Almond Orchard Recycling

Project No.: **17-PREC3-Holtz (COC)**

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Objectives:

The overall goal of this project is to comprehensively assess the implications whole orchard recycling (WOR) with the standard practice of orchard removal for energy co-generation. In addition, we will continue to monitor the impacts of WOR compared to open field burning in the 2008 experiment. Our specific objectives for WOR and conventional orchard removal are to:

1. Refine life cycle assessment (LCA) model for carbon dynamics and balance.
2. Quantify effects of the treatments on physical and chemical soil properties and nutrients.
3. Quantify effects of the treatments on biological soil properties.
4. Assess impacts of the treatments on replanted orchard growth, yield, nutrition, and water efficiency.

Interpretive Summary:

Whole orchard recycling (WOR), as an alternative to co-generation burning, could reduce net orchard greenhouse gas emissions by sequestering carbon from almond orchard biomass. The woody residue generated by WOR, estimated to be 30-65 tons per acre depending on tree size, spacing, and varieties, appears to increase soil carbon, organic matter, soil fertility, soil water infiltration rates and soil water retention. The first orchard grinding trial at Kearney, established in 2008, compared WOR of stone fruit trees with the Iron Wolf, estimated at 30

tons per acre, to burning and incorporating the ash. The orchard was replanted to almond. Ultimately, greater yields, significantly more soil nutrients, organic matter, and total carbon were observed in the grind treatment when compared to the burn. Leaf petiole analysis also revealed higher nutrients levels in trees growing in the grind treatment, thus proving that the organic matter did not stunt replanted trees. Based on positive results from this trial and the closure of co-generation plants, we estimate almond growers have chipped and incorporated 20,000 acres since 2015. A deficit irrigation trial established at Kearney has shown less water stress from trees growing where the previous orchard was ground, suggesting increased water holding capacity with additional organic matter. We also documented increased water use efficiency and stomatal conductance from trees growing in the WOR treatments when compared to the burn.

In the WOR trials established since 2015 we have already observed increased soil organic carbon, total nitrogen, and gravimetric water content in WOR soils, while observing decreased soil compaction and bulk densities. We observed that large soil macroaggregates accumulated more carbon in the WOR treatments, which means that woodchips have the potential to store and physically protect carbon from microbial breakdown. The large wood chunks of the Iron Wolf may store more soil carbon than the smaller wood chips from a horizontal chipper. Significantly higher microbial biomass carbon was observed in the WOR treatment while microbial biomass nitrogen was decreased.

Preliminary data from several of the newly established trials have suggested that our nitrogen recommendation for first year almond trees will double from 4 ounces of actual nitrogen per tree per year to 8 ounces after implementing WOR, because of the additional carbon added to the soil. The nitrogen applications are typically spread out so that no more than one ounce of actual nitrogen is applied per tree per application. After the first year we hypothesize that increased nitrogen efficiency will be observed as the wood chips decompose and release bound nitrogen from the soil organic matter. Impacts of orchard debris on incidence and severity of soil-borne diseases of almond are largely unexplored, but increases in soil organic matter have resulted in favorable soil microbial community shifts, resulting in suppression of some soil-borne diseases and improved plant nutrient dynamics.

Materials and Methods:

Six additional orchard recycling trials with almond were established in 2016. A second trial with the Iron Wolf was established with Agriland Farming in Chowchilla comparing Iron Wolf grinding (WOR-G) with a horizontal Morbark chipper (WOR-C) and complete tree removal for co-generation. WOR was estimated at 68 tons per acre. Third and Fourth trials were established with Wonderful Orchards, in Bakersfield and Shafter, where WOR was compared to complete tree removal, with and without fumigation, and estimated at 39 and 65 tons per acre respectively. A fifth trial was also established at Kearney where WOR was performed at 85 tons per acre and compared to other substrates used for anaerobic soil disinfestations (ASD). The 85 ton per acre rate of wood chips is the most we have attempted to recycle. Our sixth and seventh trials were established at the Nickel's Soil Lab in Arbuckle and at Tallerico Orchards in Manteca. At Nickel's the orchard was recycled at two rates, 54 and 25 tons per acre, while at Tallerico the orchard was recycled at 64 tons per acre. Orchard plots were fumigated in the fall and planted to second-generation almond trees in early 2017, except at

Nickel's where fumigation was delayed until 2018, while trees will be planted in spring 2019. Our eighth and ninth trials were established in 2018 at Warkentin Orchards in Parlier and California State University Fresno (CSUF). The Warkentin orchard was recycled at 40 tons per acre, and used in Dr. Culumber's greenhouse gas measuring project, while the CSUF trial was recycled at 60 ton per acre in association with Dr. Browne's anaerobic soil disinfestations (ASD) treatments. Our goal at each trial location was to spread the wood chips back onto the orchard floor at the same rate the trees were removed. In most cases a smaller (slower to chip) two inch size screen was used on the horizontal chippers to reduce chip size.

Soil carbon and physio-chemical properties. Total carbon and nitrogen in soil and tree residues are being analyzed using combustion method (Costech elemental Analyzer, Gaudin Lab) in soil micro aggregates after fractionation. Labile carbon pools (PoxC) and soil indicators of microbial functions such as total activity (FDA) and C and N cycling (microbial enzymatic activities) are measured using established colorimetric assays. Total C and N in microbial biomass is being measured using the fumigation method. Wet aggregate stability, water infiltration (double ring infiltrometer) and water holding and release dynamics (Hyprop system) are being tested using standard methods. Additional soil properties (texture, CEC and other macro/micronutrients) are being determined at UCD ANL laboratory.

N retention: Soil samples have been collected from paired plots at the Kearney site to test for NO_3^- leaching in lab setting using soil columns with fine mesh supporting a glass filter at the base. For the nitrate leaching study, the soil samples from the different field treatments will be amended with mineral fertilizer and incubated. After 2 weeks, excess water will be applied, and water flowing out from the column will be collected. The NO_3^-- content of the outflow water and soil will be determined by standard methods.

Tree-soil water relationships. A fully watered (100% ET) and deficit irrigation treatments (64% of ET at hull split for three weeks) were established in 2016 on Nonpareil rows in the 2008 tree incorporation and burned control plots. Tree water status was monitored weekly using a pressure bomb while neutron probe tubes were installed and monitored.

Results and Discussion:

Objective 1. Refine life cycle assessment (LCA) model for evaluation of carbon dynamics and balance (Gaudin, Marvinney).

