. However, the decreased effectiveness observed at the deepest injection depth in Trial 1 suggests a potential plateau in effectiveness with increasing depth for both weed and pathogen control. This finding highlights the value of optimizing steam application through targeted adjustments in depth and width, with indication that configuration can influence the temperature results thus also impact the pest control results. Our results in trial 1 are indicative that width is influential for pest control, with narrower sizes being less favorable, whereas depth needs further studies on deeper depths to solidify the findings in this study.

In commercial lettuce fields (Trials 3-5), band steaming effectively controlled weeds and the soil-borne pathogen *Fusarium oxysporum*, indicating its practical viability in commercial agriculture. This finding aligns with previous studies conducted on experimental fields, which reported a 100% reduction in *Pythium ultimum* during 2018-2020 trials (Kim et al., 2021) and several *formae speciales* of *Fusarium oxysporum* (Lu, 2009). Similarly, in the study conducted by Carlesi et al (2021) on organic fields demonstrated that steam applications significantly lowered weed emergence, benefiting organic crops. Guerra et al. study (2022) also suggests that the combination of band steaming's ability to reduce both weed and pathogen presence can greatly benefit organic fields struggling with these pests. For instance, a study conducted on steam by Michuda et al. (2021) found that organic systems had slightly higher and more significant net returns compared to conventional systems. In both scenarios, steam treatments resulted in higher net returns than the controls, with organic systems showing particularly more positive outcomes. However, band steaming trials also highlight the need to continuously improve application time and reduce fuel costs. For instance, this current band steamer takes approximately 22.4 hours to steam a hectare (Guerra et al, 2022). Despite these challenges, such

trials provide valuable insights into the potential large-scale application of band steaming and its feasibility for commercial operations.

In Trial 1, all treatments except at the shallowest depth reached the target temperature of 70°C. In Trial 2, only the deeper injectors exceeded 70°C. These findings differ from Elsgaard et al. (2010) study, where temperatures reached 75°C at 5 cm depths but decreased to 45°C within 8 minutes (Elsgaard et al., 2010). Similarly, Hansson and Svensson study (2007) found that 5 cm depth and 10 cm wide band steam treatments reduced weed emergence up to 90%, and reached 86°C. In contrast, our study analyzed greater depths (5 to 15 cm), with the higher depths (10cm, 12.7cm , and 15 cm) reaching 80°C and decreasing to 60°C within 20 minutes. Therefore, the greater depths allowed for the heat to be retained over a longer duration in the soil. Thus, this study provides a nuanced understanding of how steam banding can be modified to achieve ideal dimensions for effective pest control, such as maximizing retention of heat within the soil.

One limitation of this study was the variability in band configurations, which made direct comparisons between trials challenging. Additionally, the lack of control over experimental conditions in the commercial field during Trials 3 through 5 may have affected both weed density and pathogen assessments, despite careful monitoring. This limitation also impacted the ability to quantify *Fusarium oxysporum* f. sp. *lactucae* using the TaqMan qPCR assay, as detecting the specific pathogen formae speciales proved challenging in the large commercial field. Future research should aim to better control these variables to validate findings in commercial environments.

Nevertheless, this study clearly demonstrates that band steaming is an effective and sustainable alternative to chemical pesticides. By reducing dependence on chemical inputs, steam banding not only mitigates environmental impacts but also offers labor savings through

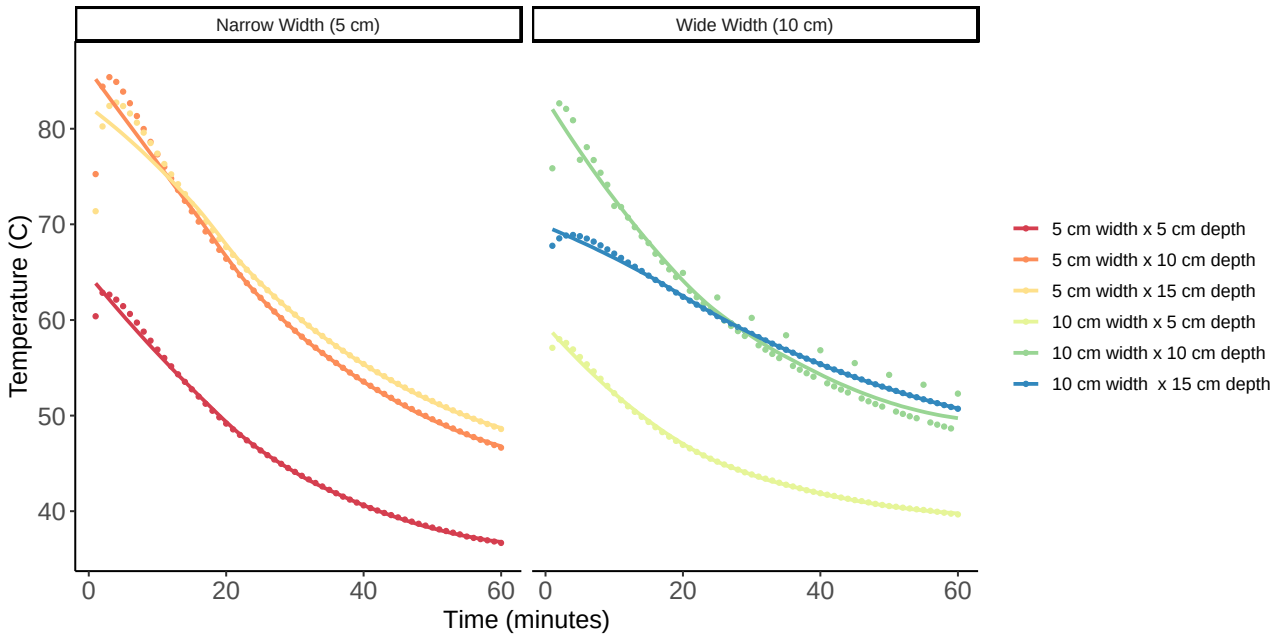
decreased hand weeding and controlling for pathogens. Moreover, the study provides valuable insights into optimizing steam banding for maximum efficacy by changing the band configurations. Furthermore, the successful reduction of both pathogen and weed pressure in commercial fields highlights the potential of band steaming for pest management. Our results indicate that band steaming is a highly promising, environmentally friendly solution that effectively controls both soil-borne diseases and weed emergence.

TABLES and FIGURES

Table 2.1 Critical management dates for trials conducted in Salinas, CA and Soledad, CA

Trial/Crop	Pre-Soil Collection	Preplant/ Steam	Post-Soil Collection	Planting	Weed density measurements/ Hand Weeding Collection
1. Lettuce	1, 2 Aug 2022	2, 3 Aug 2022	3, 4 Aug 2022	4 Aug 2022	18 Aug 2022
2. Lettuce	28 Aug 2023	29, 30 Aug 2023	29, 30 Aug 2023	31 Aug 2023	19 Sep 2023
3. Lettuce (commercial)	16 Jun 2023	16 Jun 2023	17 Jun 2023	25 June 2023	19, 31 Jul 2023
4. Lettuce (commercial)	29 Jun 2023	30 Jun 2023	30 Jun 2023	5 Jul 2023	19, 27, 31 Jul 2023
5. Lettuce (commercial)	28 Jun 2023	28 Jun 2023	29 Jun 2023	7 Jul 2023	31 July, 7 Aug 2023

a)



b)

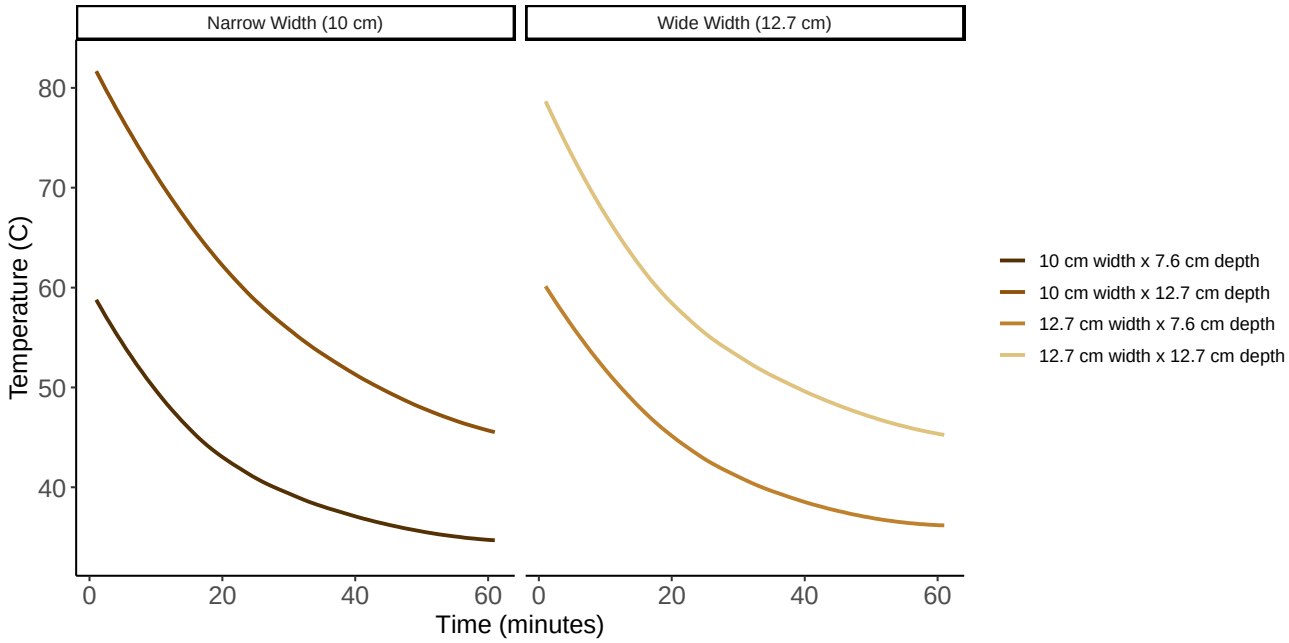


Figure 2.2: Average soil temperatures during the first hour of steam applications in Trials 1 and 2 at Salinas, CA during the 2022-2023 lettuce growing season. a) Trial 1 (2022): Six treatments comparing two steam band widths, 5 cm (left) and 10 cm (right) applied at depths of 5, 10, and 15 cm. b) Trial 2 (2023): Four treatments comparing two steam band widths, 10 cm (left) and 12.7 cm (right), applied at depths of 7.6 and 12.7 cm.

Table 2.2 The cumulative weed density and hand-weeding time in Trial 1 at Salinas, CA, in 2022.

Treatment	Steam band width and depth (cm)	Weed densities					Hand Weeding Time
		Purslane	Shepherd's Purse	Goosefoot	Burning Nettle	Total Weeds	Aug 18, 2022
		1000s/A					Hours/A
Trt 1	5 x 5	22.5 a[i]	7.5 a	15 a	0 a	162.5 a	39.1 a
Trt 2	5 x 10	22.5 a	0.0 a	5 a	10 a	72.5 a	20.6 a
Trt 3	5 x 15	10.0 a	2.5 a	7.5 a	2.5 a	50.0 a	23.5 a
Trt 4	10 x 5	0.0 a	7.5 a	0.0 a	2.5 a	52.5 a	16.6 a
Trt 5	10 x 10	0.0 a	0.0 a	0.0 a	0.0 a	17.5 a	12.4 a
Trt 6	10 x 15	10.0 a	5.0 a	0.0 a	0.0 a	65.0 a	15.2 a
Control	---	550.0 b	430.0 b	257.5 b	247.5 b	1702.5 b	154.7 b
Treatment Prob (F)		<0.001	<0.001	<0.001	<0.001	0.001	0.001

[i] Means followed by the same letters within a column are not significantly different ($P < 0.05$) according to Tukey's HSD.

Table 2.3. The cumulative weed density and hand-weeding time in Trial 2 at the Salinas, CA, in 2023.

