CALIFORNIA LEAFY GREENS RESEARCH PROGRAM ANNUAL REPORT

Project title: Application of organic soil amendments for improved soil health and management of fusarium wilt of lettuce

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Abstract:
Fusarium wilt of lettuce is a growing problem for lettuce production in California. Though this soilborne disease has been in CA since the 1990s, it is becoming more common in primary lettuce producing regions like the Salinas Valley. Fusarium wilt of lettuce is caused by a fungus that lives in soil. Once this fungus establishes in a grower's field, it is very difficult to eliminate. To reduce the negative impacts of fusarium wilt of lettuce, growers need disease management options that can be integrated into conventional and organic production systems and that complement other needs of lettuce production, such as maintaining healthy soil. Application of organic soil amendments prior to planting lettuce has potential to reduce fusarium wilt of lettuce, while providing additional benefits to soil health by increasing soil organic matter and microbial biomass and diversity. The primary goals of this project are to test if organic soil amendments reduce fusarium wilt of lettuce and improve chemical and microbial metrics of soil health. A secondary goal is to determine if the fungus that causes fusarium wilt of lettuce can infect crops that are grown in rotation with lettuce or used as green manures. Organic soil amendments tested will include composted plant waste and green manures from broccoli, oilseed radish, and sudangrass. The effect of each amendment will be compared to an unamended (fallow) soil treatment. Organic amendments will be applied to soil infested with the fungus that causes fusarium wilt of lettuce, prior to planting lettuce. The impact of organic soil amendments on disease will be determined by quantifying the abundance of the pathogenic fungus in soil using molecular methods and measuring disease symptoms and lettuce yield. The impacts of amendments on soil health will be evaluated by characterizing changes in soil organic matter and nitrogen as well as the abundance, diversity, and types of bacteria in soil. The ability of the pathogenic fungus to infect other crops will be tested by drenching plant roots with fungal spores and using molecular and microbiological methods to test for the fungus inside the plants. Tested plants will include broccoli, kale, mustard, oilseed radish, strawberry, and sudangrass. The outcomes of this project will help determine if organic soil amendments are a viable strategy for managing fusarium wilt of lettuce and soil health in CA lettuce production.

Objectives:

1) Test the effect of compost and green manure organic soil amendments on fusarium wilt of lettuce and abundance of *Fusarium oxysporum* f. sp. *lactucae* in soil.
2) Test the effect of compost and green manure organic soil amendments on chemical and microbial metrics of soil health.
3) Test the ability of *Fusarium oxysporum* f. sp. *lactucae* to infect and colonize crops used as green manures or grown in rotation with lettuce.

Procedures:

Objective 1: The effect of green manure and compost soil amendments on *F. oxysporum* f. sp. *lactucae* (causal agent of fusarium wilt of lettuce) and fusarium wilt disease were evaluated under greenhouse and growth chamber conditions. Green manure treatments included biomass from broccoli, oilseed radish, or sudangrass. Compost was derived from plant material. All amendments were added at 5% (per unit weight soil) to soil infested with *F. oxysporum* f. sp. *lactucae*, except compost which was also added at a higher (10%) application rate. Pathogen abundance in soil was measured using pathogen-specific quantitative PCR (qPCR) assay. Effects of amendments on fusarium wilt disease was tested under growth chamber conditions using susceptible lettuce cv. Patriot. Soil amendments were added to pathogen infested soil as previously described, prior to planting two-week-old lettuce seedlings. Plants were maintained under conditions conducive to disease (28°C day and 20°C night temperature)s. Disease was rated on a 0-4 scale, 21 days after transplant. Data were analyzed using analysis of variance (ANOVA), followed by TukeyHSD post-hoc comparisons.

