

FINAL PROJECT REPORT

Project Title: Greater system efficiency and fruit quality via soil microbiology

PI: Mark Mazzola
Organization: USDA-ARS
Telephone/email: 509- 664-2280 x 207
mark.mazzola@ars.usda.gov
Address: 1104 N. Western Ave
City: Wenatchee
State/Zip: WA 98801

Cooperator: James Mattheis
Organization: USDA-ARS
Telephone/email: 509- 664-2280
james.mattheis@ars.usda.gov
Address: 1104 N. Western Ave
City: Wenatchee
State/Zip: WA 98801

Other funding sources

Agency Name: USDA-NIFA
Amount awarded: \$517,798
Notes: Field trials established and maintained with funds from the USDA-NIFA funded program were used in the studies noted below. These resources were also utilized to conduct metagenome analysis of soil microbial communities.

Total Project Funding: \$208,547

Budget History:

Item	2010	2011	2012	2013
Salaries ^a	47,690	49,120	50,594	
Benefits	15,737	16,210	16,696	
Wages				
Benefits				
Equipment				
Supplies	3,500	4,500	4,000	
Travel			500	
Miscellaneous				
Total	66,927	69,830	71,790	0

The objective of this program was to obtain insight into the role of soil microbiology on resource utilization within orchard systems and to determine potential impacts of fertility management programs on the function of soil biology. The resulting effects of fertility inputs on tree growth, with particular emphasis on root development, and orchard production were also to be examined.

Specifically, this program:

- 1.) Assessed the rooting behavior of apple as affected by different resource inputs.
- 2.) Examined the effect of orchard management options on soil microbial communities and orchard productivity.
- 3.) Quantified the key genes driving microbial nitrogen cycling in the apple rhizosphere under different resource input programs
4. Determined the effect of altered soil biology on fruit quality characteristics including coloring.

SIGNIFICANT FINDINGS

2010:

- In a sandy texture orchard soil, root development of M9 rootstock was enhanced or depressed in a nitrogen amendment-dependent manner
- In this same orchard soil it was apparent that the effect of nitrogen amendments on M9 lateral root development were indirect, being determined by the orchard soil biology
- Bacteria belonging to the genus *Streptomyces* initially isolated from the rhizosphere of M9 apple rootstock were capable of enhancing root and overall plant development

2011

- The type of nitrogen amendment significantly altered abundance of microbial genes involved in N cycling, and thus could result in altered retention or loss of N from orchard soils
- The effect of N amendment on microbial cycling gene abundance was influenced by the long-term soil management system; that is organic and conventional systems differed.
- In extension of initial year findings, in an additional orchard soil the effect of nitrogen amendments on rootstock lateral root development were similarly shown to be indirect, apparently being determined by the orchard soil biology
- Through examination of a larger bacterial population, root stimulation induced by *Streptomyces* bacteria was found not to be linked directly to nitric oxide production, and could be replicated using other bacterial species.

2012

- Metagenome analysis (microbial community genomic analysis) provided new insight into the structure and function of orchard soil microbial communities. It served to effectively identify novel microbial species with the potential to affect soil health and orchard productivity.
- Yield performance and rhizosphere microbial communities were similar for both Gala/M9 and Gala/G11 rootstocks in non-treated replant orchard soil.

- In contrast, yield performance exhibited a rootstock-dependent response in Brassicaceae seed meal (BSM) amended soil; Gala/G11 outperformed Gala/M9; however, yields for trees on both rootstocks were superior to that in fumigated soils (23% increase on M9; 24% increase on G11).
- Application of BSM soil amendment did not alter fruit quality as assessed by firmness, relative to control or fumigation treatments after 4 months cold storage in air.
- Contrary to common belief, ammonia oxidation activity, the rate limiting state in the nitrification process, is dominated by the activity of archaea, rather than bacteria, in Central Washington orchard soils and these two microbial groups respond differently to the type of nitrogen input.