Treatment	Steam band width and depth (cm)	Weed densities					Hand Weeding Time
		Burning Nettle	Shepherd's Purse	Chick Weed	Purslane	Total Weeds	Sep 19, 2023
		1000s/A					Hours/A
Trt 7	10 x 7.6	4.4 a[i]	0.5 a	0.1 a	0.0 a	6.0 a	23.1 a
Trt 8	10 x 12.7	0.5 a	0.6 a	0.0 a	0.1 a	2.5 a	25.5 a
Trt 9	12.7 x 7.6	2.5 a	0.3 a	0.0 a	0.3 a	4.1 a	23.2 a
Trt 10	12.7 x 12.7	0.1 a	0.1 a	0.0 a	0.0 a	2.4 a	18.4 a
Kerb SC 3.5 p.t/A	---	5.3 b	14.9 b	0.8 a	0.9 a	28.6 b	68.0 b
Control	---	24.0b	29.1 c	4.1b	3.6 b	67.0 c	121.0 c
Treatment Prob (F)		<0.001	<0.001	0.6181	<0.001	<0.001	.001

[i] Means followed by the same letters within a column are not significantly different ($P < 0.05$) according to Tukey's HSD

Table 2.4 Effect of band width and depth on weed density in trial 1 and 2 2022-2023 lettuce growing season.

	Treatment Effect on Total Weed Densities	
	Trial 1	Trial 2
Factor	P-values	
Width (W)	<0.0001	0.15
Depth (D)	0.31	0.57
W x D	0.14	0.62

Table 2.5: The cumulative weed densities from commercial field trials at Soledad, CA

	Total weed densities		
	Lettuce trial 3[ii]	Lettuce trial 4[iii]	Lettuce trial 5[ii]
Treatments	(No. weeds/A)		
Steam Only	35.39 a[i]	192.12 a	67.69 a
Control	381.53 b	1744.14 b	305.07 b
P Value	<0.0001*	0.0182 *	0.0102*

[i]Mean separation by Tukey's HSD. Means followed by the same letter within columns do not differ significantly at 5% level.
[ii]two assessments were conducted
[iii]three assessments were conducted

Table 2.6. Abundance of *Pythium* spp. density before and 1 day after treatments for lettuce trial 1 in 2022 .

		Pythium CFU/g		
Treatment	WxD[ii]	Pre-treatment	Post-treatment	% reduction
Trt 1	5 x 5	1263.0	132.7 ab[i]	89
Trt 2	5 x 10	1101.0	18.0 a	98
Trt 3	5 x 15	1197.0	3.75 a	99
Trt 4	10 x 5	840.7	328.5 b	61
Trt 5	10 x 10	873.7	8.25 a	99
Trt 6	10 x 15	879.0	3.75 a	99
	Control	972.4	710.80 c	27
P- value			<0.001	

[i] Means followed by the same letters within a column are not significantly different ($P < 0.05$) according to Tukey's HSD.
[ii] Width (W) by depth (D) in centimeters

Table 2.7 Abundance of *Pythium* spp. density before and 1 day after treatments for lettuce trial 2 in 2023.

		Pythium spp. CFU/g			
Treatment	WxD [ii]	Pre treatment	Post treatment	% reduction	p-value
Trt 7	10 x 7.6	253.5	40.5 b[i]	84.0	0.0732
Trt 8	10 x 12.7	304.5	0.0 a	100	0.0049
Trt 9	12.7 x 7.6	201.0	39.0 b	80.5	0.2694
Trt 10	12.7 x 12.7	384.0	0.0 a	100	0.0004
	Control	246.0	169.5 c	31.3	1.000

[i] Means followed by the same letters within a column are not significantly different ($P < 0.05$) according to Tukey's HSD.
[ii] Width (W) by depth (D) in centimeters

Table 2.8 Effect of band width and depth on the *Pythium* spp. density from lettuce trials 1 and 2 in Salinas, CA.

	Treatment Effect on Total *Pythium* spp.	
	Trial 1	Trial 2
Factor	*P*-values	
Width (W)	0.00012	0.8792
Depth (D)	0.15580	0.1133
W x D	0.18636	0.4906

Table 2.9: Abundance of *Fusarium oxysporum* counts in commercial fields in Soledad, CA

	Fusarium Colony Forming Count [i]		
	Trial 3	Trial 4	Trial 5
Treatment	CFU/g		
Pre-Steam	5597.0 a [ii]	3952.6 a	3502.2 a
Post-Steam	337.7 b	168.9 b	402.9 b
Reduction %	93.9	95.7	88.5
LSD (P=0.05)	918.2	675.3	405.4
Treatment Prob (F)	0.001	0.001	0.001

[i] Contains both A and B assays for cumulative comparisons pretreatment and 1 d after steam treatment.
[ii] Means followed by the same letters within a column are not significantly different ($P < 0.05$) according to Tukey's HSD.

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Chapter 3: Exploring Bacterial Diversity in Band-Steam Treated Soils: Insights from Spinach and Lettuce Fields in the Salinas Valley

3.1 Introduction

In 2022, California led the nation in agricultural revenue, producing a diverse range of crops and generating the highest total farm receipts in the United States (California Department of Food and Agriculture, 2023). Monterey County, often referred to as the "Salad Bowl," plays a key role in the state's agriculture, particularly in leafy green production, cultivating approximately 7,029 hectares of spinach and 40,752 hectares of lettuce (Monterey County Agricultural Commissioner, 2023). However, despite the region's favorable climate, leafy greens such as spinach and lettuce face significant challenges related to weed pressure and soil-borne pathogens. Weed control is critical for these crops due to their low competitiveness against invasive species (LeStrange et al., 2012; Smith et al., 2017). The need for hand weeding remains significant, adding to high labor expenses, with costs averaging $701.56 per hectare for two weeding sessions (Martin, 2019; Tourte et al., 2023). In addition to weeds, soil-borne fungal pathogens like *Fusarium oxysporum* f. sp. *lactucae* and *Pythium ultimum* present substantial threats to crop yields. These pathogens are challenging to manage, with *Fusarium* spp. capable of persisting in the soil and infecting successive crops, while *Pythium* spp. impact a wide range of plant species, including both leafy vegetables and weeds (Martin and Loper, 1999 ;Gordon, 2017).

Chemical inputs can be used to mitigate weed and pathogen pressures, but it can also create additional challenges. Overusing pesticides in agriculture has resulted in significant problems, including the development of pesticide-resistant weeds and contamination of soil and water systems (Hicks et al., 2018; Varah et al., 2020; Syafrudin et al., 2021). For example, the United Kingdom has seen an increase in pesticide resistance due to over-reliance on chemical

management (Hicks et al., 2018; Varah et al., 2020). Additionally, pesticide runoff can contaminate nearby water sources, posing serious risks to aquatic ecosystems and human water supplies (Syafrudin et al., 2021). Moreover, chemical fumigants can disrupt soil health by negatively affecting microbial communities. Metam sodium, sometimes used to control soil-borne pathogens in leafy greens, has been shown to impair soil microbial activity, resulting in minimal recovery of heterotrophic activity and changes in microbial fatty acid composition post-application (Macalady et al., 1998). Methyl bromide, a fumigant previously used in strawberry production, was beneficial in reducing pathogen pressure for subsequent crop rotations like leafy greens (Gordon and Koike, 2014). However, due to its role in ozone layer depletion, it has since been banned (Fennimore et al., 2008). Research on this restricted fumigant has also shown significant reductions in soil microbial diversity, alongside long-term effects on soil enzyme activity and microbial respiration (Ibekwe et al., 2001).

Given the challenges of weed and pathogen control in leafy green production and the environmental drawbacks of chemical inputs, there is a growing need for sustainable pest management alternatives. Steam pasteurization has recently re-emerged as a promising solution, offering an alternative to conventional pesticide use in both organic and conventional agriculture. Historically, this technique, which utilizes heat rather than chemical inputs to disinfest soil, was first introduced in the 1880s in Germany. Early studies demonstrated its potential in effectively managing soil-borne pests and weed seeds (Baker, 1962). Additionally, it has been observed that maintaining temperatures above 70°C for at least 20 minutes is sufficient to suppress soil-borne pathogens (Baker, 1970). The earliest iteration of this method was broadcast sheet steaming, which involved applying steam to the entire soil surface, but this approach consumed excessive energy and use was limited to greenhouse soils (Gay et al., 2010a, 2010b). Modern

advancements have led to more efficient techniques such as mobile band steaming, which applies steam directly along seed lines prior to planting, resulting in reduced fuel consumption and increased precision (Xu & Goodhue, 2017). Previous studies have confirmed the efficacy of band steaming in managing weeds and reducing soil-borne diseases (Fennimore et al., 2014; Guerra et al., 2022). While steam offers several benefits, it is essential to assess any potential adverse effects, as with all pest management methods. A primary concern is the impact on the soil microbiome, particularly due to temperature fluctuations during steam treatment. In recent years, California has recognized the critical role of the soil microbiome in nutrient cycling and biodiversity, which are key for the long-term sustainability of agricultural systems (California Department of Food and Agriculture, 2023b). This acknowledgement highlights the importance of understanding steam's potential effects on soil microbiome and soil health.

Concerns regarding the impact of steam on the soil microbiome have primarily been based on sheet steam treatments. For example, Roux-Michollet and Dudal (2010) used a metal sheet to confine steam across entire treatment plots, heating the soil to 100°C. This treatment disrupted nitrogen cycling, with heterotrophic and denitrifying bacteria beginning to recover, while nitrifier activity remained significantly reduced—by 60% to 85% compared to non-treated soil—for up to 62 days post-treatment (Roux-Michollet et al., 2008). In contrast to sheet steaming and surface steam treatments, which treat the entire soil surface, targeted applications like band steaming have shown less severe effects on soil bacterial communities. According to Domsch et al. (1983), soil microbial communities can generally tolerate disturbances if recovery occurs within a defined time frame. Their concept suggests that microbial populations can typically rebound from reductions of up to 90% within 60 days under natural stresses; however, prolonged recovery may indicate potential long-term ecological impacts While this concept has

faced criticism for not accounting for compound disturbances, it provides a useful baseline for understanding microbial resilience.

Elsgaard et al. (2010) applied this concept to band steaming over a 90-day period and observed that while bacterial populations showed recovery, fungal populations remained reduced by 38% even after 90 days. Enzyme activities, such as fluorescein diacetate hydrolysis and arylsulfatase, were also inhibited, showing limited recovery during this period (Schnürer et al., 1982; Elsgaard et al., 2002, 2010). Nonetheless, the authors suggested that band steaming's localized impact could be further mitigated by annual tillage, which redistributes soil and promotes microbial diversity. Although band steaming targets a smaller soil area compared to broadcast steaming, more research is needed to fully understand its impact on soil microbiomes.

Building on prior research, our study examines the effects of band steaming on soil microbiomes in Monterey County's lettuce and spinach fields, focusing on a shorter 30-day post-treatment period and an extended 84-day period for one of the enzyme assays. Using 16S rRNA sequencing and enzyme activity assays, we assessed microbial community changes at the Hartnell College research fields near the USDA Salinas Center during the summer of 2023, using a custom-built steam applicator. Given the shorter timeframe relative to Elsgaard et al. (2010) for the16S rRNA sequencing, we aimed to evaluate whether microbial activity and diversity showed a trajectory toward recovery by 30 days, ideally reaching at least 50% of pre-treatment levels. Although complete recovery may not be expected until around 60 days, positive trends within the first 30 days would indicate resilience and suggest potential stabilization over time.

The primary objectives of this study were to evaluate the impact of band steaming on microbial communities in agricultural soils and to observe the initial recovery of soil microbiomes post-treatment. We hypothesized that band steaming would initially impact the soil

microbiome but that microbial communities would begin to recover, thereby maintaining soil health. This recovery pattern was expected to align with findings from Elsgaard et al. (2010), indicating resilience compared to the recovery observed in broadcast steaming. Insights from our study will help assess the feasibility of band steaming as a sustainable agricultural practice, especially in terms of soil biodiversity and ecosystem functionality. Ultimately, this research aims to expand our understanding of band steaming, positioning it as a viable, environmentally friendly alternative for pest control in agricultural systems.