Objective 2: Effect of green manures and compost soil amendments on chemical and microbial characteristics of soil were tested under microplot and greenhouse conditions. Amendments described in Objective 1 were added to microplot soil at 6.25 tons/acre, except compost which was also added at a 2X application rate of 12.5 tons/acre. Soil samples were collected three weeks after amendment and three-week-old lettuce cv. Salinas seedlings were transplanted into microplots. Pathogen biomass in soil was measured using methods described in Objective 1 (greenhouse experiment only). Bacterial biomass in soil was measured using qPCR. Microbial communities were characterized using metagenome sequencing as previously described (LeBlanc 2022). Soil chemical characteristics were measured using standard protocols at the University of Idaho Analytical Science Laboratory. Data were analyzed using analysis of variance (ANOVA), followed by TukeyHSD post-hoc comparisons. Metagenomic data were analyzed using a multivariate analog of ANOVA to determine if amendments altered the composition of microbial communities in soil.

Objective 3: The ability of *F. oxysporum* f. sp. *lactucae* to colonize crops potentially grown in rotation with lettuce was tested under greenhouse conditions. The following crops were included in the host range study: rye, barley, sudangrass, mustard, radish, broccoli, kale, strawberry, and lettuce (positive control). Plants were inoculated using root dips in 1×10^6 spores/mL of *F. oxysporum* f. sp. *lactucae* or water (mock inoculation) for 30 minutes. Plants were inoculated three weeks after seeding, except strawberry which used bare-root nursery stock. Plants were maintained under greenhouse conditions for one month. At harvest, each plant was sampled for DNA extractions. DNA extracted from the plants was tested for the pathogen using previously described qPCR methods (Objective 1).

Results:

Objective 1: Compost significantly ($P<0.01$) reduced the abundance of *F. oxysporum* f. sp. *lactucae* at regular and 2X application rates treatments compared to fallow soil eight weeks after application. No other treatment significantly reduced the abundance of the pathogen compared to fallow soil in the same greenhouse experiment. Under growth chamber conditions, compost had a marginally ($P<0.1$) significant effect on fusarium wilt disease severity. Radish and sudangrass significantly reduced disease severity compared to fallow soil (Figure 1). However, the green manures delayed development of lettuce seedlings which may have confounded these results.