2013

- Communities of archaeal ammonia oxidizers differed significantly between organic and conventional orchard systems highlighting the need for increased focus in research to understand and improve specificity of fertilizer application for orchard production systems.
- Long-term lesion nematode suppression in seed meal amended soils was associated with a sustained shift in soil microbial community composition. Rapid nematode re-infestation of fumigated soils was associated with a simultaneous reversion of the soil microbial community to one indistinguishable from non-treated orchard soil.
- This difference in nematode re-establishment typically corresponded with enhanced tree growth and yield in seed meal amended soils. Thus, it is plausible that the beneficial effects of Brassicaceae SM amendment in terms of overall growth and yield will persist leading to enhanced orchard economic viability.
- Overall diversity of the microbial community in the disease suppressive seed meal amended soils was reduced relative to the disease conducive non-treated or fumigated soil. This finding brings into question the widely held view that enhanced “biodiversity” is instrumental in achieving system resilience and/or pathogen suppression.
- Abundance and diversity of potential foliar and fruit pathogens, including *Penicillium* spp., was significantly lower in seed meal amended soils demonstrating that an understanding of below ground/above ground interactions may enhance orchard sustainability and fruit quality.

Results and Discussion

1a. *Effect of fertility inputs on rooting behavior.* Studies were conducted in a Sandy soil, WSU Sunrise orchard (SR), a Sandy Loam soil, RF orchard, Chelan and a Gravelly Sandy Loam, GC orchard Manson. Experiments were conducted in both the native orchard soil and pasteurized orchard soils in order to obtain an understanding of the role of soil microbiology in the observed plant growth response. Plant rooting responses were virtually identical between the orchard soils. In pasteurized orchard soils, amendments had no effect on M9 rootstock root development and there were no significant differences in the number of lateral roots per length of primary root. In contrast, when assays were conducted in the native (natural) orchard soil, the type of fertility input had significant effects on rooting behavior of M9 apple rootstock regardless of the orchard

soil. Changes in relative root proliferation were associated with qualitative differences in soil microbial communities. Fertility inputs inducing enhanced root development also elevated populations of total fungi, and bacteria belonging to the genera *Pseudomonas* and *Streptomyces*.

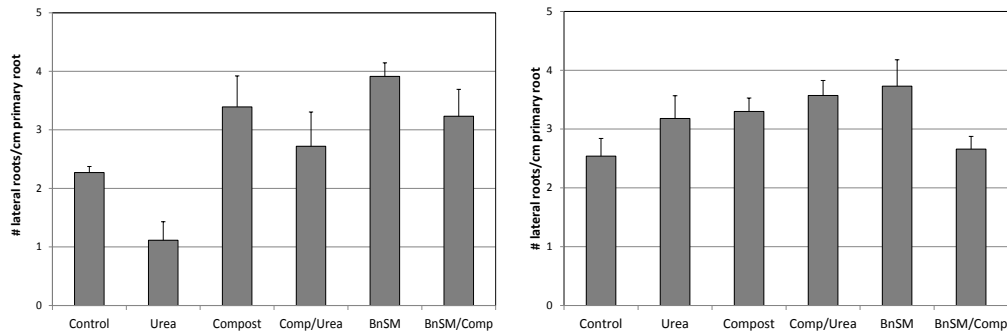


Figure 1. Effect of fertility amendments on M9 rootstock lateral root development in control (left panel) or pasteurized (right panel) sandy soil (WSU-Sunrise). BnSM=canola seed meal.

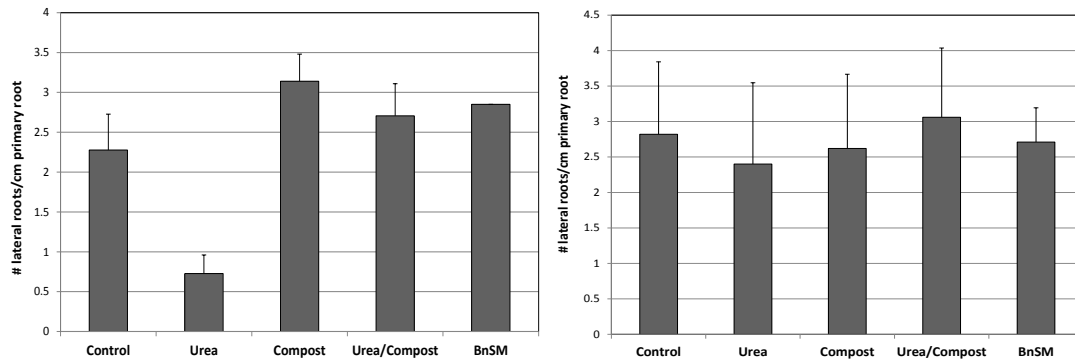


Figure 2. Effect of fertility amendments on M9 rootstock lateral root development in control (left panel) or pasteurized (right panel) sandy loam soil (RF orchard, Chelan) . BnSM=canola seed meal