3.2 Materials and Methods

3.2.1 Study site and field logistics

Two field trials were conducted in a randomized complete block design (RCBD) with four replicates for each steam-treated and untreated plot. Both trials were located at the Hartnell College Research Station in Salinas, California, on loam soil consisting of 53% sand, 32% silt, 15% clay, and 2.09% organic matter. The first trial was conducted with spinach (Spinacia oleracea L.), and the second with romaine lettuce (*Lactuca sativa var. longifolia*). Steam-treated beds in the spinach trial measured 27.4 meters in length and 2.0 meters wide. Four non-treated control beds, each 12.2 meters long and 2.0 meters wide, were included, though only two were sampled for microbiome analysis. Steam treatment was applied in bands along the seed line prior to planting on July 27, 2023, and the last soil sample was collected 30 days post-steaming. The design of the lettuce trial was like the spinach trial with steam and control treatments replicated four times within a single bed per plot. Each bed measured 36.6 meters long and 1.0 meter wide. Steam was applied in bands along the seed lines on raised beds on August 29 and 30, 2023, and the trial continued for 84 days post-treatment until harvest.

3.2.2 Soil steaming and temperature measurements

The band steam applicator was towed by a 5520 John Deere tractor with the engine set at a 1500 to 1700 RPM, moving 2.4 to 3.7 meters per minute while steaming is being injected via shank injectors. The steam applicator employed a band steaming technique based on the design in Guerra et al. (2021) study. It applied steam in narrow 10 to 12.7 cm bands along the seed line, injecting it directly into the soil within the intra-row space where the crop would later be planted. The steam generator, with a capacity of 65 BHP (boiler horsepower), was mounted on a bed shaper sled (Simox, La Forêt, France). The soil was heated to temperatures above 70°C for at least 20 minutes, and soil samples were collected both before and after steam treatment to monitor changes in microbial communities. Soil Temperature data were recorded at a depth of 10 cm using HOBO T-Type thermocouples (U12 Outdoor, Onset Computer Corp., Pocasset, MA) for the first 24 hours after steam application.

3.2.3 Soil Sampling

All soil samples were collected along the seed line in the band where the steam had been applied. In the spinach trial two soil samples were taken from each steam-treated plot at distances of 9.1 m and 18.3 m from the end of the bed, while one sample was collected from the center of each non-treated plot. In lettuce trial one soil sample was collected from each plot, both treated and non-treated, by combining samples taken from two points located 12.3 m and 24.4 m from the end of the bed. In both trials, soil samples were collected from the top 10.7 cm. In the spinach trial, soil samples were collected one day before the steam treatment and one day after the treatment (Table 3.1). Similarly, in the lettuce trial, soil samples were collected following the same schedule, with additional collections 30 d and 84 d after the steam treatment (Table 3.1). The 84 d soil samples were utilized for the enzyme assays only.

3.2.4 Enzyme Activity

The enzyme assays targeted seven key enzymes involved in major nutrient cycles, including C-cycling enzymes such as β-glucosidase (BG), cellobiohydrolase (CB), β-xylosidase (XYL), α-glucosidase (AG), and N-acetyl-β-D-glucosaminidase (NAG); the P-cycling enzyme phosphatase (PHOS); and the N-cycling enzyme leucine aminopeptidase (LAP) (Table 2). These enzymes play critical roles in the breakdown of organic matter and nutrient availability within the soil ecosystem. The assessment of the latter enzymes followed the protocol described by Bell et al (2013). Substrate solutions using 4-Methylumbelliferone (MUB) and 7-Amino-4-methylcoumarin (MUC**)** standards were prepared and protected from light exposure by covering them with foil. In preparation for the enzyme assays, soil pH was determined following the protocol outlined by Thomas (1996), and soil water content was measured to calculate dry weight based on Gardner's (1986) method. On the first day of the assays, enzyme activities were measured using 2.75 g of moist soil, combined with the appropriate substrate at its optimal pH, as described by Bell et al. (2013). The soil-substrate mixtures were dispensed into labeled deep-well plates and incubated at 25°C for 3 hours. After incubation, samples were centrifuged and transferred to black 96-well plates, and fluorescence was measured using a BioTek fluorometer (BioTek Instruments, Winooski, VT, USA). Enzyme activity was calculated using fluorescence readings, incubation time, and dry soil weight, with results expressed in nmol/g dry soil/hr. Standard curve fluorescence values MUB and MUC were converted from µM to µmol, and analyzed using linear regression to calculate the slope, y-intercept, and R^2 values (R^2 >0.98). Sample fluorescence values were corrected by subtracting the y-intercept and dividing by the slope. The resulting µmol values were multiplied by 91 mL (buffer volume), normalized by incubation time and dry soil weight, and converted to nmol activity per gram of dry soil per hour.

3.2.5 DNA extraction

DNA was extracted from all soil samples using the Qiagen DNeasy PowerSoil Pro Kit (QIAGEN, Venlo, Netherlands). The DNA extracts were quantified using a Thermo Scientific NanoDrop 2000C Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V4 region of the 16S rRNA was amplified using bacterial/archaeal universal primers 515F (5′ -GTG YCA GCM GCC GCG GTA A- 3′) and 806R (3′ -GGA CTA CNV GGG TWT CTA AT-3′)73. . PCR amplification was performed with an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 60 seconds, and extension at 72°C for 90 seconds, with a final extension step at 72°C for 10 minutes. All reactions were carried out using a high-fidelity polymerase to minimize amplification errors (Caporaso et al., 2011). The resulting amplicons were then purified and paired end sequenced on the MiSeq platform following standard Illumina protocols at the University of California, Davis Genomic Center.

3.2.6 Sample processing 16S Amplicon Sequencing

Following base calling, paired-end amplicon sequences were generated from the Illumina MiSeq system, producing individual fastq files for both forward and reverse reads of each sample (Fadrosh et al 2014). The forward and reverse adapter sequences were removed using the DADA2 pipeline, and the resulting sequences were processed through QIIME2 to produce a taxonomic table (Bolyen et al., 2019). In the spinach trial based on the quality plots from the DADA2 pipeline, forward and reverse reads were truncated at 250 bp and 140 bp, respectively, ensuring a median sequence quality score above 97.13%. After truncation, filtering, merging, and removal of chimeric sequences, 20,484 unique amplicon sequence variants (OTUs) were obtained for all samples with varying lengths. In the lettuce trial forward and reverse reads were truncated at 275 bp and 165 bp, respectively, ensuring a median sequence quality score above

91.19%. After truncation, filtering, merging, and removal of chimeric sequences, 2,412,015 unique amplicon sequence variants (OTUs) were obtained for all soil samples in the lettuce trial. The amplicon libraries were prepared and sequenced at University of California, Davis Host Microbe Systems Core Biology Core (https://health.ucdavis.edu/medmicro/hmsbcore/).

3.2.7 Data Analysis

Statistical analyses were conducted using the microeco and emmeans packages in R. Additional analyses and plotting were done with R (R Core Team, 2022) in RStudio, using packages phyloseq (McMurdie and Holmes, 2013), dplyr (Wickham et al., 2021), and ggplot2 (Wickham, 2016). Statistical analyses were conducted using the microeco (Hu, 2022) and emmeans (Lenth, 2022) packages in R. Beta diversity was assessed using Principal Components Analysis (PCA) based on Euclidean distances to visualize the dissimilarity among samples with unique ASVs. To reduce the impact of zero values, Bray-Curtis distance was also used to calculate dissimilarity between microbial communities across different treatments and time points (Paliy & Shankar, 2016). The Bray-Curtis dissimilarity values were then ordinated using Principal Coordinates Analysis (PCoA**)** to better represent the variation in microbial community composition (Figures 1 and 2). Alpha diversity was evaluated using the Shannon diversity index, which measures species diversity at the local level by accounting for the abundance and evenness of species (Whittaker 1972). The Shannon index reaches zero when all individuals in a sample belong to the same species, indicating no diversity. Microbial community composition was further analyzed at the phylum level across both trials to assess shifts in taxa distribution.

Additionally, Random Forest analysis was used to examine taxa at the genus level. This method implements the Random Forest algorithm by bootstrapping and using significant features

as input, with MeanDecreaseGini selected as the indicator value in the analysis (Yatsunenko et al., 2012). Random Forest, a supervised machine learning technique, identifies the most important microbes for accurate predictions regarding specific groups by evaluating the drop in accuracy when each microbe is removed. This approach indicates the importance of each microbe in distinguishing between groups, such as steaming or non-treated. In our study, a microbe was considered very important if its MeanDecreaseGini score was significant at the 0.05 p-value. The relative abundance of these selected predictors was then plotted. To ensure the reliability of our results and avoid data variation influence, the program was run 1,000 times. The predictors were selected to steam side if significantly enriched ("positive response") or to the nontreated side if depleted ("negative response") due to steam treatment.

Functional diversity was evaluated through a differential analysis of OTU abundances from the lettuce trial. This analysis utilized predicted functional profiles of prokaryotic communities based on sequencing results in FAPROTAX (Louca et al., 2016). By deriving these functional profiles, we gain deeper insights into the structure and dynamics of microbial communities. FAPROTAX leverages a database of traits and functions associated with known prokaryotes, based on extensive research documented in scientific literature (Louca et al. 2016). Integrating taxonomic data with this database enables the tool to predict microbial traits and their specific roles in soil biogeochemical processes. This approach enhances the understanding of microbial ecological processes that drive biogeochemical cycling within soil ecosystems (Louca et al., 2016).

Enzyme activity effects were analyzed by comparing trends between steam-treated and non-treated samples at different time points: DAT0 to DAT1, DAT0 to DAT30, and DAT0 to DAT84 (the latter only for the lettuce trial). Mean differences were calculated using the

emmeans package, with significance assessed at a p-value of 0.05 and Bonferroni adjustment for 2 and 3 comparisons. For both non-treated and steam-treated soils, enzyme activity means at DAT1, DAT30, and DAT84 were compared to the initial enzyme activity at DAT0. These trends, defined as changes in enzyme activity over time, were then expressed as mean differences and compared to non-treated samples to determine whether the observed changes were statistically significant. A significant difference would indicate that enzyme activity was impacted by the steam treatment. By examining the mean differences between steam-treated and non-treated enzyme activities relative to DAT0, we could further understand the reasons for the observed differences between the steam and non-treated soils.

3.3 RESULTS

3.3.1 Effects of band steaming on soil enzyme activities

The two trials yielded contrasting results: in the spinach trial, there was no significant difference in mean enzyme activities, while the lettuce trial had differences between enzyme activities in steam-treated and non-treated soils across several timepoints (Table 3.3). The enzymes analyzed included five involved in carbon cycling (β-glucosidase [BG], cellobiohydrolase [CB], β-xylosidase [XYL], α-glucosidase [AG], and N-acetyl-β-D-glucosaminidase [NAG]), one related to phosphorus cycling (phosphatase [PHOS]), and one associated with nitrogen cycling (leucine aminopeptidase [LAP]).

In the lettuce trial, the first significant difference in enzyme activity trends between treatments (non-treated and steam-treated) was observed in BG activity when comparing changes from the day before treatment (DAT0) to the day after treatment (DAT1). This difference was due to a decrease in mean BG enzyme activity in steam-treated soils by approximately 303.9 nmol/g dry soil/hr at DAT1 compared to DAT0 ($p = 0.0270$) (see Supplementary Figure 3.1.2).

Differences in BG activity trends between treatments reemerged 84 days post-treatment (DAT84), even though no difference was observed 30 days post-treatment (DAT30). This later difference can be attributed to BG activity in steam-treated soils, which was 275.8 nmol/g dry soil/hr lower at DAT84 than at DAT0 ($p = 0.0494$) (supplementary figure 3.1.2).

Additional change in trends were observed for CB, AG, and NAG. For CB and AG, the distinction in trends between steam-treated and non-treated soils was primarily due to an increase in enzyme activity at DAT30 in the steam-treated soils, while enzyme activity decreased in the control soils. For NAG, activity in the steam-treated soils declined at DAT30 by approximately 150.3 nmol/g dry soil/hr relative to DAT0 levels (supplementary figure 3.1.2). However, this difference did not persist 84 days post-treatment.

3.3.2 Effect of band steaming on soil microbial diversity

In both trials, the Shannon diversity index showed a decrease in alpha diversity immediately following steam treatment. In the spinach trial, the Shannon index dropped from 5.3 to 4.3 after steaming (Figure 3.1). Similarly, in the lettuce trial, a significant reduction was observed, with the Shannon index decreasing from approximately 5.5 to 2.5 the day after treatment. However, the soils in the lettuce trial showed recovered by 30 days, with the Shannon index returning to approximately 4.7, indicating a positive trajectory toward full alpha diversity recovery within 60 days (Figure 3.1).