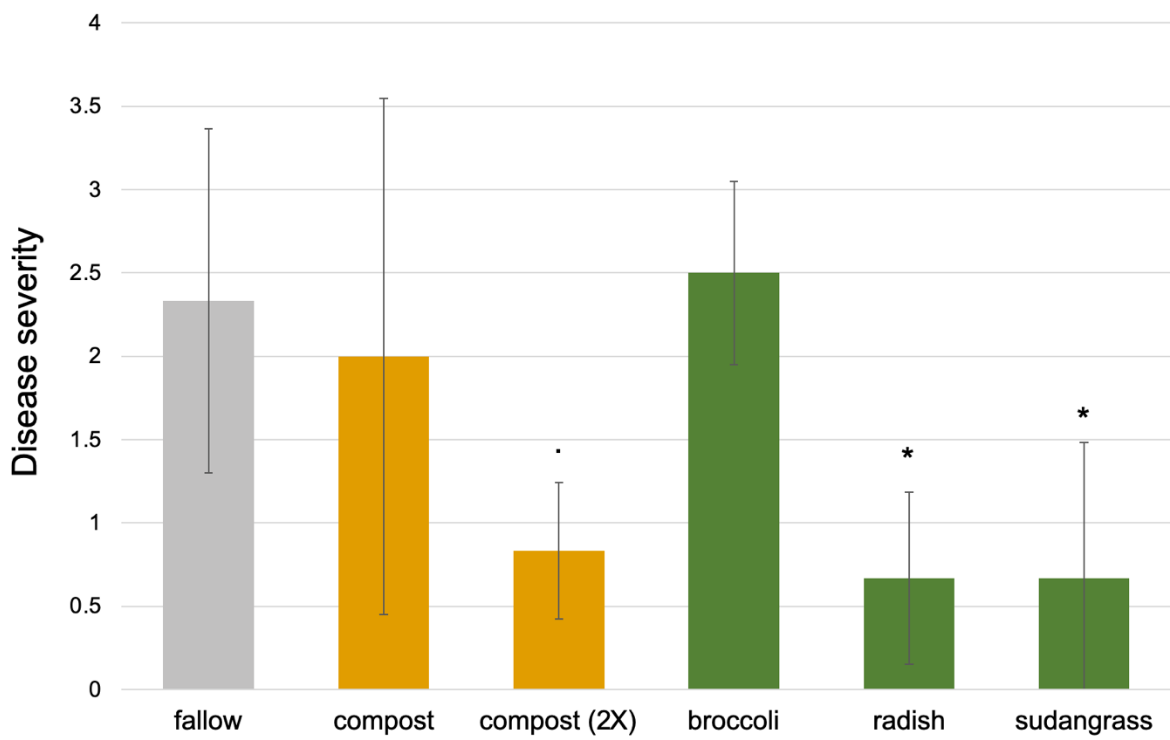

Figure 1. Suppression of fusarium wilt of lettuce disease severity on lettuce cv. Patriot under growth chamber conditions. Treatments labelled with "." are marginally significantly different ($P<0.1$) compared to the fallow treatment and treatments with "*" are significantly different ($P<0.05$) compared to fallow treatment.

Objective 2: Compost was the only amendment that significantly ($P<0.05$) increased bacterial biomass in soil compared to fallow soil. This effect was observed under field microplot conditions for the high (2X) compost application rate and for the regular and high application rate under greenhouse conditions (Figure 2). Green manures and compost soil amendments had a significant ($P<0.05$) effect on microbial community composition in both experiments. As shown in Figure 3, this is reflected in clustering of soil microbial communities in each soil by different soil amendment types.

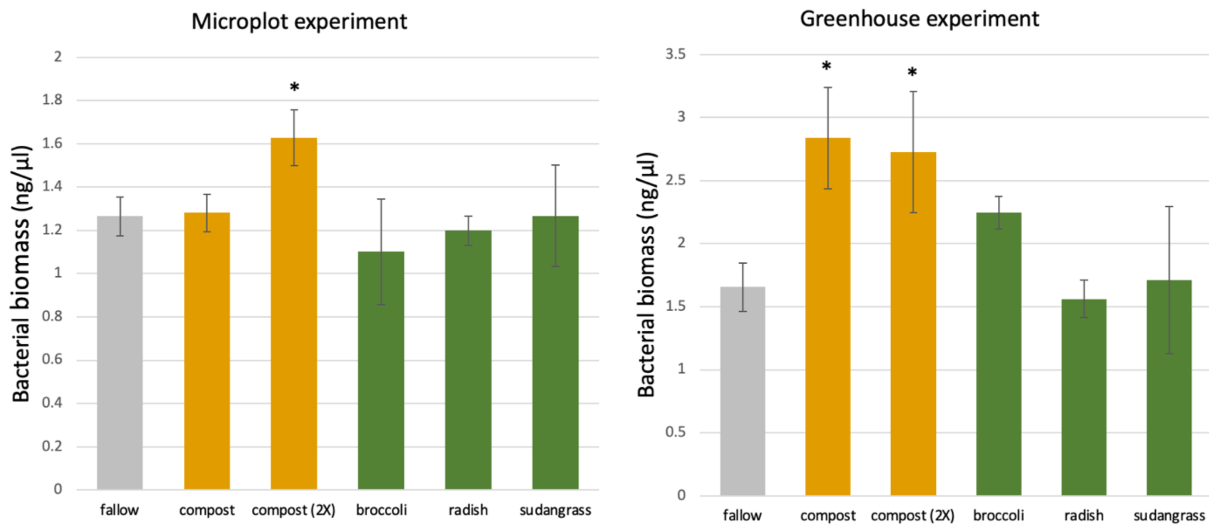

Figure 2. Bacterial biomass across treatments in field microplot and greenhouse experiments. Each experiment represents a different environment and soil type. "*" indicate treatment is significantly (P<0.05) greater than fallow treatment.