Significance: Based upon the similar findings in disparate orchard soils, tree root development in response to organic/mineral amendments appeared to be consistent across soil systems, which indicates that the materials are likely to have predictable outcomes for use to enhance tree establishment and long-term performance. The findings also demonstrate that the positive and negative effects of these fertilizer inputs are not direct but likely function through their effects on orchard soil biology.

1.2 Effect of bacterial strains on root development. Root development in response to soil amendments was associated with a significant increase in the density of bacteria belonging to the genus *Streptomyces*. In initial studies we demonstrated that among four individual *Streptomyces* isolates, only those producing nitric oxide (NO) stimulated apple root development in sterile orchard soil. However when a larger bacterial population composed of *Streptomyces* and *Pseudomonas* isolates were screened using the model plant *Arabidopsis thaliana* (Fig. 3), no association was detected between NO production and induction of lateral root development. In addition isolates were further characterized for the root induction hormone IAA and again there was no association between IAA production and capacity to stimulate lateral root formation.



Figure 3. Effect of bacterial isolates on root proliferation by *Arabidopsis thaliana*.

2.1. Effect of soil management practice on orchard microbiology. The effect of soil treatment and rootstock on composition of the rhizosphere fungal and bacterial community was assessed through metagenome analysis. The study utilized trees (Gala on G11 or M9) that were planted at WSU-Sunrise in May 2010 into replant soil that was not treated (control), fumigated (Telone-C17) or amended with *Brassicaceae* mustard seed meal (*Brassica juncea/Sinapis alba*). Samples were collected in October, 2011 and DNA isolation and sequence analysis was conducted through 2013. The analysis yielded approximately 1 million fungal sequences representing 568 different fungal species and greater than 1 million bacterial sequences which represented 1219 different bacterial species.

There were clear associations between bacterial/fungal community composition and relative tree growth and yield performance as influenced by soil treatments. For both rootstocks, yields attained in seed meal amended soils were greater than that attained in fumigated soils (**Fig. 4**, left panel). Differences in growth and yield were associated with rapid re-infestation of fumigated soil by replant pathogens (e.g. lesion nematode; **Fig. 4**, right panel), while seed meal amended soils were suppressive toward soil-borne pathogens. Correspondingly, within two years of planting the bacterial/fungal communities detected in the rhizosphere of trees cultivated in fumigated soil were indistinguishable from the no treatment control while the same communities in seed meal treated soils were highly dissimilar (**Fig. 5**).

A significant finding relative to long-term orchard resilience to disease development was the observation that members of the genus *Oidiodendron* were only detected in the rhizosphere of apple cultivated in seed meal amended soil (**Fig. 6**). The importance of these fungi resides in the fact that they have been reported to provide biological control of oomycetes including *Pythium* spp. and *Phytophthora cactorum*, causal agent of Phytophthora crown and root rot of apple. In addition, soil treatments also displayed significant effects on populations of multiple fungi known to incite foliar and fruit diseases of apple including species that incite apple scab, fly speck, and blue mold (*Penicillium*; **Fig. 6**). This result indicated that the seed meal amendment suppressed potential inoculum of these aerial pathogens which may reduce disease incidence.