This project will provide input data for use in the revised almond LCA model under development. Measurement of soil carbon additions in response to WOR will allow refinement of current model parameters for temporary carbon storage in orchard floor soils as well as allowing the parameterization of alternative management scenarios for uncertainty and sensitivity analysis. Standing biomass will be quantified in order to estimate potential carbon stock added to orchard floor soil in the WOR alternative scenario, and used to compute potential temporary carbon storage credits based on prior reports analyzing rates of wood decomposition in soil and establishing a methodology for calculation of time adjusted warming potential as well as data collected in the proposed study. Soil carbon and wood chip observational data from long-term barrel and surface wood decay experiments were incorporated into the almond life cycle model using the Time-Adjusted Warming Potential metric (reported as kg CO2 equivalent), treating woodchips in orchard floor soils as an

additional pool for temporary carbon storage - effectively extending the carbon storage potential of standing biomass into a subsequent orchard life cycle (**Figure 1**). In order to utilize these data, it was assumed that observed rates of carbon loss from chips and net soil carbon accumulation were independent of both woodchip application rate (kg per cubic meter of soil) and enclosure in barrels. In order to account for the possibility that these assumptions will not hold true in a field setting and result in significantly lower carbon storage than observed in the barrel experiments, a soil carbon cap of 1.2% total C (calculated based on soil particle size distribution) was applied as a model scenario. Results from ongoing field trials will further inform the development of this aspect of the almond LCA model.

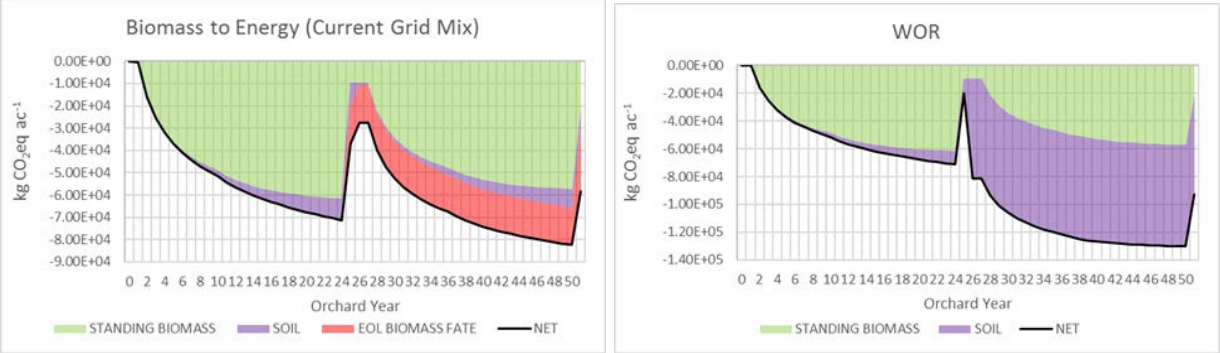


Figure 1. Soil carbon and wood chip observational data from long-term barrel and surface wood decay experiments were incorporated into the almond life cycle model using the Time-Adjusted Warming Potential metric (reported as kg CO2 equivalent), treating woodchips in orchard floor soils as an additional pool for temporary carbon storage - effectively extending the carbon storage potential of standing biomass into a subsequent orchard life cycle.

Objective 2. Quantify the short and long-term effects of the treatments on physical and chemical soil properties and tree essential nutrients (Holtz, Gaudin, Jahanzad, Culumber)

Soil samples were taken from the Kearney, Chowchilla and Wonderful Orchard (WO3371 and WO3381) sites to measure the effects of WOR on the physical and chemical soil health characteristics. Samples were taken from top 6 inches of the soil (on the berm, between trees) where woodchips were incorporated. Soil cores from each plot were combined to obtain a uniform and representative sample per treatment/replication. Depending on the sites, 4 to 7 replications were used. In addition to the WOR treatment, soil was also sampled from the control plots where wood chips were either burned and surface applied (Kearney) or exported from the field (3381, 3371, and Chowchilla). Results showed that soil total carbon content ranged from 0.71% to 0.92% in the woodchips plots, which indicated significant increase compared to the control (burn or no woodchip) treatments (**Tables 1 – 4**). Woodchip incorporation resulted in increasing soil organic matter content compared to the control in all of the recycled orchards; however, significant differences were observed only at the Kearney and 3371 sites (**Tables 1 and 4**). Increasing trends were observed in soil organic carbon and total nitrogen with WOR at all of our sites, but only Kearney and 3371 had significant differences. Treatments did not impact pH, EC, CEC, P, K, and other soil minerals. Gravimetric water content increased in the WOR treatment compared to the control, however, treatments were not significant (**Table 5**). In addition, soil compaction, as indicated by resistance, decreased with WOR; however, the only significant difference was observed at 3371 (**Table 6**). Bulk

density decreased in all of the sites but 3381 was the only site where significant differences between treatments were observed (**Table 7**).

Table 1. Soil chemical properties at the 3371 site.

3371	OM (%)	Org C (%)	TC (%)	TN (%)	NO_3^- (ppm)	pH	EC (ds/m)	CEC (meq)	Na (ppm)	Cl (ppm)
Control	1.02	0.59	0.53	0.05	52.02	6.67	0.54	8.37	137	59.92
Woodchips	1.52	0.88	0.75	0.07	56.82	7.15	0.59	10.37	73	30.22
P Value	0.01	0.01	0.02	0.22	0.91	0.12	0.87	0.22	0.18	0.12
	K	P	S	Zn	Fe	Mn	Cu	Ca	Mg	B
	ppm									
Control	169	16.82	29.02	10.77	13.3	14.37	0.81	1196	121	1.03
Woodchips	210	18.87	21.17	13.96	9.87	12.75	0.97	1614	144	1.06
P Value	0.49	0.65	0.43	0.32	0.37	0.63	0.37	0.2	0.22	0.87

Table 2. Soil chemical properties at the 3381 site.

3381	OM (%)	Org C (%)	TC (%)	TN (%)	NO_3^- (ppm)	pH	EC (ds/m)	CEC (meq)	Na (ppm)	Cl (ppm)
Control	1.2	0.7	0.55	0.04	23.15	6.87	0.22	8.45	26.75	6.92
Woodchips	1.42	0.82	0.71	0.06	34.4	6.5	0.34	8.85	46.5	11.77
P Value	0.22	0.23	0.01	0.06	0.59	0.37	0.53	0.5	0.38	0.28
	K	P	S	Zn	Fe	Mn	Cu	Ca	Mg	B
	ppm									
Control	114	17.17	20.52	15.54	13.17	15.02	1.83	1365	131	0.51
Woodchips	126	16.45	22.8	16.16	18.85	15.4	1.66	1273	125	0.62
P Value	0.49	0.82	0.77	0.74	0.3	0.94	0.53	0.54	0.48	0.57

Table 3. Soil chemical properties at the Chowchilla site.