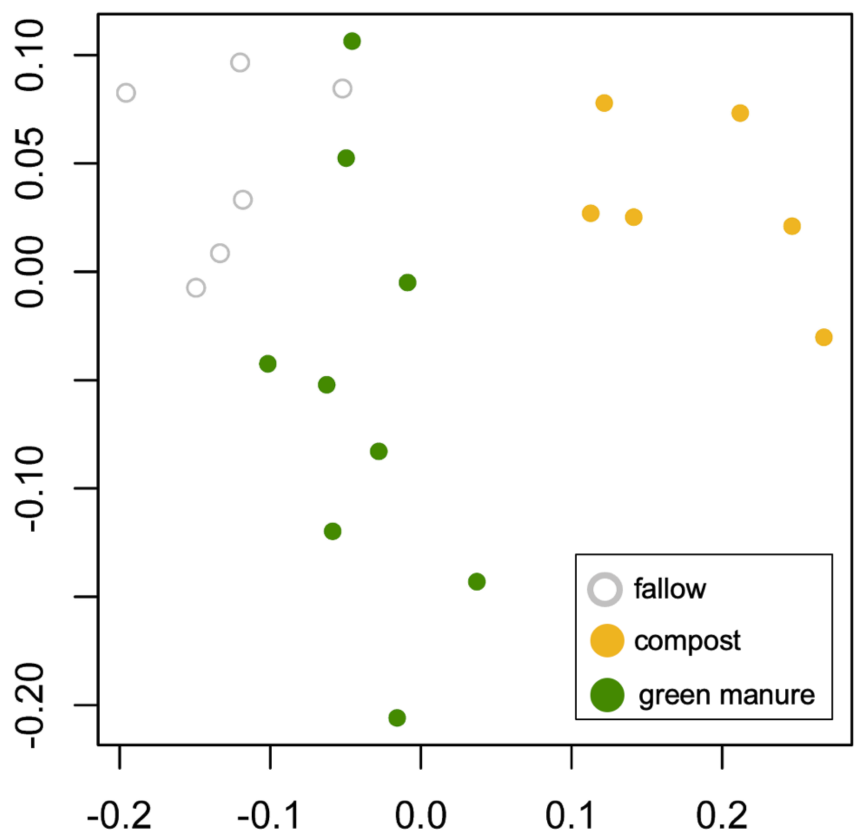

Figure 3. Graph showing changes in soil microbial communities following amending soil with green manures or compost, relative to fallow soil. Points represent individual soil samples from a greenhouse experiment. Points clustering together (closer together in the graph) contain more

similar groups of microorganisms. Inset legend shows which samples correspond to which experimental treatments. Multivariate ANOVA analog showed treatments had a significant ($P<0.05$) effect on microbial communities in this greenhouse experiment and field microplot experiment.

Chemical analyses of samples from the greenhouse experiment showed soil amendments had different effects on soil chemical characteristics. Both application rates of compost and radish green manure treatments significantly increased potassium. All amendments, except the regular application rate of compost significantly increased phosphorus. Radish and sudangrass treatments significantly decreased nitrogen. Broccoli increased soil pH. Both compost treatments and sudangrass increased soil organic matter (Table 1).