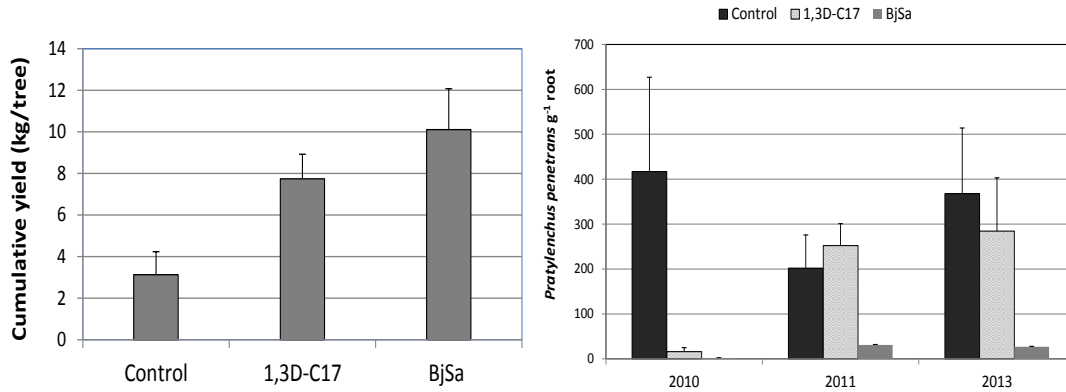


Figure 4. Cumulative yield (2012-13; left panel) and lesion nematode root densities (right panel) of Gala/G11 planted at the WSU Sunrise orchard in May 2010. 1,3D-C17=Telone-chloropicrin soil fumigation; BjSa=*Brassica juncea/Sinapis alba* seed meal.

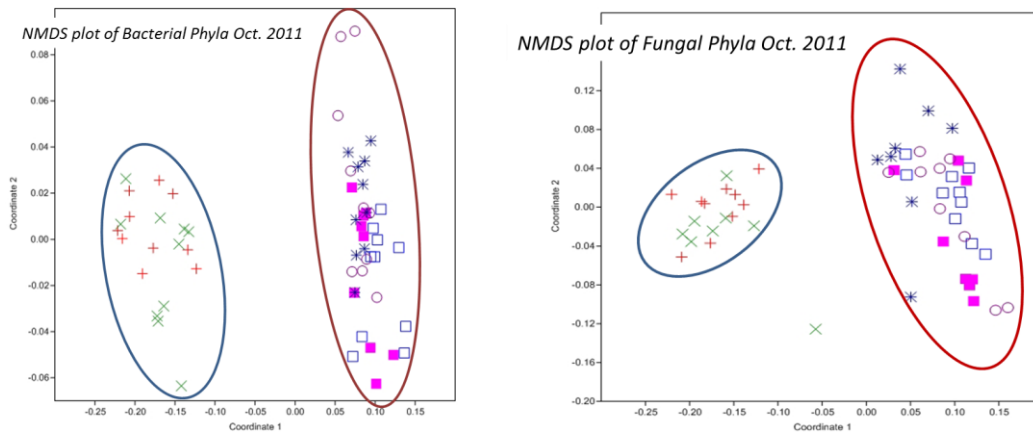


Figure 5. Effect of soil treatments on bacterial community (left panel) and fungal community composition (right panel). Treatment symbols: Control: M9 = ○; G11 = □; Telone-chloropicrin fumigation M9 = *; G11 = ■; *Brassica juncea/Sinapis alba* seed meal M9 = ×; G11 = + .

2.2. Effect of nitrogen input on dynamics of nematode communities. Nematode communities are commonly reported to serve as indicators of soil health and composition is influenced by orchard practices including tree row management systems. In the current study, type of nitrogen input had only a short-lived effect on nematode community composition as determined by examination of nematode community DNA using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis. Although the nematode communities from soils treated with nitrogen in the form of *Brassica juncea* or *Brassica napus* seed meal appeared to form a cluster at three weeks after fertilizer application (Fig. 7), no such trend was observed at 6, 9, 12 or 15 weeks after application.