In terms of beta diversity, principal component analysis (PCA) of Bray-Curtis distances for 16S bacterial composition revealed significant shifts in bacterial community structure, indicating changes in species abundance within microbial communities before and after steam treatment (Figures 3.2 and 3.3). In the spinach trial, the microbial community composition post-steam treatment was distinctly different from the pre-treatment community (Figure 3.2). The

lettuce trial exhibited a similar trend, with notable differences between the microbial community immediately following steam treatment and 30 days later, suggesting that the community continued to evolve after the treatment rather than remaining static (Figure 3.3).

3.3 3 Effect of band steaming on the taxonomic composition of the soil microbial community

At the phylum level, there was a reduction in the relative abundance of several phyla, including Proteobacteria, Acidobacteriota, and Myxococota in both trials (Figures 3.4 and 3.5). Conversely, the Firmicutes phylum increased in abundance the day after steam treatment but was less dominant 30 days post-treatment, resembling levels observed in the pre-treatment soil samples in the lettuce trial (Figures 3.4 and 3.5). In the lettuce trial, the phylum composition shifted between the day immediately after treatment and 30 days post-treatment, moving towards a more diverse state that more closely resembled the pre-treatment soil sample compared to the day after treatment (Figure 3.5).

Random Forest analysis identified three significant genus predictors associated with steam treatments in the spinach trial (Figure 3.6). The other twelve predictors were more indicative of control treatments, suggesting these genera were notably reduced by steam treatments (p-value < 0.05). Among the reduced genera, most were Gram-negative and non-spore-forming bacteria, with three belonging to the Proteobacteria phylum. Notably, *Fictibacillus*, *Ferruginibacter*, and *Micromonospora* showed significant enrichment after steam treatment, with higher relative abundance than in control conditions (p-value < 0.05). Among the enriched genera, two were Gram-positive and spore-forming (Euzéby, 1997; NCBI, 2024). *Fictibacillus* is associated with the Firmicutes phylum, which saw a significant increase in the spinach trial's phylum-level composition (Figure 3.6).

In the lettuce trial, the top 15 predictors the day after steam treatment included *vadinHA49, Limibaculum, PB-19, A4b, Limnobacter, mle1-27, Aquisphaera, KD3-10, Fimbrimonadaceae, Burkholderia-Caballeronia-Paraburkholderia, ADurb.Bin063-1*, *Cnthonomonas*, and *Nitrosospira*. *Nitrosospira*, a known nitrogen-fixing genus, was among the predictors for control treatments, indicating a decrease in relative abundance following steam treatment (Figure 3.7) (Daims and Wagner, 2016). Most depleted genera were Gram-negative and non-spore-forming, with four belonging to the Proteobacteria phylum. The steam treatments had two specific predictors: *Fictibacillus* (also observed in the spinach trial) and *Gracillibacter*, a thermotolerant genus (Figure 3.7).

Thirty days after treatment, the predictors shifted, with the control treatments showing ten key predictors, including *Skermanella*, *Phycicoccus*, *MND1*, *Hyphomicrobium*, *Geodermatophilus*, *Saccharothrix*, *Nonomuraea*, and *OM190* (Figure 3.8). *Cnthonomonas* was consistently a predictor across both time points for the control group and is known to be thermophilic (Figure 3.7 and 3.8). Among these predictors, four belonged to Actinomycetota and two to Proteobacteria phylums. Most were non-spore-forming, and four were Gram-negative. For steam treatments at 30 days post-treatment, the five predictors were *Bacillus*, *Pseudomonas*, *Bosea*, *Sediminibacterium*, and *Pontibacter*, encompassing both Gram-negative and Gram-positive bacteria. Notably, *Bacillus* and *Pseudomonas* are recognized for containing plant growth-promoting strains (Lyng & Kovács, 2023; Radhakrishnan et al., 2017; Saxena et al., 2019; Ait Tayeb et al., 2005). Among the steam treatment predictors, two belonged to the Bacteroidota phylum, two to Proteobacteria, and one to Firmicutes. In both trials and at both time points, steam treatment consistently included a genus predictor from the Firmicutes phylum

(*Fictibacillus* and *Bacillus*), which saw a notable increase in phylum composition (Figures 3.6 and 3.7).

3.3.4 Effect of band steaming on the functional diversity of the soil microbial community

The functional diversity of the soil microbiome in the lettuce trial was analyzed to assess recovery in soil ecological processes (Figure 3.9). Functional diversity analysis was conducted only in the lettuce trial to visualize the recovery within the soil, focusing on the functional state 30 days post-treatment. The primary functions detected included methanotrophy, methanol oxidation, nitrification, denitrification, and chitinolysis.

The day after steam treatment, there was a reduction in methanol oxidation, nitrification, denitrification, and chitinolysis, although these functions were not eliminated. Methanotrophy was the only function that remained stable across all time points following steam treatment. Thirty days post-treatment, partial recovery was observed in nitrification and denitrification, indicating that the microbial communities responsible for these functions were not entirely lost and were able to recover within this period. Additionally, methanol oxidation and chitinolysis showed notable enrichment, with approximately a two-fold increase in activity 30 days after treatment (Figure 3.9).

3.4 Discussion

Monterey County is a critical agricultural hub for leafy greens, where conventional pest management often relies on chemical pesticides that can contaminate soil and water systems and damage soil microbial communities (Hicks et al., 2018; Varah et al., 2020; Syafrudin et al., 2021). For example, the phased-out fumigant methyl bromide was effective in pest control but caused ozone layer depletion, and reductions in soil enzyme activity and microbial diversity as well as being very toxic to bystanders (Ibekwe et al., 2001; Fennimore et al., 2008). Given these

challenges, band steaming has emerged as a sustainable alternative, providing targeted soil disinfestation with fewer adverse effects on microbial communities compared to traditional broadcast steaming. Unlike broadcast steaming, which heats the entire soil surface, band steaming applies heat in narrow bands along the seed line, allowing for faster cooling and minimizing disruption to non-target soil organisms. According to Domsch et al. (1983), microbial communities can generally tolerate disturbances if recovery occurs within 60 days; however, prolonged recovery may suggest long-term ecological impacts. Supporting this concept, Elsgaard et al. (2010) observed that while bacterial populations in band-steamed soils showed recovery within 90 days, certain enzyme activities and fungal populations exhibited slower recovery (Schnürer et al., 1982; Elsgaard et al., 2002, 2010). With our shorter 30-day timeframe and extended 84-day period, we aimed to assess whether microbial activity and diversity showed early signs of recovery, ideally reaching at least 50% of pre-treatment levels and eventually full recovery. While full recovery might not be expected until around 60 days, positive trends within the first 30 days would indicate resilience and suggest potential stabilization over time. Our findings indicate that, although band steaming initially disrupted soil microbiomes, microbial communities showed early signs of recovery within 30 days based on 16S rRNA gene sequencing. Additionally, enzyme activity results indicate minimal inhibition in the spinach trial and recovery in the lettuce trial. In the lettuce trial, most enzyme levels returned to levels comparable to those in non-treated soils by 84 days post-treatment, with the exception of BG enzyme activity. These positive trends in depleted microbial communities support the hypothesis that band steaming is a viable, eco-friendly alternative for pest control with minimal long-term effects on soil health.

3.4.1 Enzyme Activities

Our enzyme activity findings revealed that steam treatments did not impact all enzyme activities analyzed, partially diverging from the results of Elsgaard et al. (2010) and suggesting potential for recovery and preservation of enzyme activity in band-steamed soils. In the spinach trial, there were no differences in enzyme activity between steam-treated and control soils. In contrast, the lettuce trial displayed notable differences in enzyme activity between steam-treated and non-treated soils across various time points. The first difference was observed in β-glucosidase (BG), an enzyme involved in carbon cycling. When comparing trends between treatments (non-treated and steam-treated) from DAT0 to DAT1, BG activity showed a substantial decrease in steam-treated soils. BG activity fluctuated by DAT30, and trends were no longer different from those in non-treated soils. However, by DAT84, BG activity in steam-treated soils was less than at DAT0, distinguishing it from the trends observed in non-treated soils. Additional differences were detected in the carbon-cycling enzymes α-glucosidase (AG), cellobiohydrolase (CB), and N-acetyl-β-D-glucosaminidase (NAG) between DAT0 and DAT30 time intervals. For NAG, enzyme activity decreased between DAT0 and DAT30 in steam-treated soils, showing a distinct trend compared to the non-treated soils over the same interval. In contrast, CB and AG activity increased relative to non-treated soils, indicating a unique pattern in enzyme activity between DAT0 and DAT30. By DAT84, all enzyme activities in the lettuce trial returned to levels similar to those in non-treated soils, except for BG, whose activity decreased at DAT84. This contrasts with findings by Roux-Michollet et al. (2008), who observed a 72% reduction in denitrifying enzyme activity immediately after broadcast steam treatment with limited recovery of nitrifying enzyme activity by 62 days later. Although our study also noted reductions in nitrogen-related enzyme activity, these reductions were not significant when compared to non-treated soils and had recovered by DAT84. Our findings align with Roux-

Michollet et al. (2010) concerning carbon enzymes, as their study reported an increase in carbon enzyme activity post-treatment. Similarly, we observed distinct increases in CB and AG activities in steam-treated soils days after treatment, though BG showed fluctuating activity patterns.

The discrepancies between the spinach and lettuce trials emphasize the need for further studies that assess a broader range of enzymes and monitor over extended timeframes for both trials. Our focus on carbon-related enzymes (five out of seven measured) may have limited the range of observed changes. Future analyses that incorporate enzymes involved in nitrogen, sulfur, and phosphorus cycling could yield a more comprehensive view of steam's impact on soil functionality.

Moreover, our field-based study differed from the controlled conditions of Elsgaard et al. (2010), who conducted their trials without active crop growth. This difference may explain some of the observed variations in our study, particularly between the spinach and lettuce results. Spinach has a shorter growth cycle than lettuce and is planted at much higher densities, which can impact the microbial community and enzymes by promoting rapid ground cover and introducing a greater abundance of roots into the soil. Spinach has a shorter growth cycle than lettuce and is planted at much higher densities, leading to rapid ground cover and a larger volume of roots in the soil, which can influence the microbial community and enzyme activity Additionally, Elsgaard et al. (2010) study noted significant inhibition of enzymatic activity post-steaming, particularly in indicators like fluorescein diacetate hydrolysis and arylsulfatase (linked to sulfur mineralization), with limited recovery. In contrast, our field conditions with active crop growth may have mitigated inhibitory effects.

3.4.2 Bacterial Diversity

Initial results indicated steam caused a decline in alpha diversity in both trials, reflecting reduced microbial diversity in terms of species richness and evenness immediately following the treatment. This finding aligns with previous studies that have shown similar impacts of steam treatments on soil microbiomes (Elsgaard et al. 2010; Roux-Michollet and Dudal, 2010 Roux-Michollet et al., 2008). However, at the 30-day recovery point, our results showed a significant rebound in microbial diversity, with the Shannon index approaching pre-treatment levels more closely than the day after treatment. This finding contrasts with earlier studies, such as Roux-Michollet and Dudal (2008), which reported slower recovery rates of enzyme activities and significant, long-lasting impacts on the genetic structure of both eubacterial and denitrifying communities, persisting up to two months after disturbance. In contrast, our beta diversity analysis revealed substantial shifts in microbial community composition before and after treatment, with further changes still evident 30 days post-treatment. These results suggest that, although steam treatments initially disrupt the soil microbiome, there is a trend towards recovery, like the findings of Elsgaard et al. (2010). This was particularly evident in the lettuce trial, where, 30 days post-treatment, the microbial community did not remain static but showed further changes in species abundance and composition. This dynamic recovery in both alpha and beta diversity supports the conclusions of Elsgaard et al. (2010), indicating the resilience of bacterial communities following band steam treatments. However, it is also important to note that, although our use of 16S rRNA gene sequencing provided valuable insights into species/genera-level shifts, this method may not capture every microbial change at finer taxonomic levels (Fadrosh et al., 2014).