Treatment	**Average±SD**	**Measurement**
fallow	180±17.321a	potassium (ppm)
compost	**263.333±15.275b**	potassium (ppm)
compost (2X)	**406.667±37.859c**	potassium (ppm)
broccoli	220±20ab	potassium (ppm)
radish	**273.333±5.774b**	potassium (ppm)
sudangrass	226.667±11.547ab	potassium (ppm)
fallow	52.333±0.577a	phosphorus (ppm)
compost	61.333±3.215ab	phosphorus (ppm)
compost (2X)	**69.667±3.786b**	phosphorus (ppm)
broccoli	**66±6.245b**	phosphorus (ppm)
radish	**67±6b**	phosphorus (ppm)
sudangrass	**67.333±5.859b**	phosphorus (ppm)
fallow	19±1c	nitrogen (ppm)
compost	18±1c	nitrogen (ppm)
compost (2X)	19.667±1.155c	nitrogen (ppm)
broccoli	16.333±1.155c	nitrogen (ppm)
radish	**6.033±3.592b**	nitrogen (ppm)
sudangrass	**0.72±0a**	nitrogen (ppm)
fallow	7.5±0a	pH
compost	7.533±0.058a	pH
compost (2X)	7.633±0.058ab	pH
broccoli	**7.767±0.153b**	pH
radish	7.667±0.058ab	pH
sudangrass	7.533±0.058a	pH
fallow	1.433±0.058a	organic matter (%)
compost	**2.267±0.115c**	organic matter (%)

compost (2X)	**2.867±0.153d**	organic matter (%)
broccoli	1.533±0.058ab	organic matter (%)
radish	1.667±0.058ab	organic matter (%)
sudangrass	**1.767±0.153b**	organic matter (%)

Table 1. Effect of organic soil amendments on chemical characteristics of soil. Treatments that are significantly ($P<0.05$) different from each other for a given measurement are indicated by letters in the "Average±SD" column. Treatments that are significantly different from the fallow treatment for a given measurement are also highlighted as bold text.

Objective 3: Inoculation of eight different crops under greenhouse conditions showed *F. oxysporum* f. sp. *lactucae* could infect non-lettuce crops. Eight months after inoculation, lettuce control plants were the only plants that displayed stunting symptoms of fusarium wilt disease. Non-lettuce plants did not show any overt symptoms of disease. Use of qPCR to test for the presence of *F. oxysporum* f. sp. *lactucae* in plant DNA showed clear positive signal for the pathogen in the following plants: barley, lettuce, mustard, and sudangrass (Figure 4).