Chowchilla	OM (%)	Org C (%)	TC (%)	TN (%)	NO_3^- (ppm)	pH	EC (ds/m)	CEC (meq)	Na (ppm)	Cl (ppm)
Control	1.1	0.64	0.57	0.05	23.03	7.13	0.27	8.63	47.66	25.7
Woodchips	1.73	1.0007	0.92	0.08	14.04	7.33	0.18	8.5	37.66	31
Iron wolf	1.23	0.72	0.65	0.05	19.4	7.26	0.24	8.3	45	20.36
P Value	0.0007	0.0006	0.006	0.03	0.04	0.63	0.25	0.72	0.32	0.36
	K	P	S	Zn	Fe	Mn	Cu	Ca	Mg	B
	……ppm……									
Control	132	34.16	9.33	14.88	14.06	15.66	4.9	1372	150	0.43
Woodchips	148	35.63	7.9	16.78	14.03	19.46	6.09	1334	154	0.74
Iron wolf	111	35.6	6.86	14.7	14.5	14.96	4.98	1319	145	0.63
P Value	0.16	0.91	0.24	0.39	0.96	0.32	0.19	0.71	0.72	0.13

Table 4. Soil chemical properties at the Kearney site.

Kearney	Total C	Org. C	OM	Total N	Labile C (mg/kg)	K (mg/L)	EC	pH
	……%.....						ds/m	
Grind	0.79	0.88	1.52	0.07	238	11.06	0.57	6.94
Burn	0.55	0.62	1.07	0.06	166	11.68	0.58	7.02
P Value	0.001	0.001	0.001	0.05	0.16	0.39	0.45	0.39
	Mg (meq/L)	Ca (meq/L)	Na (meq/L)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)	B (mg/L)
Grind	1.46	3.02	0.89	9.69	9.25	9.03	33.23	0.3
Burn	1.43	3.05	0.72	9.64	9.26	6.79	28.01	0.31
P Value	0.47	0.48	0.03	0.47	0.5	0.01	0.11	0.4

Table 5. Soil gravimetric water content at the 3371, 3381, and Chowchilla sites.

Treatment/ Site	3371	3381	Chowchilla
	……. g water/g soil…….		
Control	0.062	0.060	0.059
Woodchip	0.069	0.062	0.063
Ironwolf	-	-	0.068
P Value	0.22	0.63	0.55

Table 6. Soil compaction at the 3371, 3381, and Chowchilla sites.

Treatment/ Site	3371	3381	Chowchilla
	……psi …….……..……		
Control	175	180	180
Woodchip	139	165	160
Ironwolf	-	-	186
P Value	0.002	0.24	0.08

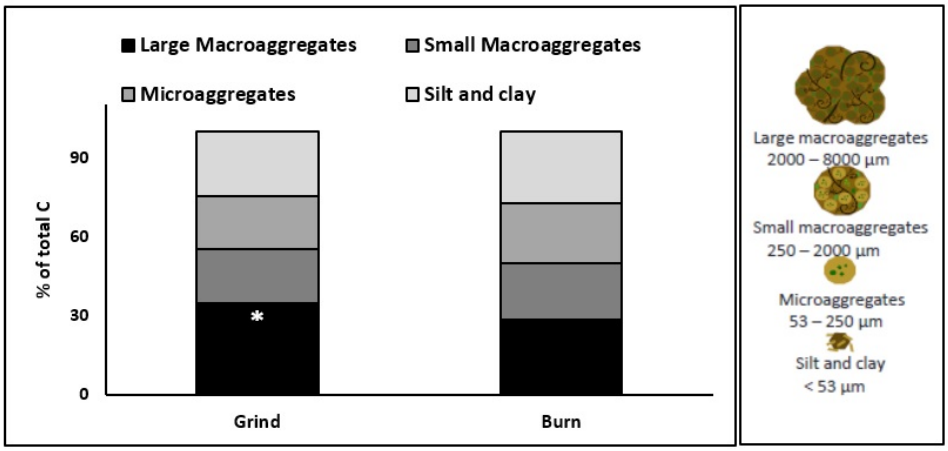
Table 7. Soil bulk density at the 3371, 3381, and Chowchilla sites.

Treatment/ Site	3371	3381	Chowchilla
	……..……g/cm3…….		
Control	2.02	1.89	1.61
Woodchip	1.82	1.42	1.49
Ironwolf	-	-	1.68
P Value	0.06	0.01	0.09

Long term C sequestration (Holtz, Gaudin, Jahanzad, Culumber)

In addition to regular soil sampling at the Kearney site incorporated ten years ago, we sampled deep soil cores using a Geoprobe in October 2017. Soil cores are currently being processed to evaluate long term effects of whole orchard recycling on carbon pools and carbon sequestration in aggregate fractions. After analyzing samples from the topsoil (6 inches), we found that large macroaggregates accumulated 14% more carbon in the woodchip plots (Grind) compared to the control (Burn). However, small macroaggregates, microaggregates, and silt and clay did not show a significant difference in terms of TC storage (**Figure 2**). This means that the grind treatment has potential to store and physically protect carbon from microbial breakdown in macroaggregates. More total carbon was observed in the grind treatment over the last decade (**Table 4**). In addition to the soil organic matter content and total carbon, samples from three different soil depths will be processed to measure other parameters such as particulate organic matter, labile carbon, dissolved organic carbon, as well as aggregate protected carbon. We also measured total C and N in microbial biomass (the main components of this labile pool) using the fumigation method, which indicated significantly

higher microbial biomass carbon in the soil sampled from the grind plots compared to the burn. However, treatments were not significantly treatments in terms of microbial biomass nitrogen (**Figure 3**).



* indicates significant difference ($P \leq 0.05$).

Figure 2. Percentage of total carbon in different soil aggregate sizes in the Burn and Grind treatments.

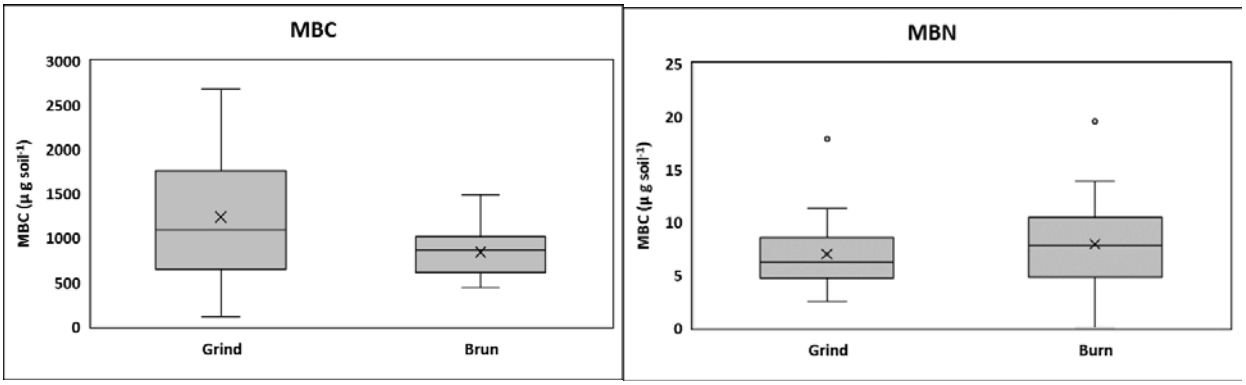


Figure 3. Soil microbial biomass carbon (MBC) and nitrogen (MBN) as influenced by woodchip incorporation. Treatments differed significantly ($P \leq 0.05$) in terms of MBC.