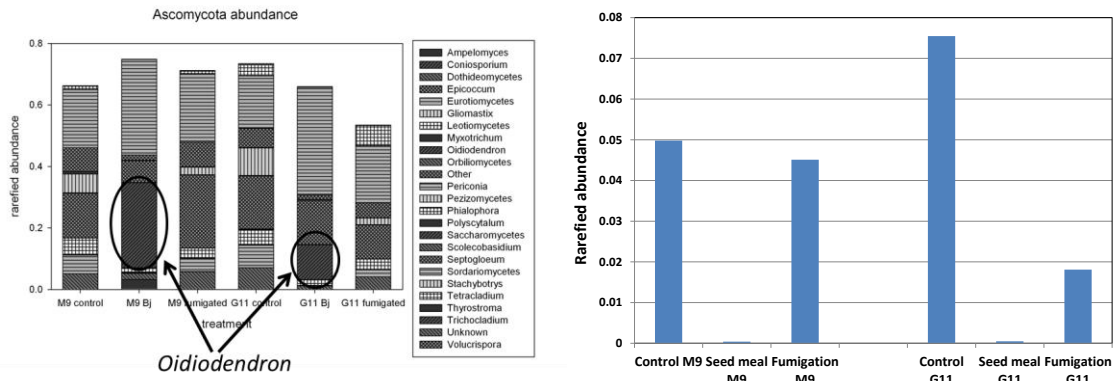


Fig. 6. Effect of soil treatments on composition of the ascomycete fungal community (left panel) and relative abundance of *Penicillium* spp. (right panel) detected in the rhizosphere of apple at the WSU-Sunrise orchard after two growing seasons as influenced by rootstock and soil treatment.

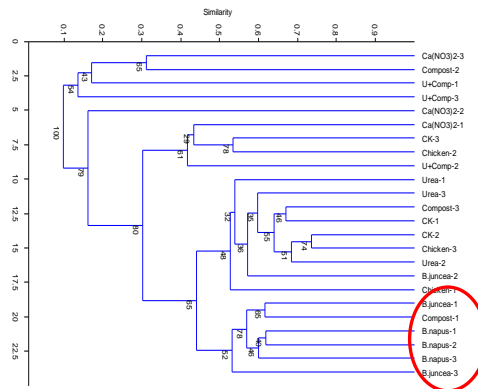


Figure 7. Cluster analysis of T-RFLP derived data for the nematode community in RF orchard three weeks after treatment with different nitrogen inputs.

Significance: These studies demonstrate that specific pre-plant soil treatments can modify the resident soil microbial community in a manner that limits re-infestation of pre-plant treated orchard soils by soil-borne pathogens resulting in significant benefits to long-term orchard health and productivity. The findings also indicate that rapid re-infestation of fumigated soil by “replant” pathogens likely is responsible for the limited (1-year) benefit of soil fumigation that has been commonly reported.

3.1 Effect of fertility inputs on N cycling gene abundance. The three orchard soils of diverse texture yielded varied responses in terms of N-cycling gene abundance; outcomes which appear to have a greater relationship with long-term management practices/soil type rather than the consequence of short-term fertility amendments. Of particular note were observations concerning the size of the denitrifying bacterial communities based upon abundance of the *nirK* gene; this gene encodes an important step in the denitrification process leading to loss of nitrogen from orchard soils through volatilization. Abundance of bacterial *nirK* was 1 to 2 orders of magnitude lower in the high organic matter (OM) RF orchard soil than in the low OM content SR orchard soil (**Fig. 8**). In addition, fertility amendments had no significant impact on bacterial *nirK* gene abundance detected in the RF soil but urea amendment resulted in a significant (two orders of magnitude) increase in *nirK* abundance in the SR soil. Co-application of nitrogen as either urea or

B. napus seed meal with compost significantly reduced *nirK* abundance in the SR orchard soil. In terms of orchard soil and inputs, fungal *nirK* gene abundance exhibited the same pattern. Thus, adding carbon as a strategy to retain N thru decreased volatilization may be of significant benefit in low organic matter orchard soils.