3.4.3 Bacterial taxonomy compositional shifts

The taxonomic analysis at the phylum level supports the observation of bacterial recovery 30 days after steam treatment in more detail. In the lettuce trial, phyla that were initially reduced by the steam treatment, such as Proteobacteria, showed an increase in relative abundance 30 days after steaming compared to the reduced levels observed one day post-treatment. At the genus level, the dynamics of microbial communities revealed that steam treatments favored the survival of gram-positive bacteria, while gram-negative bacteria were more negatively impacted.

Heat-tolerant genera like *Bacillus* and *Fictibacillus*, known for their roles in carbon cycling and biodegradation, increased following treatment (Saxena et al., 2019; Chen et al., 2020). This aligns with previous studies on broadcast sheet steaming treatments in soil, where *Bacillus* and *Paenibacillus* species consistently thrived due to their ability to form endospores, which tolerate heat and acidic conditions (Li et al., 2022). For instance, Li et al. (2021) demonstrated that alpha-type bacteria, including *Bacillus*, increased in abundance after steaming, particularly at 80°C, which significantly promoted seedling survival, growth, and disease suppression in *Panax notoginseng*.

Similarly, Richardson et al. (2002) found that broadcast sheet steaming increased *Bacillus* abundance, suggesting that heat promoted the growth of thermotolerant spores coupled with the improved soil conditions provided by steaming, allowed these bacteria to thrive. Notably, *Bacillus* is not solely activated by heat; it can also survive in harsh conditions, such as drought and extreme temperatures, due to its spore-forming ability—a trait common among enriched phyla in this study (Cutting & Ricca, 2014; Toyota, 2015). This spore-forming capacity enables *Bacillus* to persist in extreme environments and exploit nutrient-rich conditions when they arise. The study further observed that the surviving bacterial community demonstrated resilience and bioremediation post-steaming. In contrast, *Proteobacteria*, typically associated

with nutrient cycling but less tolerant to heat, consistently declined immediately after steaming (Spain et al., 2009). However, in the lettuce trial, *Proteobacteria* began to recover within 30 days, with genera such as *Pseudomonas* and *Bosea* becoming enriched (Euzéby, 1997; NCBI, 2024). This partial recovery suggests that certain microbial groups are capable of recolonizing and adapting following steam treatment, potentially supporting the restoration of critical soil functions.

Earlier studies have demonstrated varying degrees of microbial recovery at different steaming temperatures. Li et al. (2021) reported optimal recovery at 80°C, whereas Richardson et al. (2002) observed successful microbial resurgence at a lower temperature of 55°C. Li et al (2021) found that higher steam temperatures, such as 80°C, not only facilitated microbial recovery but also significantly enhanced disease suppression and plant growth. However, both studies noted that excessively high temperatures could potentially limit recovery, highlighting the need to optimize steam treatments for balancing soil recovery, plant health, and disease control.

Our study at 70°C demonstrated significant microbial recovery, highlighting the potential of band steaming for effective bioremediation. These results align with findings from broadcast steaming, showing that even localized steam treatments can enhance soil health. By fine-tuning the temperature and duration of application, band steaming offers a promising approach to improve soil recovery, support plant growth, and suppress soil-borne diseases.

3.4.4 Bacterial functional diversity for ecological process

Comparing our findings with previous studies on broadcast steaming at 120°C further illustrates the potential benefits of band steaming. Roux-Michollet et al. (2008) reported significant decreases in nitrogen cycling following broadcast steaming, with nitrifiers showing

little recovery even after 62 days. In contrast, our study using band steaming observed reductions in nitrifiers and denitrifiers, but also noted at least 50% recovery in nitrogen cycle processes within 30 days, including nitrous oxide denitrification, aerobic ammonia oxidation, and nitrate denitrification (data not shown). This suggests that band steaming, with its targeted and lower-temperature approach, may be less disruptive to the soil microbiome compared to broadcast steaming, which affects the entire soil profile and leaves fewer refuges for microbial recovery (Hoffmann et al., 2017; Roux-Michollet & Dudal, 2010; Elsgaard et al., 2010).

Thirty days post-treatment, there was also a notable increase in carbon-cycling bacteria, which aligns with previous findings (Roux-Michollet & Dudal, 2010). Additionally, chitinolysis, the breakdown of chitin from fungal cell walls, surged, indicating a potential benefit in reducing fungal pathogens (Swiontek Brzezinska et al., 2013). Enhanced methanol oxidation and methylotrophy were observed, contributing to the reduction of methanol emissions and the release of nutrients that can support plant growth (Kolb, 2009; Conrad, R., 2009). Interestingly, the genera *Bacillus* has been associated with methanol metabolism thus can gather atmospheric methanol concentrations and dissimilate in the soil (Stacheter et al, 2013; Arfman et al., 1989). These functional changes demonstrate the potential of band steaming to positively influence soil health by enhancing some ecological functions.

3.4.5 Implications for further research: comparing band steaming with traditional pesticides

Future research should focus on directly comparing band steaming with pesticides to evaluate its viability as an alternative to conventional pest control methods commonly used in agriculture. Currently, no studies have specifically compared band steaming with chemical treatments; however, prior research has examined the effects of steam sterilization alongside chemical inputs. For example, Tanaka et al. (2003) compared the effects of steam sterilization

with the fumigants, methyl bromide and chloropicrin on soil microbial communities. In their study, a mobile steam applicator was used to raise the soil temperature at a depth of 30 cm above 60°C, maintaining it for several hours before the treatment was concluded. The study found that all treatments led to the disappearance of ammonia- and nitrite-oxidizing bacteria within four months, with methyl bromide having a comparatively milder impact. Steam sterilization and chloropicrin caused the most severe reductions in microbial biomass C and N, which remained low for at least 120 days. In addition, steam sterilization initially reduced metabolic diversity, but a rapid increase followed as surviving microbes began re-establishing a broader range of metabolic functions. The study also observed an initial increase in ammonia levels and a decrease in nitrite, with the most vigorous plant growth occurring in soils treated with steam sterilization and chloropicrin. The additional nitrogen introduced to the soil by these treatments likely contributed to enhanced plant growth. Similar studies have shown that pesticides such as glyphosate, chloropicrin, and methyl bromide can significantly impact microbial communities by reducing bacterial populations and inhibiting critical processes like nitrogen and methane cycling (Tanaka et al., 2003; Ibekwe et al., 2001; Nguyen et al., 2016). However, gram-positive bacteria often demonstrate resilience. Band steaming, by avoiding long-term residues, may enable quicker microbial recovery compared to fumigants. Additionally, band steaming targets specific areas, maximizing treatment effectiveness while causing less disruption to soil ecosystems compared to broadcast steam and steam sterilization. Future studies should evaluate the effects of band steaming on soil microbial health compared to other inputs like pesticides, providing insights into management practices.

5. Conclusion

Our findings suggest that although band steaming initially disrupts the soil microbiome, significant recovery occurs within 30 days, supporting its potential to preserve long-term soil health while suppressing soil pests. Importantly, all major soil ecological cycles demonstrated signs of resilience, with soil functions showing at least 50% recovery and even greater recovery observed in alpha diversity. In addition to restored functions and taxonomic diversity, certain microbial functions and taxa were enriched 30 days after treatment, suggesting that steam treatments may enhance aspects of the soil microbiome that benefit plant growth while reducing disease pressure. The enzyme assays found no enzyme activity inhibition in the spinach trial. In the lettuce trial, all enzymes except one showed comparable activity to non-treated soils by 84 days which suggests that band steaming meets the Domsch et al. (1983) resilience criteria for recovery within 60 days. The novelty of band steaming lies in its precision, targeting soils only where needed in the seedline where crops will be planted. This focused approach minimizes the overall impact on the soil, allowing unaffected areas to support microbiome recovery, as highlighted by Elsgaard et al. (2010), who noted that annual tillage can help mitigate changes in the treated soil. Further research is necessary to confirm these findings, delve deeper into the effects of band steaming on the soil microbiome, and understand its implications for plant growth. This research reinforces the viability of band steaming as a sustainable and environmentally friendly alternative for pest control in agriculture, while maintaining soil biodiversity and functionality.

3.6 Tables and Figures

Table 3.1: Critical dates for both trials conducted in Salinas, CA.

Trial/Crop	Pre-Soil Sample	Preplant/Steam	Post-Soil Sample	Planting	Additional Post-Soil sample
1. Spinach	7/26/23	7/27/23	7/28/23	7/30/23	8/28/23
2. Lettuce	8/28/23	8/29/23	8/30/23	8/31/23	10/4/23, 11/21/23

Table 3.2: Substrates used for enzyme assays (Bell et al., 2013)

Soil Nutrient Cycling	Enzyme Assay	Substrate
Carbon Cycle	beta-glucosidase	4-Methylumbellliferyl B-D-glucopyranoside **(BG)**
	cellobiohydrolase	4-Methylumbellliferyl B-D-cellobioside **(CB)**
	beta-xylosidase	4-Methylumbellliferyl-B-xylopyranoside **(XYL)**
	alpha-glucosidase	4-Methylumbellliferyl-a-D-glucopyranoside **(AG)**
	N-acetyl-beta-D-glucosaminidase	4-Methylumbellliferyl N-acetyl-B-glucosaminide **(NAG)**
Phosphorus Cycle	phosphatase	4-Methylumbellliferyl phosphate **(PHOS)**
Nitrogen Cycle	leucine aminopeptidase	L-Leucine-7-amido4-methylcoumarin hydrochloride **(LAP)**

Table 3.3: Trends in enzyme activity (nmol/g dry soil/hr released) between non-treated and steam-treated plots in the spinach trial.

	Timepoint Comparisons with non-treated and steam soils					
	DAT0 - DAT1		DAT0 - DAT30		DAT0 – DAT84	
	Spinach Trial	Lettuce Trial	Spinach Trial	Lettuce Trial	Spinach Trial	Lettuce Trial
Enzyme[ii]	*P* value		*P* value		*P* value	
BG	0.4095	0.0104*	0.3145	0.1053	--	0.0234*
CB	0.0851	0.3277	0.1512	0.0373*	--	0.3313
XYL	0.1724	0.8222	0.1689	0.3794	--	0.7086
AG	0.1686	0.4639	0.5539	0.0282*	--	0.1665
NAG	0.2579	0.1404	0.7069	0.0480*	--	0.2518
PHOS	0.4553	0.3658	0.1595	0.8870	--	0.5464
LAP	0.6302	0.1202	0.1454	0.0828	--	0.8028

[i]Data compared non-treated and steam treatments means (non-treated – steam) between timepoints compared to the day before: DAT0-DAT1, DAT0-30, and DAT0-DAT84
[ii]Seven enzymes: β-glucosidase (BG), cellobiohydrolase (CB), β-xylosidase (XYL), α-glucosidase (AG), N-acetyl-β-D-glucosaminidase (NAG), phosphatase (PHOS), and leucine aminopeptidase (LAP).
[iii]Statistical significance between treatments at confidence level used with Bonferroni adjustment .95 : ***P < 0.001; **P < 0.01; *P < 0.05