N retention (Gaudin, Jahanzad, Holtz, Culumber):

To continue our investigation of shifts in N dynamics in situ using 15N labeled fertilizer to track N addition to different soil pools and qualify total microbial biomass and immobilization potential in both short and long-term. Treatments included soil with woodchips incorporated (WC1), soil without woodchips (WC0), fumigated (F1), and non-fumigated (F0) soil, replicated three times for two orchards: one newly recycled and one recycled 10 years ago, both located at Kearney. Soil was sampled from two different depths (0-15 cm and 15-30 cm) and all of the sampling and measurements will include both soil depths. 15N nitrogen fertilizer was applied to the columns according to the recommended fertigation rate and leachate was collected. We

are currently processing the soils cores to determine nitrogen immobilization, mineralization, and retention using 15N percentage recovery from the soil and leachate samples.

Objective 3. Quantify effects of the treatments on biological soil properties (Browne, Westphal, Yaghmour, Doll, Holtz, Culumber).

We monitored effects of WOR treatments on populations of plant pathogenic nematodes and root and soil microbial communities including bacteria, fungi, and oomycetes. We completed total DNA extraction, amplification, and sequencing of rRNA gene fragments from bacterial, fungal, and oomycete root and soil samples (**Table 8**). We are still completing "bioinformatics" (sequence quality filtering, sequence identifications in National Center for Biotechnology Information and Barcode of Life Datasystem databases, statistical analyses of relationship of organisms to soil treatments and tree growth). Some highlights of microbial community analyses completed to date are given below. In our Bakersfield (WO3371) and Shafter (WO3381) 2017 trials, WOR had little initial impact on soil bacterial or fungal communities, but subtle, significant differences in soil or root communities were measured in just a few months after WOR. For bacteria in soil, the proportion community variation explained by WOR near planting time was not statistically significant (R2 values representing the proportion explained by WOR treatment were at 0.03 for both trials, P=0.12 to 0.14); and for fungi in soil at the same time, the corresponding R2 values were 0.02 to 0.03; P=0.8 to 0.4). After planting, in June however, small but significant proportions of bacterial community diversity were explained by WOR treatment in soil (R2 = 0.04, P=0.001) or roots (R2=0.04, P=0.0002); the same was true for June fungal community diversity (R2 = 0.04 to 0.09, P= 0.01 to 0.001).

In the Parlier 2017 trial, at planting time, the preplant fumigated soil was enriched in identified members of class Actinobacteria, while having lower relative abundances of most other bacterial classes compared to the other treatments. At the same time, soils that had been fumigated or amended with ground rice bran or almond hull material were enriched in class Bacilli, compared to WOR and check treatments. The relative abundances of several operational taxonomic units (OTUs) of genus Bacillus near planting time were positively correlated with subsequent positive growth responses in the replanted trees, whereas the abundances of a few OTUs classified as unknown members of Actinobacteria correlated negatively with tree growth. The class Actinobacteria includes both plant pathogens and biocontrol agents for plant pathogens, and this may be an explanation for the seeming paradox that fumigated soil, which stimulated tree growth, was elevated in identified Actinomycetes at planting time. Clearly it will be important to learn more about how specific members of this class affect almond trees.

In the Manteca 2017 trial: In May, after planting, bacterial communities differed significantly according to whether or not they were in WOR plots or not (R2=0.22, P=0.02) and whether they were from soil or roots (R2=0.47, P=0.001). Two bacterial OTUs (one in genus Cellulomonas another in class Chloroflexi) were enriched in WOR soil compared to non-WOR soil. The OTU identified as class Chroroflexi was also enriched in roots from WOR plots, compared to non-WOR plots. Growth of trees was negatively correlated with relative abundance of the Chloroflexi and Cellumonas OTUs in soil (r= -0.78 to -0.94, P=0.006 to 0.01) and in roots (r= -0.79 to -0.92; P=0.06 to 0.0009). We will complete the bioinformatics analyses we have undertaken during the continuation of this project. Technologies and bioinformatics methods are advancing rapidly in microbial ecology and may be very helpful for

this project in resolving individual microorganisms and quantifying them. For example, in 2018, an approach for simultaneously quantifying individual members of microbial communities, taking into account differing efficiencies in sample extraction, PCR, and sequencing, was described (2). Also, an algorithm to more precisely resolve between closely related taxa was described (1).

Table 8. Experiments, treatments, and microbial sampling in whole orchard recycling trials

Experiment (Yr planted)	Treatment			Microbial community monitoring?	
	Mainplot	Subplot	Rootstock(s)	Soil	Roots
Shafter 1	No WOR	Control	H536, NG	+4	+3
(2017)		ASD	H536, NG	+4	+3
		Strip fumigation	H536, NG	+4	+3
		Spot fumigation	H536, NG	-	-
	WOR	Control	H536, NG	+4	+3
		ASD	H536, NG	+4	+3
		Strip fumigation	H536, NG	+4	+3
		Spot fumigation	H536, NG		
Shafter 2	No WOR	Control	H536, NG	+2	+1
(2017)		ASD (Ahs 9 t/a)	H536, NG	+2	+1
		Strip fumigation	H536, NG	+2	+1
		Spot fumigation	H536, NG	-	-
	WOR	Control	H536, NG	+2	+1
		ASD (Ahs 9 t/a)	H536, NG	+2	+1
		Strip fumigation	H536, NG	+2	+1
		Spot fumigation	H536, NG		
Parlier (KARE)	Control		NG	+4	+2
(2017)	Fumigation		NG	+4	+2
	WOR		NG	+4	+2
	Rice bran+pre-irrigation		NG	+1	-
	Almond hull+pre-irrigation		NG	+1	-
Manteca	Control+fumigation		Viking	+2	+2
(2017)	WOR+fumigation		Viking	+2	+2
Parlier Lincoln	No WOR	Control	Viking	+4	+3
(2018)		Fumigation	Viking	+4	+3
	WOR	Control	Viking	+4	+3
		Fumigation	Viking	+4	+3
Fresno (CSU)	Control		NG	+5	+4
(2018)	Control + preirrig		NG	+1	-
	Control + preirrig + TIF		NG	+5	-
	WOR + preirrig + TIF		NG	+5	+4
	ASD: Ahs 9t/a + preirrig + TIF		NG	+5	+4
	ASD: Ahs 9 t/a + preirrig + WOR		NG	+1	-
	ASD: Ahs 9 t/a + preirrig + WOR + TIF		NG	+5	+4
	ASD: Ahs 9 t/a + preirrig + WOR + TIF + N		NG	+5	+4
	ASD: Tom 9 t/a + preirrig + TIF		NG	+1	-
	ASD: RBr 9 t/a + preirrig + TIF		NG	+5	+4
	Fumigation		NG	+5	+4

[a]All treatments monitored for increases in trunk cross section area. In last two columns, "-" indicates no monitoring, and "+" indicates monitoring, followed by number of sampling dates. "ASD" indicates anaerobic soil disinfestation; "Ahs" indicates ASD carbon source of finely ground almond hull + shell; "preirrig" indicates drip irrigation for ASD, added sufficient water to saturate soil profile to 5 ft, kept at or above field capacity for 4 to 6 weeks; "TIF" totally impermeable film, clear, used to cover ASD plots. For rootstocks "H536" = Hansen 536, "NG"= Nemaguard.