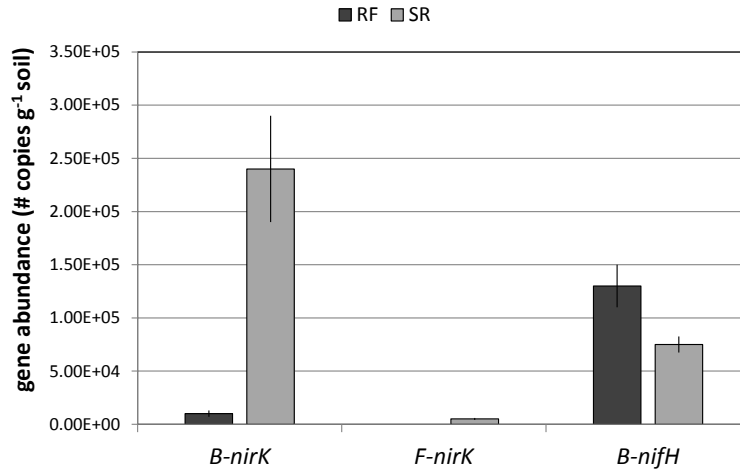


Figure 8. Relative abundance of the bacterial (B) and fungal (F) denitrification gene *nirK* and nitrogen fixation gene *nifH* in the RF and SR orchard soils.

Despite the large additions of N to these soils, bacterial N₂-fixation genes (*nifH*) were present in both soils (**Fig. 8**), though regardless of amendment, they were significantly more abundant in the high organic matter RF than SR orchard soils. At the SR orchard, the free-living N₂-fixing bacterium *Azospirillum brasilense* was commonly detected in the apple rhizosphere and abundance did not differ among soil treatments.

Soil amendments had significant effects on the abundance of the ammonia monooxygenase gene (*amo*) detected in the apple rhizosphere. This gene encodes the enzyme involved in the first step in the process of nitrification, resulting in the conversion of ammonia to nitrate. Initial soil geochemistry appeared to influence the ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) community response to fertilizer treatments. The GC soil microbial community appeared to be more limited by inorganic N than the RF soil microbial community as AOB abundance significantly increased only when additional inorganic N in the form of urea was added. This limitation is not surprising given the initial low soil inorganic N concentration of the GC orchard soil compared to the RF orchard soil. In the RF soils, AOB abundance increased compared to the no-treatment control only with the addition of both urea and compost. In contrast to AOB, fertilizer additions had little effect on AOA gene abundance in the orchard soils, thus illustrating again the complexity and variety of responses of the nitrogen cycling microbial community to fertilizers. Most short-term, fertilizer applications had no significant effect on genetic composition of the AOA or AOB community. The sole exception was observed in the conventionally managed GC orchard soil where a significant shift in genetic composition of the AOB community was detected in response to application of urea (**Fig. 9**).

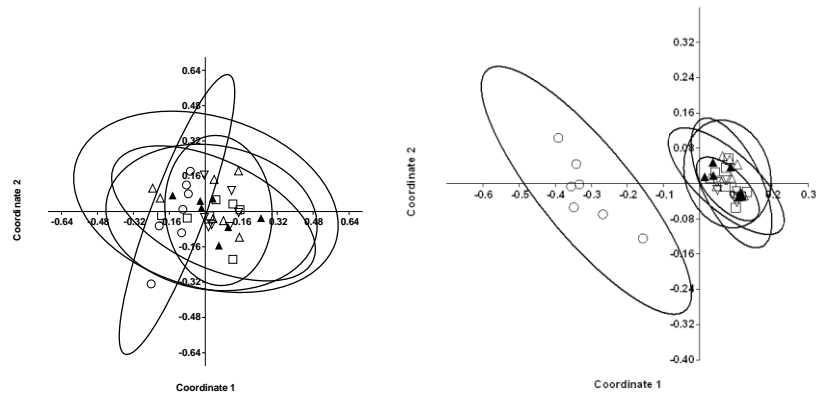


Fig. 9. Effect of fertilizer treatments on ammonia-oxidizing bacterial community composition in RF (left) and GC (right) orchard soils as assessed by non-metric multidimensional scaling of T-RFLP data. Treatments include non-amended control (Δ), *Brassica napus* seed meal (\blacktriangle), plant-based compost (\square), urea (\circ), and urea with compost (∇). Ellipses represent the 95% confidence region.

Significance: Overall, the relative abundance of the NifH, AOB, and AOA genes compared to the NirK genes indicates a potentially greater capacity for N retention, and thus plant availability, in the high OM RF than the low OM SR orchard soil system. The relative differential abundance of AOB and AOA in soil systems is of significance because AOA have been reported to respond preferentially to organic nitrogen inputs over inorganic N fertilizer. Fumigation will adversely affect the process of nitrification in soil systems and could lead to significant N losses.