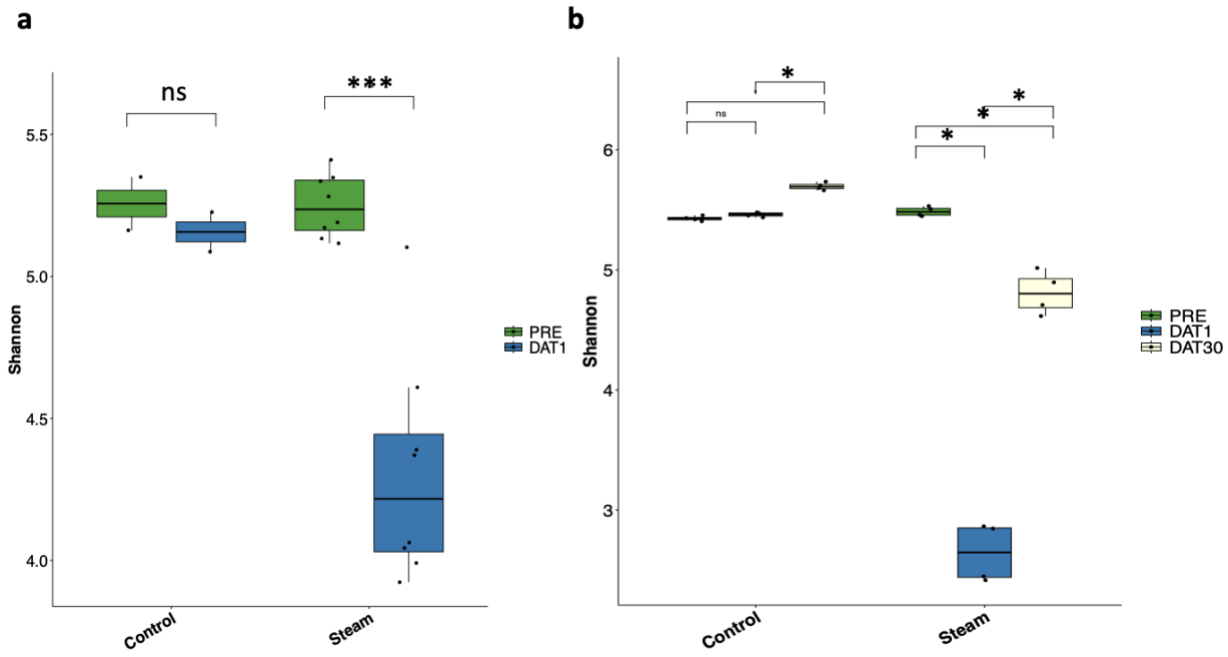

Figure 3.1 Alpha diversity in soil samples of the spinach and lettuce trial using the Shannon Index. a. Spinach Trial: The Shannon index of control vs steam treatments in spinach fields before and after treatment. **b.** Lettuce Trial: The Shannon index of control vs steam treatments in lettuce fields a day before, a day after, and 30 days after treatment. Statistical significance between treatments is given as : ***P < 0.001; **P < 0.01; *P < 0.05, ns, not significant (P > 0.05)

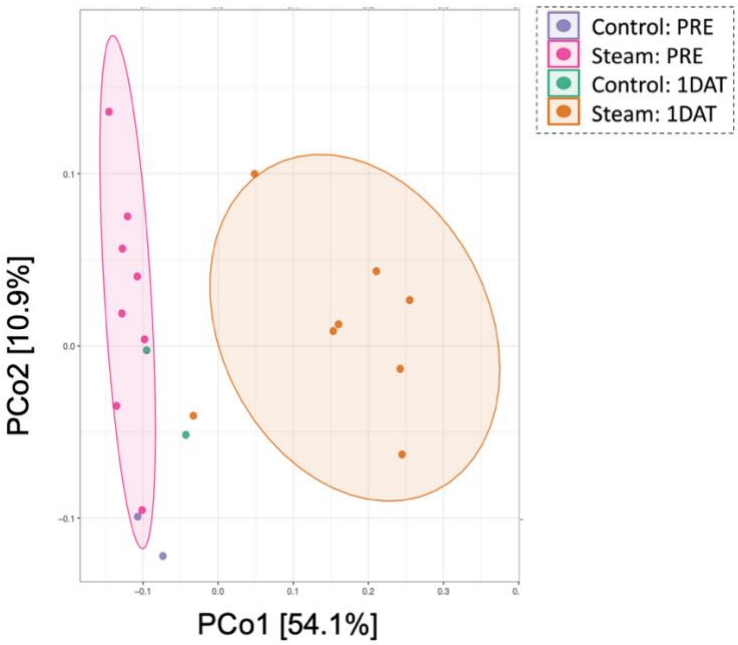

Figure 3.2. PCoA plot for the Spinach Trial's beta diversity. Principal coordinates ordination of a Bray Curtis dissimilarity matrix. Objects that are closer together have smaller dissimilarity than those ordinated apart. Steam treatments (n=8) the day before and after treatment are plotted in the pink and orange color respectively. Control treatments (n=2) the day before and after treatment are plotted in the purple and green color respectively. Boxplots of coordinate scores are displayed parallel to the respective PCoA axis.

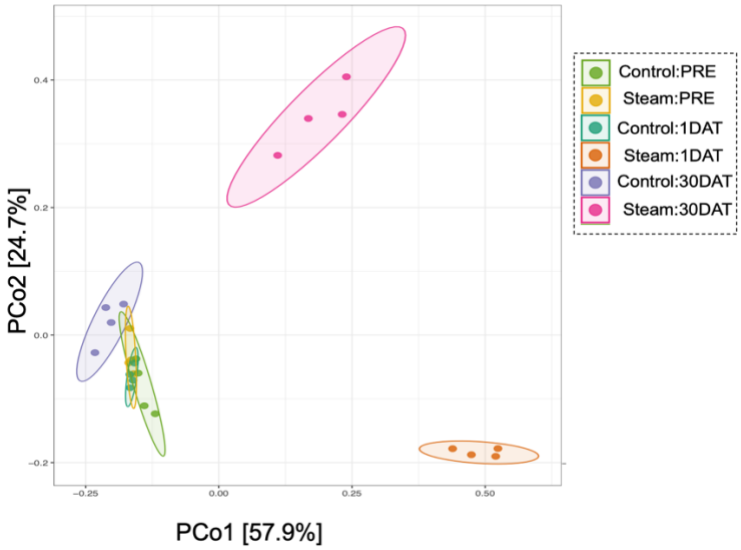

Figure 3.3 PCoA plot for the Lettuce Trial's beta diversity. Principal coordinates ordination of a Bray Curtis dissimilarity matrix. Objects that are closer together have smaller dissimilarity than those ordinated apart. Steam treatments (n=4) the day before, a day after, and 30 days after treatment are plotted in the yellow, orange, and pink color respectively. Control treatments (n=4) the day before, a day after, and 30 days after treatment are plotted in the green, purple, and teal color respectively. Boxplots of coordinate scores are displayed parallel to the respective PCoA axis.

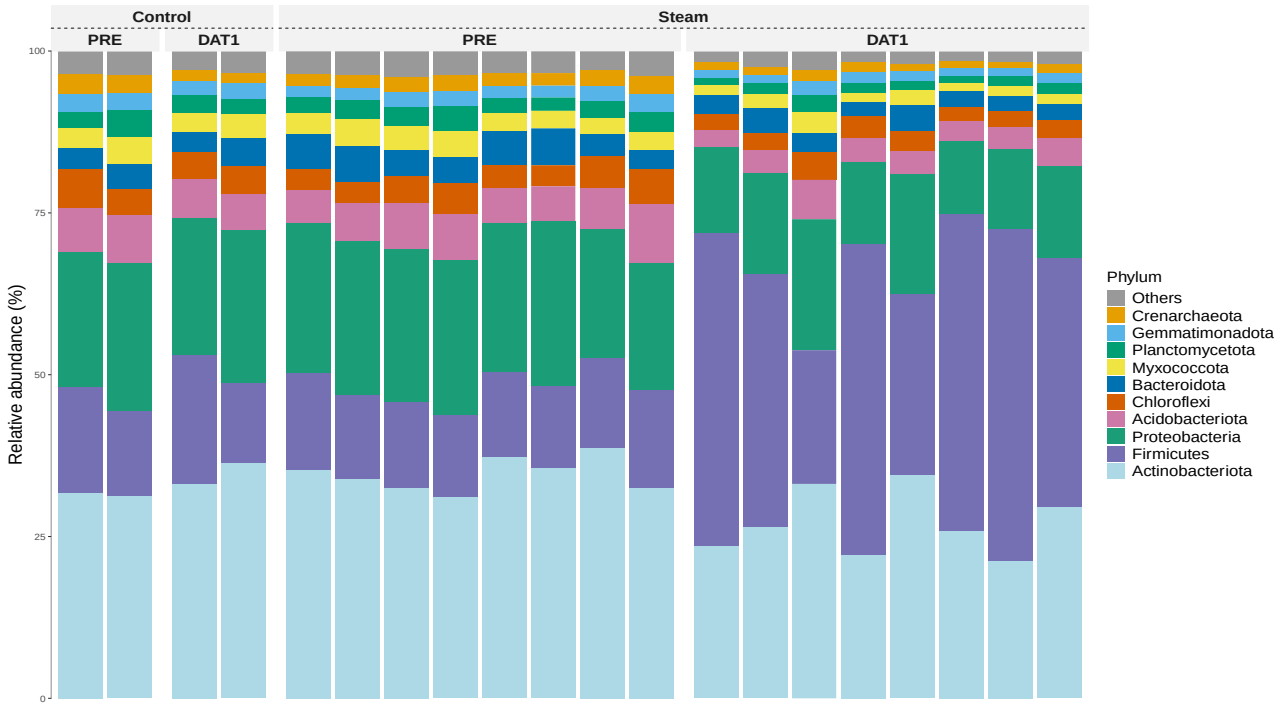

Figure 3.4 Taxa composition at the phylum level for the Spinach Trial. The top ten phyla are shown, with the remaining phyla categorized as "others."

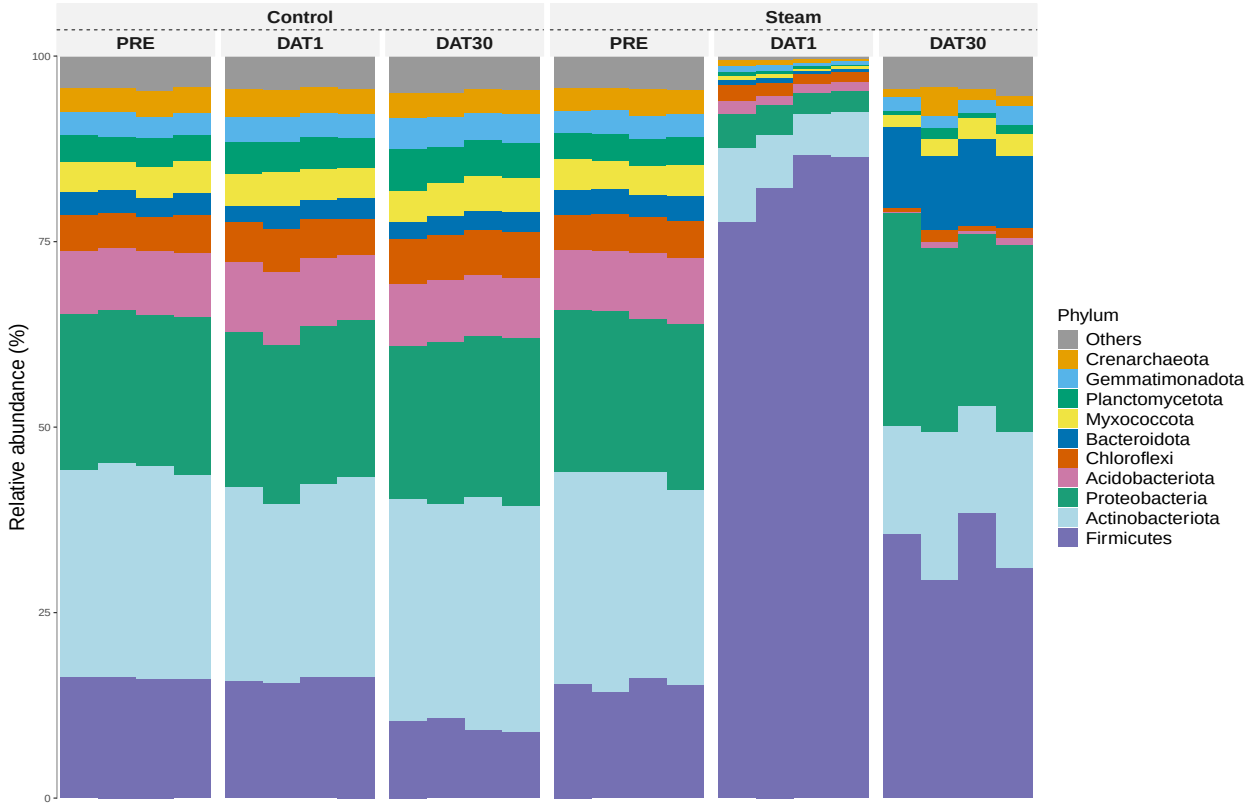

Figure 3.5 Taxa composition at the phylum level for the Lettuce Trial. The top ten phyla are shown, with the remaining phyla categorized as "others."