Identification of plant pathogenic and free-living nematodes (Westphal).

It is the aim of this project to monitor population densities of plant-parasitic nematodes in plots amended with WOR and equivalent control plots. Two orchards were sampled during late

winter of 2018/early summer and examined for the presence of plant-parasitic nematodes. One site was near Chowchilla and a second site near Bakersfield (field 3371). Soil samples from the upper 18 inch of soil were collected in bulk, and examined with nematological methods. Root-knot nematodes were detected at Chowchilla at 3.7 J2/250 ml in WOR and at 1.3 J2/250 ml in non-amended plots. There were 43.7 pin nematodes/250 ml of soil in non-WOR plots but this high number was contributed by one replication only. In contrast, there was consistently throughout the replications 1 pin nematode per 250 ml soil in the chip-amended treatment. At Chowchilla, there were 645 free-living nematodes/250 ml of soil in the amended, and 325 free-living in the non-amended plots. At field 3371, there were some plant-parasitic nematodes detected: 6.2 and 5.7 vermiform root lesion nematodes in the WOR and non-WOR plots, respectively. Pin nematodes were 33 and 23.5 per 250 ml of soil in the amended and non-amended treatments. There were 341.7 in the WOR and 173.2 free-living nematodes per 250 ml of soil in the non-WOR plots. Observations at these two sites seem to prove the hypothesis that free-living nematodes increase after the chip-amendment in early years after amendment. This appears a common trend in most trials of the overall program in the first years after orchard establishment. We hypothesize that such increase is influenced by the food source amendment of the soil system in the form of the chips. Free-living nematodes consume primary decomposers, bacteria and fungi, and an increased activity of these is expected to increase numbers of these nematode species.

To further investigate if these population changes may impact the suppressiveness of the soil, a series of greenhouse experiments was initiated. Each of the soils was screened under maintenance of the field replications, and used for greenhouse experiments. Each of the samples was then divided into one portion to be left untreated and a second portion being autoclaved before potting in 4-inch pots in the greenhouse. Arranged in randomized complete block design (under observance of the original blocks), three treatments per origin were established: (i) non-treated, non-inoculated, (ii) non-treated, inoculated with root lesion nematode, *Pratylenchus vulnus* (RLN), and (iii) autoclaved, inoculated with RLN. All pots were planted to ‘Nemaguard’ seedlings and arranged on a greenhouse bench. Controls were soils from (a) chip-amended plots, and (b) burnt residue-amended plots of a long-time experiment at Kearney. These control soils each had the same i-iii treatments. A second experiment was conducted with *Mesocriconema xenoplax* (ring nematode) as the test pest.

Objective 4. Assess impacts of the treatments on replanted orchard growth, health, nutrition, and water relations (all team members). Soil hydraulic properties, tree water status, water use efficiency

We monitored effects of WOR treatments on parameters that can be associated with soil health and long and short-term orchard productivity, including soil chemical and physical properties and tree nutritional status. We measured soil hydraulic properties (water holding capacity, infiltration rates, and water retention curves) at the old Kearney site. Although not statistically analyzed yet, moisture retention curves showed higher field capacity in the grind treatments and potentially larger water conservation potential without shifts in permanent wilting point (**Figure 4**).

Grind plots also had greater infiltration rates as measured with hydraulic conductivity compared to the Burn (0.003 vs 0.001 cm/s, respectively) (**Figure 5**). We finished the analysis of the 2017 nonpareil kernel yield data from the deficit irrigation trial at the old Kearney site. Deficit irrigation to 80%ET for 28 days did not cause any significant yield loss in both

treatments. However, we show large yield benefits of the grind treatment 9 years after establishment under both adequate and deficit irrigation. Benefits were up to 20% under regular irrigation scenario (**Figure 6**). This led to an increase in 20% increase in irrigation water use efficiency in the Grind treatment compared to the Burn (1.25 vs 1.04 kg/m^3, respectively) (**Figure 7**). Stomatal conductance was not affected by the deficit irrigation treatment in both treatments but larger conductance was consistently observed in trees planted after WOR (613.34 mmol $m^{-2}s^{-1}$ in grind vs 558.98 in burn, + 9.7%) (**Figure 8**). Stem water potential was slightly less negative at all time points in the grind compared to burn treatment. This was significant on the most stressed day (28 days after onset of the DI treatment) and a week after regular irrigation was resumed (**Figure 9**). We did not detect any significant effects of WOR or irrigation regimes on tree canopy temperature. The neutron probe readings of soil moisture content at different soil depth increments (0, 9, 18, 30, 42, 52 inches) did not reveal a significant difference between the two treatments at the Kearney site.