3.2 Effect of physical/chemical properties on community of nitrogen cycling bacteria and archaea. Initial composition and activity of the ammonia oxidizer community was evaluated in three orchard systems. Interestingly, AOA abundance was higher in all systems, but the difference was amplified in soils of higher organic matter content (GC and RF orchards). While ammonia oxidizing archaea (AOA) were marginally higher than bacteria (AOB) in the SR soil, AOA abundance was over 10 times greater than AOB in the RF soil system and nearly 100 times greater in the GC orchard soil. (**Fig. 10**). The abundance of ammonia oxidizers in the low OM SR soil was 1 to 2 orders of magnitude lower than that in the high OM content GC and RF soils.

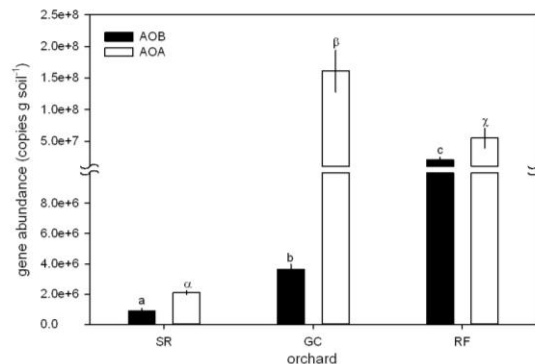


Figure 10. Bacterial (AOB) and archaeal (AOA) ammonia-oxidizing *amoA* gene abundance in non-amended SR, GC and RF orchard soils. Values are mean \pm SE (n = 7). Means with different letters are significantly different at $P < 0.05$ based upon Tukey's honest significance test for comparisons between soils.

Significance: AOA abundance was greater than abundance of AOB in orchard soils and genetic composition of AOA communities differed significantly between the organic (RF) and conventional (GC) orchard (**Fig. 11**). These microbial groups largely determine levels of nitrate in soils, the preferred N form for plant uptake. Previous studies indicate that archaea respond less favorably to inorganic forms of N. Correspondingly, in this study, AOA abundance increased in response to the organic N input in the form of brassica seed meal but not urea. This result would suggest inefficient use (and potential loss) of N from these orchard systems. These findings highlight the need for an increased focus in agricultural research to understand and improve the specificity of fertilizer application for orchard production systems.

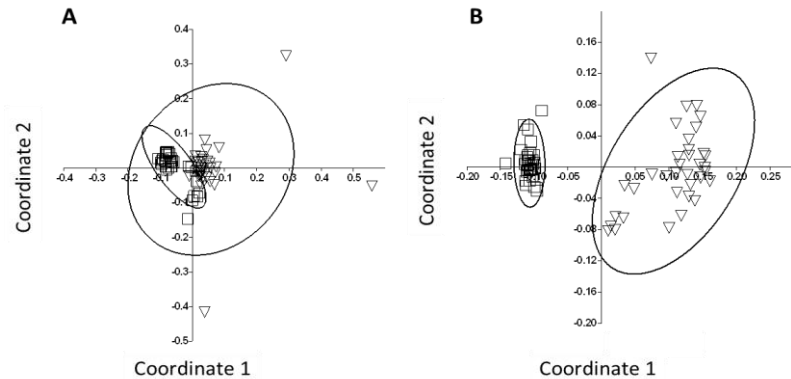


Figure 11. Composition of the ammonia-oxidizing bacterial community (A) and archaeal community (B) in the RF (∇) and GC (\square) orchard soils as assessed by non-metric multidimensional scaling analysis of T-RFLP data. Ellipses represent the 95% confidence region.

4.1. Effect of altered soil biology on fruit quality. The effect of soil treatments on fruit quality was assessed. Seed meal amendment at planting did not alter fruit quality in terms of firmness. After 4 months storage under normal atmosphere at 40 F, no significant differences in Gala fruit firmness were observed among soil treatment or rootstock (**Table 1**). Differences in fruit color (hue high to low = green to yellow; chroma high to low = increased red) were observed. Gala fruit from trees grown in the non-treated control replant soil exhibited enhanced color development relative to fruit from either the fumigated or seed meal amended soil. This finding indicated that the difference in fruit color may have resulted from a stress response or differences in canopy development rather than differences in fertility management as fumigated and non-treated plots received the same fertility input. Ongoing studies are examining the potential effects continued urea, calcium nitrate, compost and seed meal fertility inputs on nematode community structure and fruit quality.