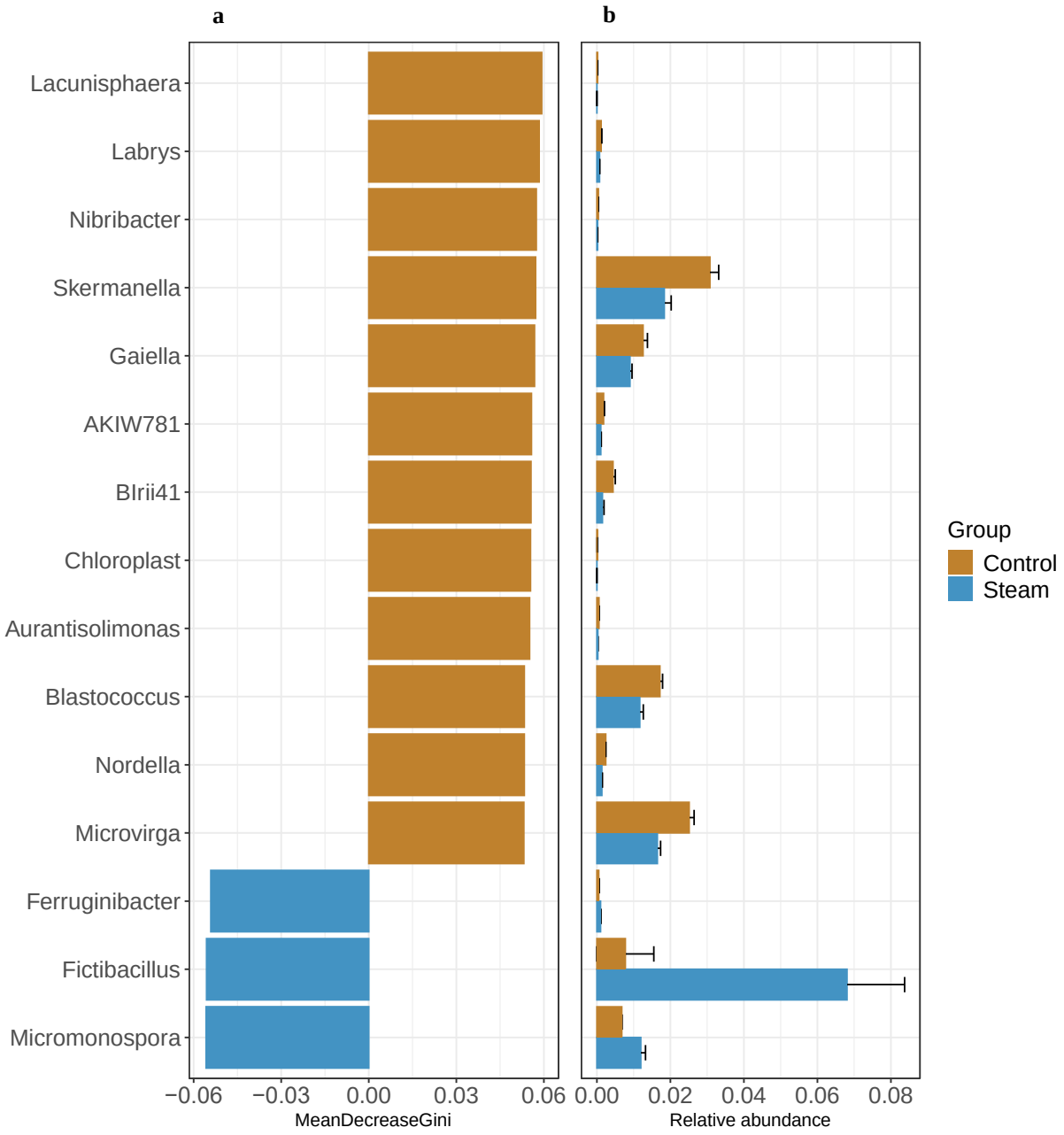

Figure 3.6. Random Forest's top 15 genus-level predictors for the spinach trial, 1 day after treatment. MeanDecreaseGini was used as the indicator value for the analysis. Microbes were considered highly important if their MeanDecreaseGini score was significant at p-value < 0.05.**a.** MeanDecreaseGini values for the selected genera, illustrating their importance in either the steam treatment (blue) or the control treatment (orange).**b.** Comparison of the relative abundance of the top predictors between the steam and control treatments.

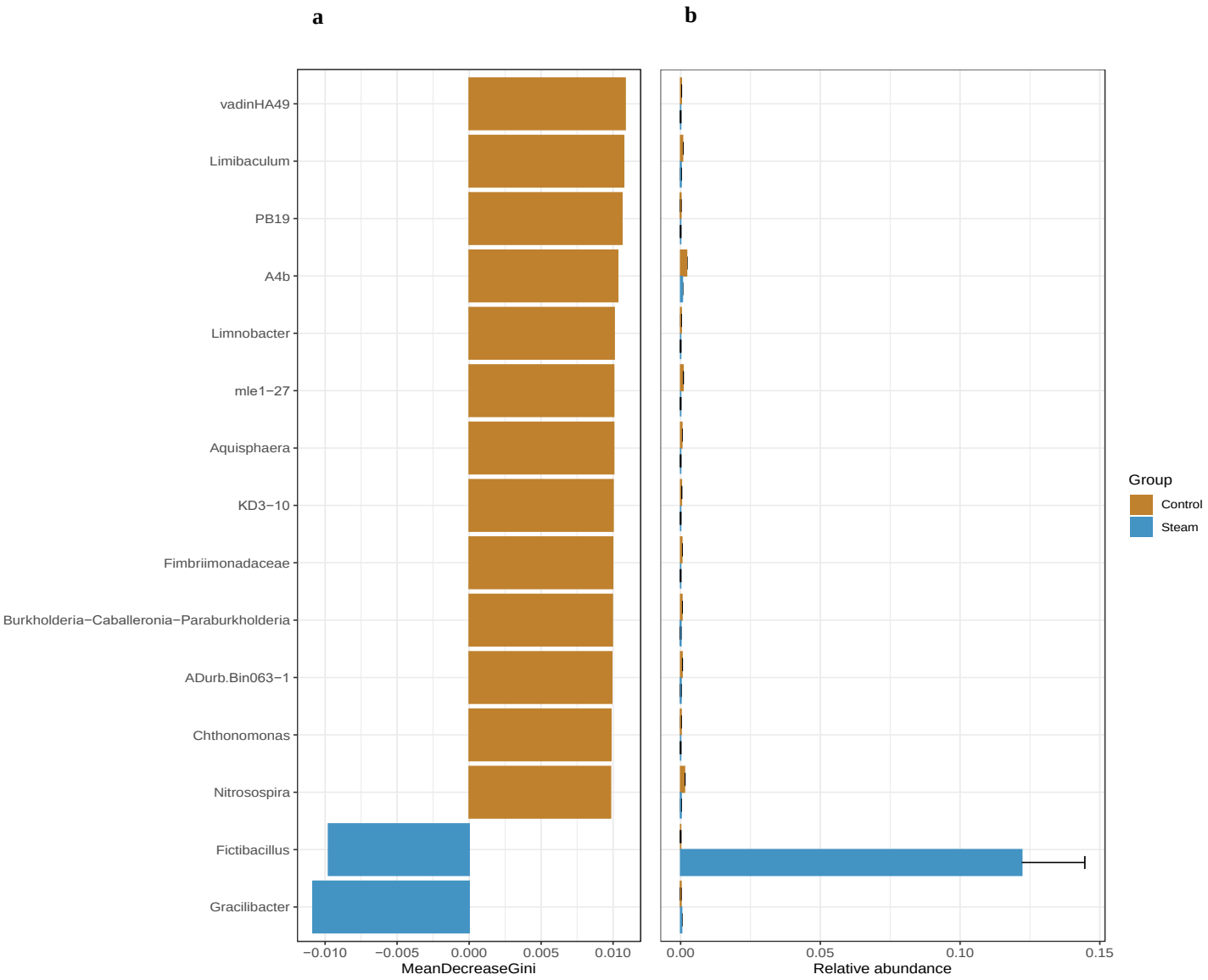

Figure 3.7. Random Forest's top 15 predictors at the genus level for the Lettuce Trial 1 day after the treatment. MeanDecreaseGini was selected as the indicator value in the analysis. In this study, a microbe was considered very important if its score was significant at the 0.05 p-value. **a.** MeanDecreaseGini values for the selected genera, indicating their importance in either the steam (blue) or control treatment (orange). **b.** Comparison of the relative abundance of the top 5 predictors between the steam and control treatments.

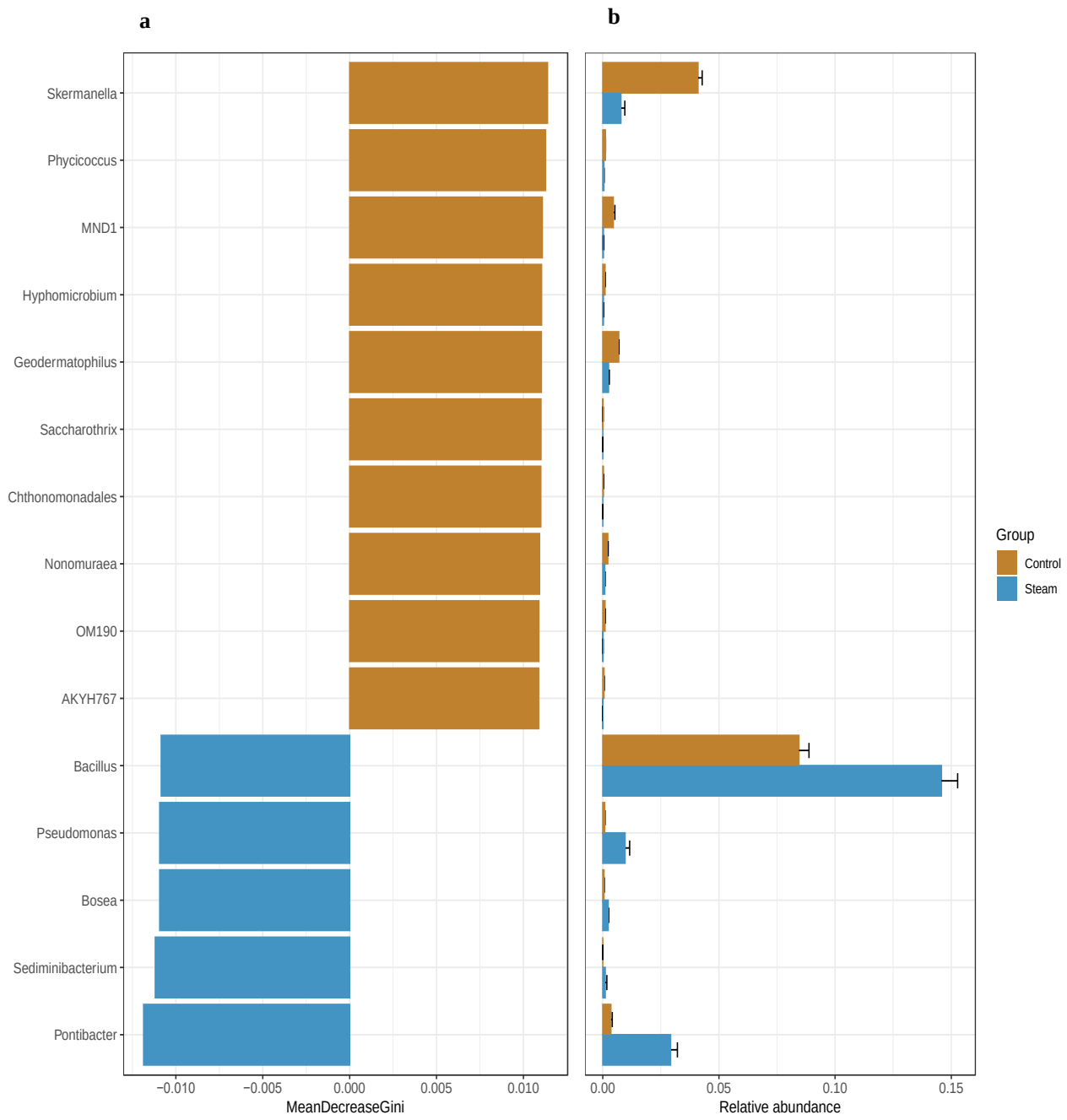

Figure 3.8. Random Forest's top 15 predictors at the genus level for the Lettuce Trial 30 days after the treatment. MeanDecreaseGini was selected as the indicator value in the analysis. In this study, a microbe was considered very important if its score was significant at the 0.05 p-value. **a.** MeanDecreaseGini values for the selected genera, indicating their importance in either the steam (blue) or control treatment (orange). **b.** Comparison of the relative abundance of the predictors between the steam and control treatments.