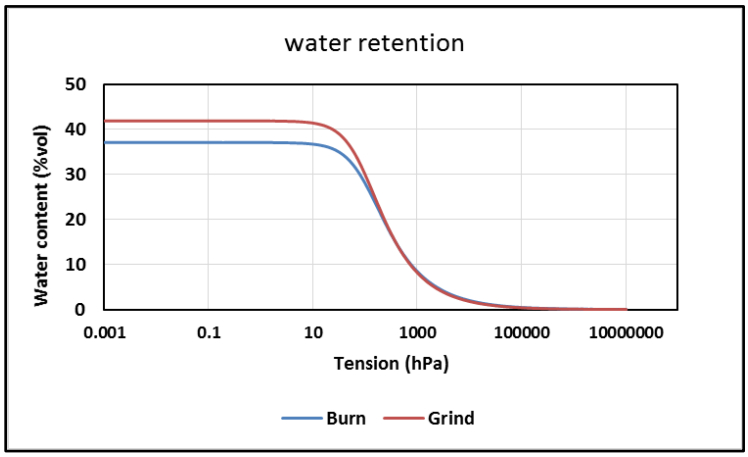
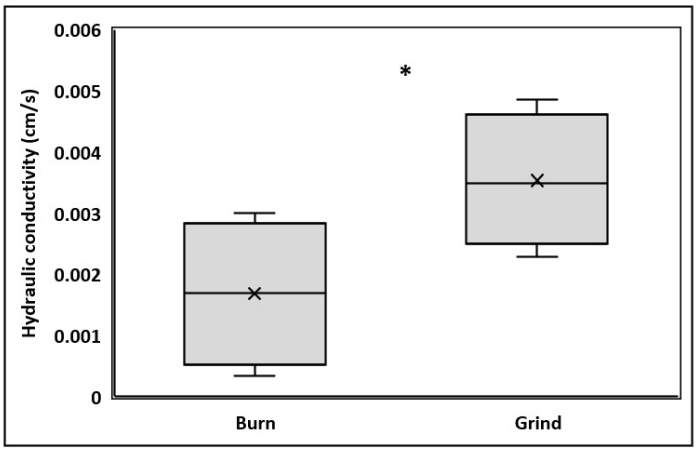
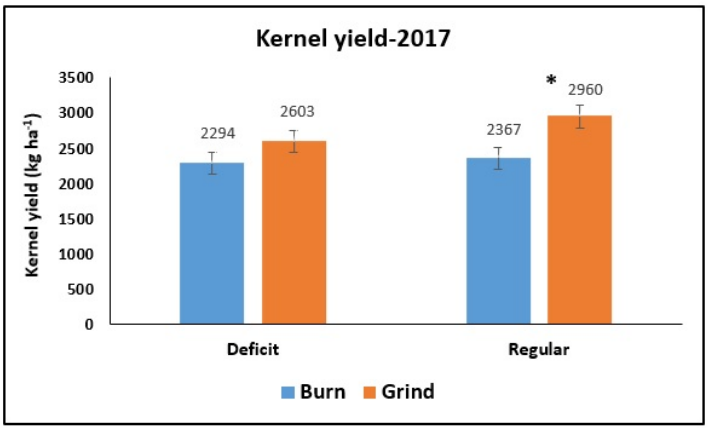


Figure 4. Water retention curves [volumetric water content (%)] as affected by Grind and Burn treatments at the old Kearney trial.



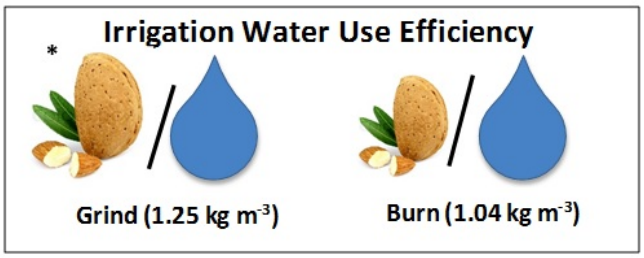
* indicates significant difference ($P \leq 0.05$).

Figure 5. Hydraulic conductivity (kfs = cm/s) differences between the Grind and Burn treatments at the old Kearney site.



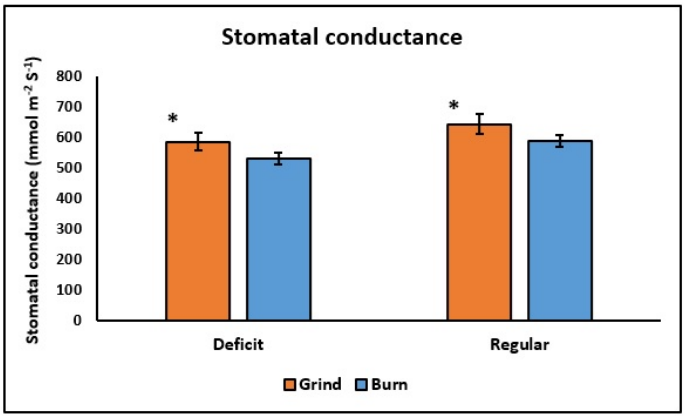
* indicates significant difference ($P \leq 0.05$). Bars are standard error.

Figure 6. 2017 Kernel yield data from the deficit irrigation trial at the Old Kearney site.



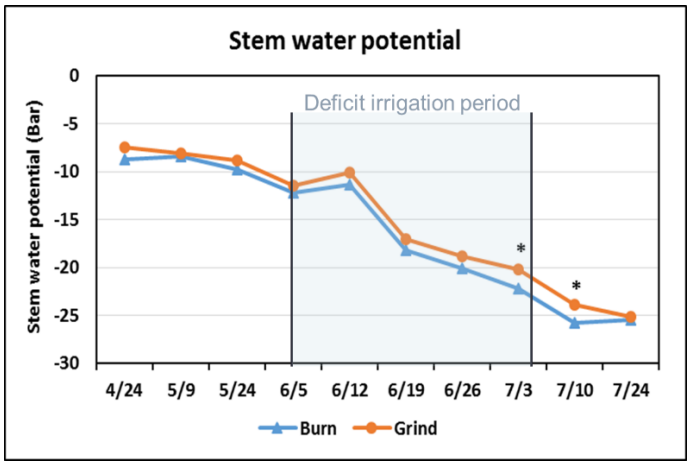
* indicates significant difference ($P \leq 0.05$)

Figure 7. Irrigation water use efficiency (kg m-3) difference between the Grind and Burn treatments at the old Kearney site.



* indicates significant difference ($P \leq 0.05$).

Figure 8. Stomatal conductance of almond leaves as influenced by the WOR treatments as well as irrigation regimes.



* indicates significant difference ($P \leq 0.05$).

Figure 9. Stem water potential differences between the Grind and Burn treatments.

Tree growth and nutrition (Browne, Holtz, Yaghmour, Culumber, Gordon, Doll)

We monitored effects of WOR treatments on tree growth and parameters that can be associated with soil health and long and short-term orchard productivity. Tree growth in response to the preplant treatments was assessed by measuring increases in trunk cross sectional area (TCSA) at the end of the first growing season. For microbial community examinations, fine roots (< 1 mm diameter) and surrounding bulk soil were collected (sampled treatments indicated in **Table 8**) from 1 to 3 ft horizontal distance from the almond tree trunks and at a depth of 4 to 24 inches. The root and soil samples were frozen on dry ice within minutes of collection and transported within 24h to long-term storage at -80 °C until DNA extraction. After total DNA extraction from the soil and roots, diagnostic rRNA gene fragments were amplified using PCR with primers for bacteria, fungi, and oomycetes (primers amplified 16S rRNA gene regions v4 and v5-v7 of bacteria, ITS1 and ITS2 of fungi, and ITS1 and ITS2 of oomycetes). An Illumina Miseq platform was used to sequence the amplicons, and after filtering for quality the sequences were linked to their identities in sequence databases and examined as a function of the treatments that they originated from.