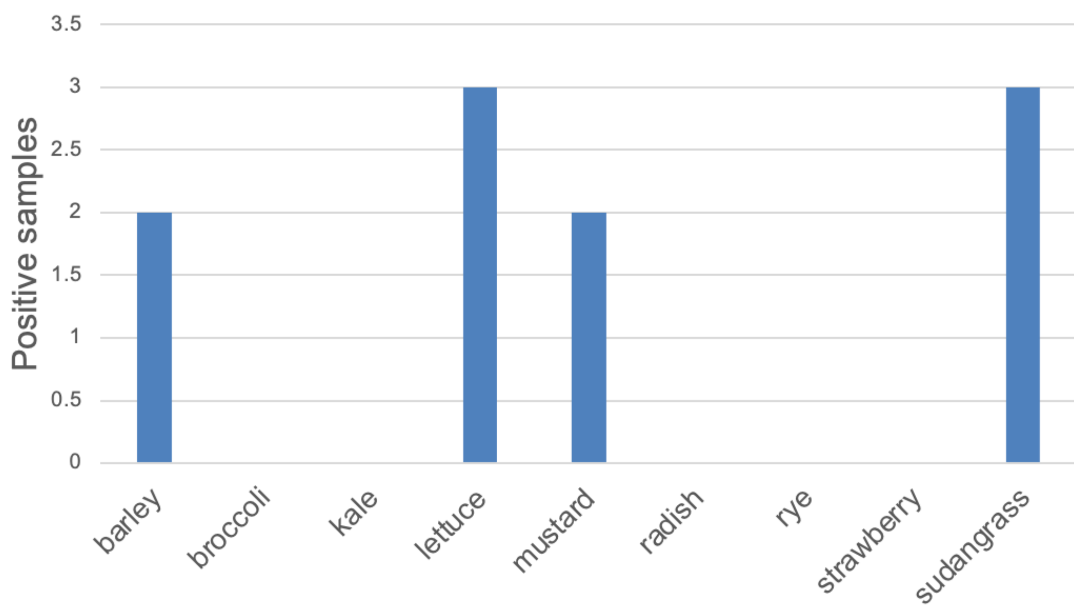

Figure 4. Detection of *F. oxysporum* f. sp. *lactucae* in plant tissue from host range greenhouse experiment. Five total samples from individual plants per crop were tested for the pathogen using qPCR. "Positive samples" indicate the number of samples where the pathogen was detected. The pathogen was not detected in any mock inoculated plant material (data not shown).

Discussion:

Soilborne diseases like fusarium wilt of lettuce are becoming more frequent in primary commercial lettuce production regions like the Salinas Valley. Though genetic resistance is a long-term solution for disease management, there is a pressing need for immediate solutions that growers can use to mitigate negative effects of this and other soilborne diseases. Prior research has shown application of organic soil amendments like green manures and compost can suppress soilborne diseases and improve other characteristics of soil health (Rosskopf et al. 2020).

Results from the past year of this new project demonstrate pre-plant application of organic soil amendments has potential to suppress fusarium wilt disease and the abundance of the fusarium wilt pathogen in soil. Among the soil amendments tested, plant-based compost displayed the greatest potential since this was the only substrate that significantly reduced the abundance of the fusarium wilt pathogen in soil and showed marginal evidence of suppressing disease severity under conditions conducive to fusarium wilt disease. Sudangrass and radish green manures also reduced disease severity. However, these substrates delayed lettuce seedling development which may explain the reduced disease severity and would be problematic for a lettuce grower. A limitation to these results is that pathogen and disease suppression from compost was observed at high application rates using plant-based compost, which may not reflect standards used in commercial lettuce production. It is also still unknown if plant-based compost will have similar effects on other soilborne pathogens and diseases impacting lettuce production in California.

The second objective of this project focused on the effects of organic amendments on broader microbial and chemical characteristics of soil that relate to the concept of soil health. Compost was the only amendment that increased bacterial biomass in soil, which was observed under field and greenhouse conditions. Application of all organic soil amendments altered microbial communities in soil compared to fallow soil. Effects of amendments on microbial communities was substrate specific based on the different microbial responses to green manures versus compost. Similar organic amendment-specific effects were observed for chemical characteristics of soil. Sudangrass was the only green manure to increase soil organic matter and two brassica green manures had different effects on soil nitrogen, green manure from broccoli did not influence soil nitrogen and green manure from radish significantly reduced soil nitrogen. The implications of these amendment-specific effects on microbial and chemical characteristics of soil are that different amendments, even similar green manures, cannot be assumed to have the same effects on soil health.

Fusarium oxysporum f. sp. *lactucae* is only known to cause disease on lettuce. However, there is growing evidence that non-lettuce crops can serve as asymptomatic hosts for this pathogen. Asymptomatic infection of non-lettuce crops could contribute to disease outbreaks by serving as a reservoir for pathogen populations. This research showed *F. oxysporum* f. sp. *lactucae* can infect barley, mustard, and sudangrass asymptomatically in a single greenhouse experiment. This suggests similar infection of these crops could occur under field conditions suggesting use of barley, mustard, or sudangrass as rotational crops should be avoided in fields with a history of fusarium wilt of lettuce.

The practical implications for this work are that organic amendments, especially compost, have potential to reduce the abundance of the fusarium wilt pathogen in soil and suppress fusarium wilt disease, while altering microbial and chemical characteristics of soil. Future research will need to evaluate if compost application provides similar benefits to plant and soil health at the field scale in the Salinas Valley climate and integrate use of this amendment with other disease and soil health management practices.

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endorsement by the US Department of Agriculture. The USDA is an equal opportunity provider and employer.

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