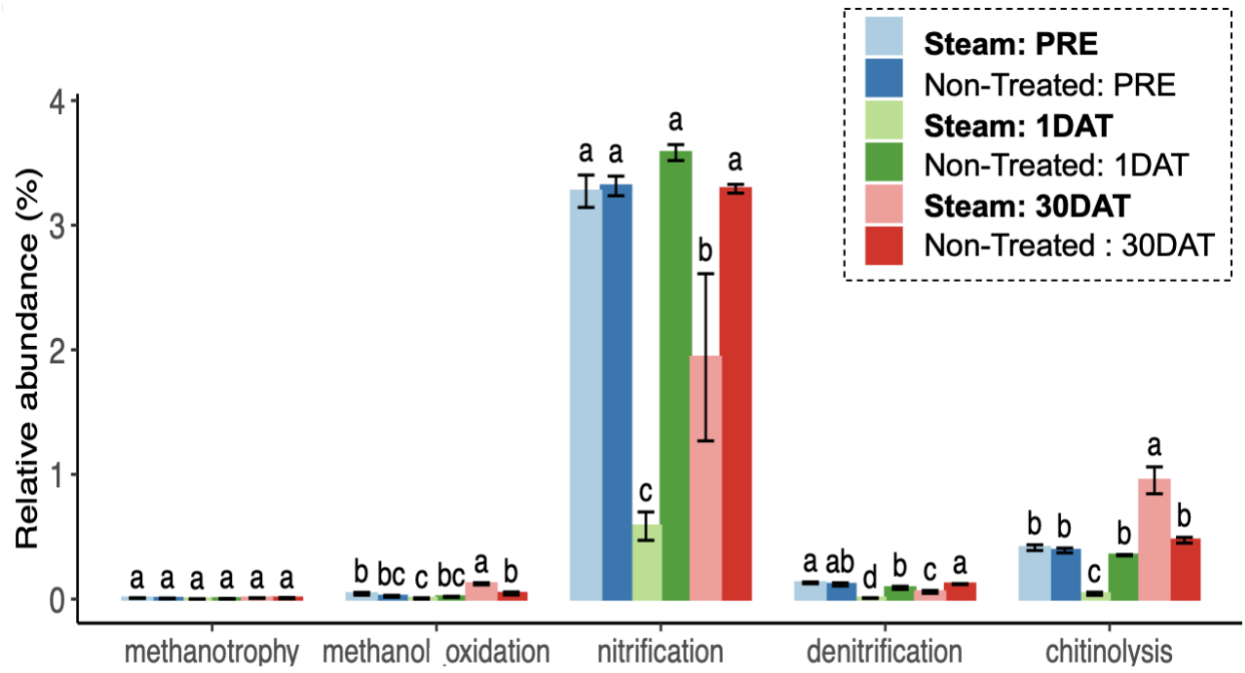

Figure 3.9. Differential abundance analysis in the lettuce trial at the species level. Functional profiles of prokaryotic communities were predicted from microbiome sequencing data and analyzed using FAPROTAX (Louca, 2016). Means followed by the same letters within a group indicate no significant differences (p-value < 0.05), as determined by ANOVA with Bonferroni adjustment.

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Supplementary Figures

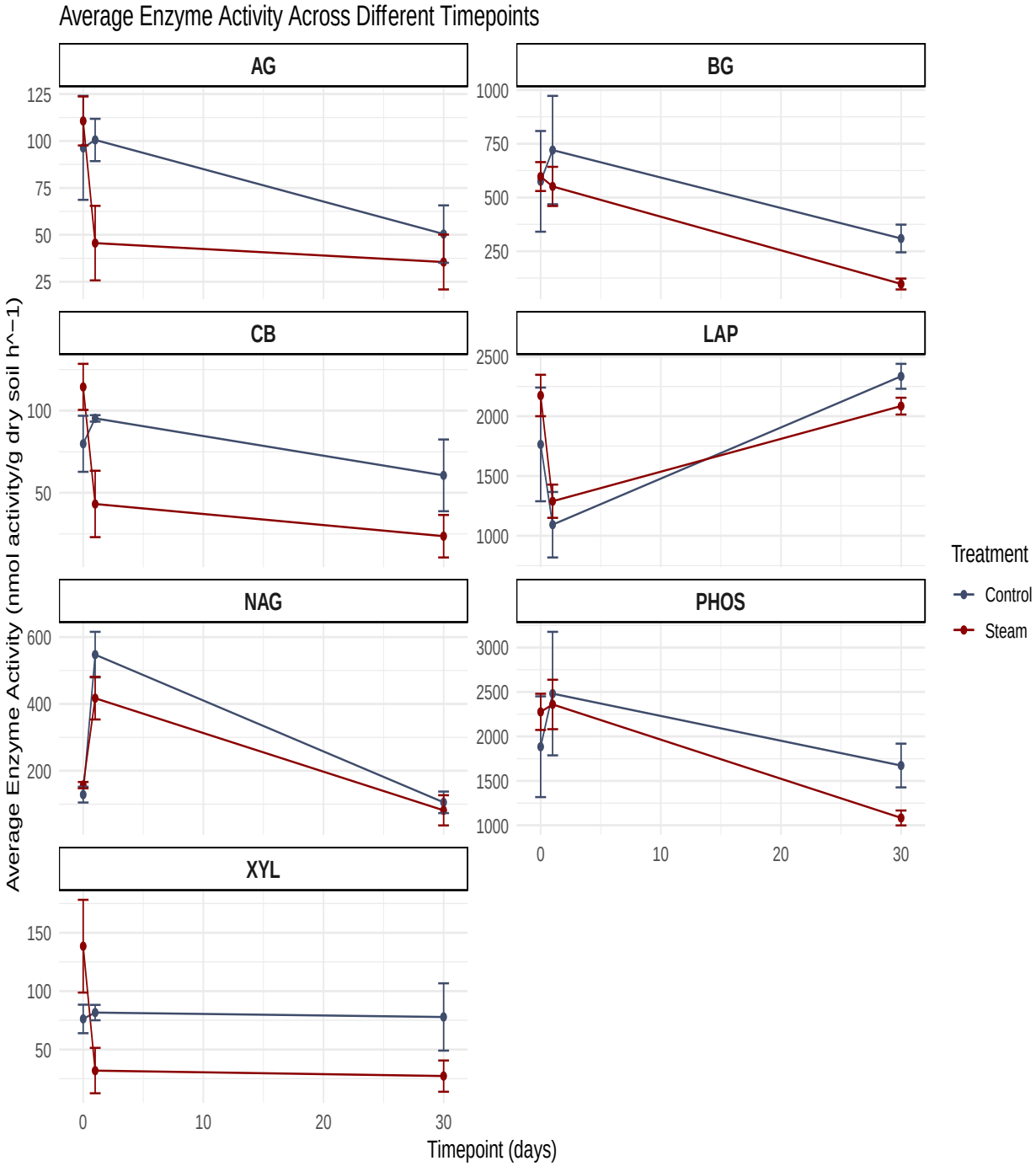

Figure 3.1.1 Mean enzyme activities (nmol/g dry soil/hr released) in soil for each enzyme in the spinach trial, comparing steam (n=2) and control treatments (n=8). The timeline is displayed on the x-axis, where time 0 is before any treatment, day 1 is the day after treatment, and day 30 is 30 days after treatment

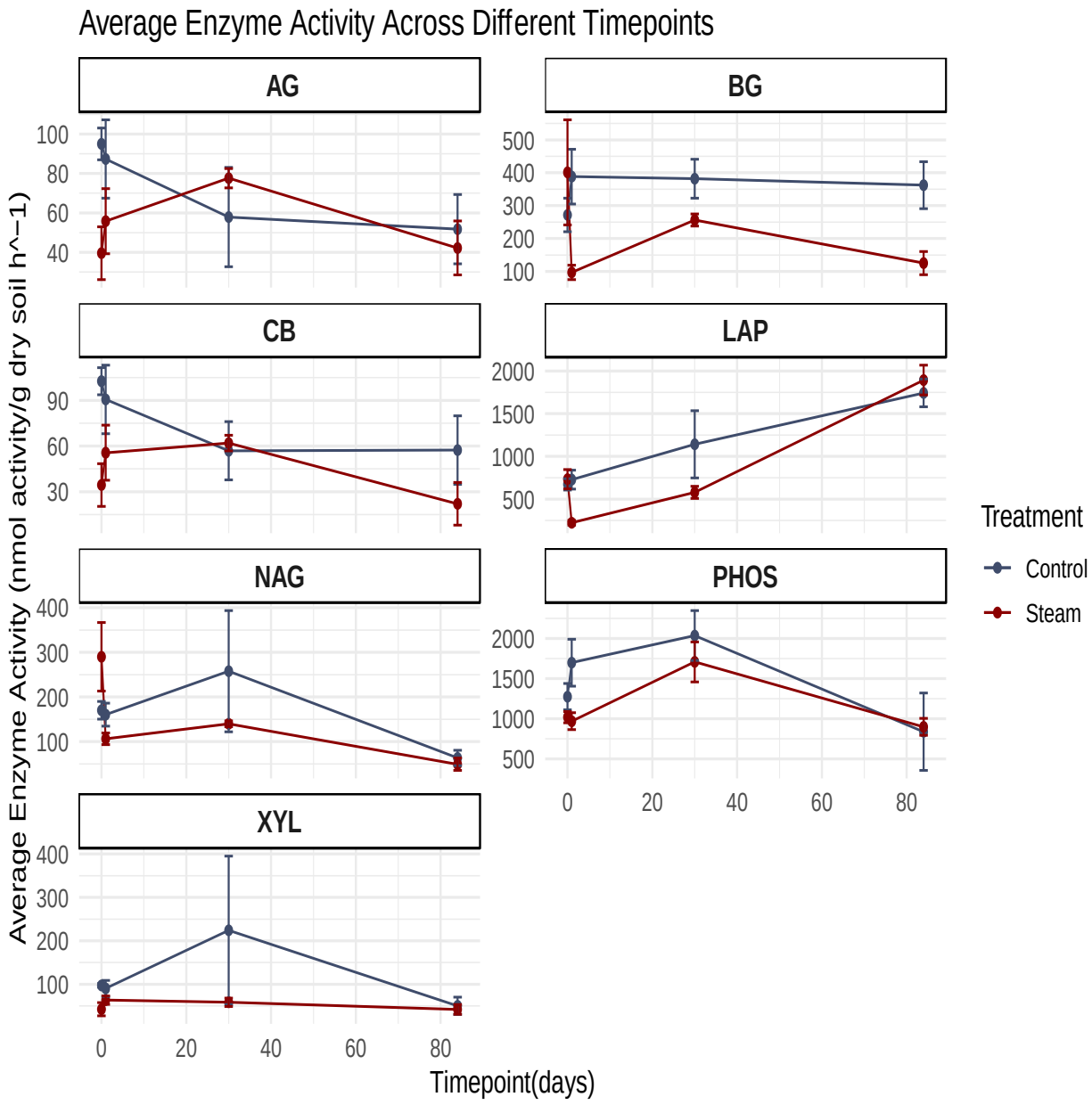

Figure 3.1.2. Mean enzyme activities (nmol/g dry soil/hr released) in soil for each enzyme in the lettuce trial, comparing steam (n=4) and control treatments (n=4). The timeline is displayed on the x-axis, where time 0 is before any treatment, day 1 is the day after treatment, 30 is 30 days after treatment and 84 is 84 days after treatment.