"First-leaf" tree growth responses varied among the orchard trials. At the Bakersfield (WO 3371) trial, there was no response to preplant soil disinfestation treatment, but potted trees on Hansen 536 rootstock generally had greater increase in TCSA than bare root trees on Nemaguard rootstock (**Table 9**, P=0.01) (**Figure 10 A**). In this trial, soil disinfestation treatment interacted significantly with WOR (40 t/a) treatment (P=0.02; **Table 9**), and WOR chips slightly inhibited tree growth in control and ASD treatments, but not in the two fumigation treatments. None of the other treatments or treatment interactions significantly affected tree growth in the.

In the Shafter trial (WO 3381), there was a highly significant effect of soil disinfestation treatment (P<0.0001; **Table 9**). Spot and strip fumigation improved the growth of trees, compared to the control, whereas ASD generally suppressed the growth of trees. WOR chips (60 t/a) inhibited tree growth, but the effect was most pronounced in the potted trees on Hansen 536 rootstock. Compared to trees without WOR chips, trees on Hansen 536 in WOR plots appeared nitrogen

deficient early in the growing season, whereas trees on Nemaguard in WOR plots did not (**Figure 10 B**); we hypothesized that the bare root trees, which had thicker trunks at planting, had greater amounts of stored nitrogen, making them less sensitive to potential nitrogen immobilization in the WOR degradation process.

In the Parlier 2017 KARE trial, preplant soil amendment treatments significantly affected tree growth (P<0.0001). The WOR treatment (WOR chips 80 t/a, applied with preplant irrigation as done for ASD, but without tarp), suppressed tree growth significantly, compared to the control (**Figure 11**). Conversely, preplant fumigation (Telone 340 lb/a + chloropicrin 200 lb/a), rice bran (9 t/a, applied as for ASD, but without tarp), and almond hull (9 t/a, applied as for ASD, but without tarp) significantly improved tree growth (**Figure 11**).

In the Manteca trial, in which all plots were fumigated, WOR chips at 64 t/a suppressed trunk circumferences, but the effect was not highly significant (P=0.10). After the first growing season, trees in WOR plots had a mean trunk TCSA of 12 cm2, whereas those in control plots had a mean TCSA of 15 cm2. At the CSUF trial, mid-August measurements of trunk circumference increases revealed significant preplant soil treatment effects (P<0.0001, **Table 9**). The addition of WOR chips to soil, whether combined with: (i) preplant irrigation as used for ASD, (ii) almond hull + shell ASD at different rates, or (ii) addition of ammonium sulfate at the time of wood chip application generally did not have large impacts on tree growth, compared to controls or similar treatments without WOR chips (**Figure 12**). Relatively large improvements in tree growth occurred following preplant fumigation (Telone 340 lb/a + chloropicrin 200 lb/a), rice bran ASD, and almond hull + shell ASD (**Figure 12**).

Research Effort Recent Publications:

Holtz, B.A., Doll, D.A, and Browne, G. 2016. Whole orchard recycling and the effect on second generation tree growth, soil carbon, and fertility. Acta Horticulturae 1112: 315-319.

Holtz, B.A. 2016. Almond orchard recycling. West Coast Nut, November, pages 8-13, www.wcngg.com.

Yaghmour, M., Holtz, B., and Browne, G., 2017. Considering new orchard replacement options—Whole orchard recycling and anerobic soil disinfestation. West Coast Nut, March, pages 14-20, www.wcngg.com.

Holtz, B. 2017. Almond Orchard Recycling: The Slasher could improve soil fertility. Resource magazine, May/June, pages 8-11, American Society of Agricultural and Biological Engineers.

Hearden, T. Orchard Recycling: newly planted almond trees benefitting from incorporated biomass. Western Farm Press, March 15, 2017.
https://www.westernfarmpress.com/tree-nuts/orchard-recycling-newly-planted-almond-trees-benefitting-incorporated-biomass

Hearden, T. 2018. Whole almond orchard recycling has promise for new almond plantings. Western Farm Press, March 14, 2018.
https://www.westernfarmpress.com/tree-nuts/whole-orchard-recycling-has-promise-new-almond-plantings

Table 2. Results of analyses of variance, increase in trunk cross section area, first growing season, by trial[a]

Experiment	Year planted	Treatment factor	F value	P value
Shafter 1	2017	Soil disinfestation method (Control, Spot fum Strip Fum, or Almond hull+shell ASD)	1.77	0.20
		Rootstock (NG or H536)	6.85	*0.01*
		WOR chips (WOR Chips 40 t/a or no chips)	0.25	0.63
		Soil disinfestation method x rootstock	0.84	0.48
		Soil disinfestation method x WOR	3.54	*0.02*
		Rootstock x WOR	0.7	0.4
		Soil disinfestation method x rootstock x WOR	0.01	0.99
Shafter 2	2017	Soil disinfestation method (Control, Spot fum Strip Fum, or Almond hull+shell ASD)	29.96	*<0.0001*
		Rootstock (NG or H536)	0.03	0.86
		WOR chips (WOR Chips 60 t/a or no chips)	14.51	*0.003*
		Soil disinfestation method x rootstock	2.01	0.12
		Soil disinfestation method x WOR	0.99	0.40
		Rootstock x WOR	6.13	*0.02*
		Soil disinfestation method x rootstock x WOR	0.68	0.57
Parlier (KARE)	2017	Preplant soil treatment (disinfestation or WOR 85 t/a)	40.33	*<0.0001*
Manteca	2017	WOR (WOR Chips 64 t/a or no chips)	8.69	0.10
Fresno (CSU)	2018	Preplant soil treatment (combinations of disinfestation treatments, WOR 60 t/a, and ammonium sulfate)	16.1	*<0.0001*

References Cited:

1. Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13:581.
2. Tkacz, A., Hortala, M., and Poole, P. S. 2018. Absolute quantitation of microbiota abundance in environmental samples. Microbiome 6 (1):110.