Table 1. Effect of soil treatment on Gala fruit quality parameters

Soil treatment/rootstock	lbs	hue	chroma
Control M9	16.33±1.6	68.5±15.1a	33.9±2.6a
Seed meal M9	16.20±2.1	71.3±23.3ab	35.5±3.9b
Fumigation M9	16.39±2.0	76.2±17.6b	35.5±3.3b
Control G11	16.71±1.9	62.0±26.4a	36.1±4.0a
Seed meal G11	16.21±1.8	72.6±20.2b	35.1±3.4a
Fumigation G11	16.78±1.5	80.3±18.8c	34.8±3.5a

Values are means \pm one standard deviation; within a rootstock, means followed by a different letter are significantly different.

Executive summary

The unseen realm of soil microbiology has not been widely examined when considering contributions of the overall orchard biology to the function and productivity of orchard ecosystems. This study identified previously overlooked microorganisms including archaea, which were shown to have a dominant role in nitrogen cycling in orchard soils, as well as fungal elements that may contribute to long-term system resilience to pathogen infestation. Our findings indicate that there exist numerous opportunities to obtain benefits of the soil microbiological community that will 1) enhance utilization of fertility inputs, 2) promote tree root development, 3) improve initial tree growth and orchard productivity relative to soil fumigation, 4) suppress re-infestation of orchard soils by soil-borne pathogens and 5) enhance long-term orchard resilience and productivity.

Long-term management programs and soil physical properties were shown to influence efficiency of nitrogen utilization and loss from orchard systems. In general, abundance and function of nitrogen cycling microbial communities were superior in high organic matter soils and those under organic management. The relative abundance of the *nifH*, AOB, and AOA genes compared to the *nirK* genes in these soils indicated a potentially greater capacity for nitrogen retention, and thus plant availability, in the organic relative to the conventionally managed orchard soil system. In addition, it was demonstrated that ammonia oxidizing archaea, rather than ammonia oxidizing bacteria, dominated in all orchard soil systems regardless of management system. As AOA have been reported to respond primarily to organic nitrogen inputs, it demonstrates the need to obtain a greater understanding of how these communities function in order to efficiently utilize fertility inputs.

Based upon findings obtained in orchard soils of very dissimilar texture and organic matter content, tree root development in response to organic/mineral amendments appears to be consistent across soil systems, which indicates that soil amendments are likely to have predictable outcomes for use to enhance tree root development and establishment. In terms of root initiation, the findings also demonstrate that the positive and negative effects of these inputs are not direct but rather function through their effects on orchard soil biology. It was apparent that various bacteria could enhance root development and it is likely that multiple mechanisms contributed to the response. The input of carbon to the soil system was critical to achieving enhanced rootstock root development.

Metagenome analysis of orchard soil bacterial and fungal populations demonstrated that the soil microbial community could be efficiently managed to obtain a more resilient and productive orchard system. Specific pre-plant soil treatments, a Brassicaceae seed meal formulation, transformed the resident soil microbial community in a manner that limited re-establishment of soil-borne pathogens. Interestingly, the altered soil microbial community in seed meal amended soils was maintained over multiple growing seasons and created a soil environment that suppressed pathogen re-infestation. This altered soil microbial environment resulted in significant benefits to long-term orchard health and productivity. In contrast, fumigated soils were highly conducive to re-infestation by soil-borne pathogens. In particular, lesion nematode populations increased dramatically in the second growing season to root densities that were as high or higher than that detected in non-treated orchard soil. The findings also indicate that rapid re-infestation of fumigated soil by “replant” pathogens likely is responsible for the limited (~1-year) benefit of soil fumigation that has been reported. Such a response indicates that continued management of the soil microbial community beyond the initial application of soil fumigation, will significantly improve orchard productivity. Our continued studies will be directed by this question with a focus on reduced seed meal inputs in concert with rootstock genotype as a means to improve orchard system resilience and productivity.