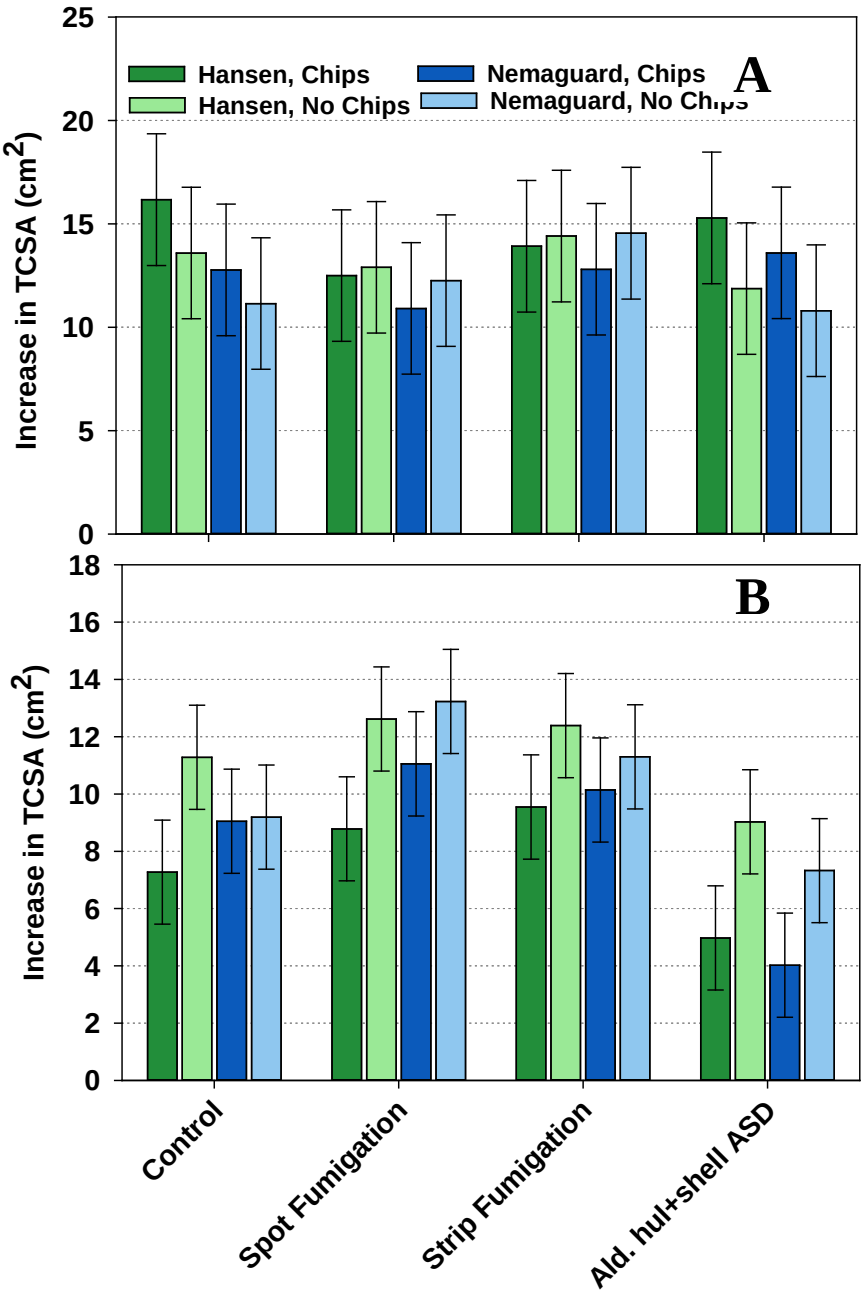


Figure 10. A, first-year growth of trees in replanted almond orchards A, in Shafter trial 1 (WO3371), and B, in Shafter trial 2 (WO3381), as a function of preplant soil disinfestation treatment, rootstock, and whole orchard recycling treatment (chips vs. no chips). The treatments were applied in fall 2016, trees were replanted in winter 2017, and trunk cross sectional area (TCSA) increase was measured between planting and mid-November 2018. Error bars are 95% confidence intervals.

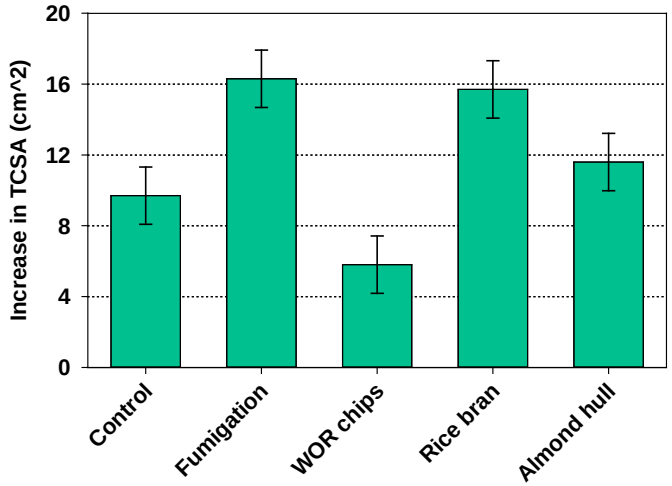


Figure 11. First-year growth of trees in replanted almond orchard of trial Parlier (KARE) 2016, as a function of preplant soil treatment. WOR, rice bran, and almond hull incorporations (at 80, 9, and 9 t/a, respectively) into soil were followed immediately by full wetting of soil profile and maintaining soil moisture at or above field capacity for 6 weeks. No tarp was used. The treatments were applied in fall 2016, trees were replanted in winter 2017, and trunk cross sectional area (TCSA) increase was measured between planting and mid-November 2018. Error bars are 95% confidence intervals.

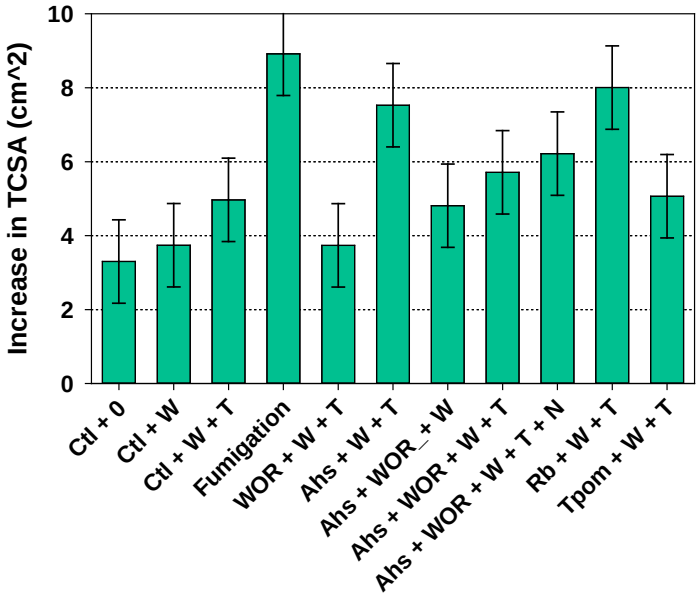


Figure 12. First-year growth of trees in replanted almond orchard of trial CSUF 2017, as a function of preplant soil treatment. The treatments were applied in fall 2017, trees were replanted in winter 2018, and trunk cross sectional area (TCSA) increase was measured partway through the first growing season, August 14, 2018. Error bars are 95% confidence intervals. “Ctl” = control; WOR = whole orchard recycling chips added at 60 t/a, “Ahs”= ground almond hull and shell mixture added at 9 t/a, “Rb” = ground rice bran added at 9 t/a, “Tpom” = tomato pomace added at 9 t/a, “0”= no additional treatment component, W=irrigation as for ASD, including complete water saturation of soil profile, followed by daily to every-other-day irrigations to maintain soil moisture at field capacity.