Project Name: **Wood chipping almond brush to reduce air pollution and to study the effect of wood chips on harvest, soil nutrients, soil aggregation, and the microbial community**

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Abstract
The wood chipping of almond prunings could provide an alternative to burning that would not contribute to air pollution and add valuable organic matter to soils. The success of wood chipping depends on whether the wood chips interfere with harvest or delete the soil of critical nutrients necessary for tree growth. An average of 502 pounds per acre of wet weight prunings were pruned in our orchard trial in the fall of 2004. Harvest wind-rows in the wood chipped treatments had significantly more (0.136 kg wood/22 ft row) wood debris than the wind-rows of nuts in treatments without wood chips (0.073 kg of wood/22 ft row). Ten-pound bulk inshell almond samples taken from harvest carts harvested from both the wood chipped and non-wood chipped treatments were analyzed by the USDA with respect to foreign material consisting of wood debris. The wood chipped treatments averaged 1.49 % wood debris and were significantly greater than non-wood chipped treatments that averaged only 0.79 % wood debris. Leaf analysis showed no effect of the wood chips on tree nutrient status in the orchard trial. There were more free-living bacterial (bacterivorous) and fungal feeding (fungivorous) nematodes in the wood chipped soils when compared to non-chipped soils. Rhizosphere inhabiting bacteria from wood chipped soils were found to be distinctive from bacteria from non wood chipped soils based on analysis of 95 tests for their utilization of carbohydrate, polymers, organic acids, and other compounds. Significantly more soil aggregating basidiomycetes fungi in water stable aggregates were found in wood chipped soils when compared to non-wood chipped soils.

INTRODUCTION
In the San Joaquin Valley of California almond trees are pruned every year after harvest in the late fall or early winter. Prunings are typically removed from orchards with a "buck-rake" mounted on a tractor. Prunings are usually placed in burn piles and burned green, generating smoke. In 2003, the San Joaquin Valley had 550,000 bearing acres (214,574 hectares) of almonds. Preliminary studies have shown that approximately 1997 lbs/ acre (2,240 kg/h) of prunings are removed annually. This would result in the burning of approximately 1 billion pounds of green almond prunings per year. The San Joaquin Valley Unified Air Pollution Control District restricts the burning of agricultural wastes and further restrictions have recently been approved (Senate Bill 700) due to worsening air pollution. Since the passing of The Federal Clean Air Act Amendments of 1990 the San Joaquin Valley of California has not met national ambient air quality standards for particulate matter 10 microns (PM-10) or less.

The wood chipping or shredding of almond prunings could provide an alternative to burning that could add valuable organic matter to San Joaquin Valley soils typically low in organic matter. A small percentage of almond growers have been chipping or shredding their prunings, some for over 15 years, because they are farming on the agricultural-urban interface where brush burning is prohibited because of its close proximity to urban housing. Other growers have chipped or shredded their prunings solely to add organic matter to their soils. But many growers fear that wood chips or shreddings will take valuable nutrients away from the trees because of their high carbon to nitrogen ratio. If wood chips can be shown not to interfere with harvest or take valuable nutrients from trees, then growers would be more likely to adopt chipping or shredding as an alternative to burning, especially if advantages to soil health and nutrition could also be demonstrated.

MATERIALS AND METHODS

Wood chipping orchard trial

Almond pruning was performed in October 2003 and 2004 in an almond orchard in Madera, County. The pruning wet and dry weights were determined. The shredding trial was divided into two treatments, trees that received prunings and trees that did not receive prunings. Four quarter-mile Nonpareil rows received prunings while another four Nonpareil rows did not. The prunings from the rows that did not receive prunings were added to the rows that did receive prunings. After the prunings were placed on the orchard floor they were shredded with a Diana Shredder in 2003 and a Rears Pull Behind Shredder in 2004.

Wood debris in the orchard at harvest

After the nuts had been shaken to the ground, dried, and wind rowed, the amount of wood debris was determined per 22 feet of wind-row sorting and weighing of the debris by hand. At the time the nuts and debris were picked up from the orchard floor during harvest, five 10 pound sub samples were removed from harvest carts of both wood chipped and non wood chipped treatments and sent to the USDA in Kerman, CA for a Federal State Inspection Certificate.

Isolation of bacteria

Samples of soil were taken at two intervals of depth, ca 1-75 cm and ca 76-150 cm using a coring tool. Each of three samples each taken from chipped or nonchipped soils consisted of pooling three subsamples. Samples processed to determine the composition of the bacterial community consisted of 1g of soil. Soil aliquots were agitated in MgSO4 buffer (0.1 M, pH 7.3) in the presence of glass beads (0.5 mm diameter) for 16 h at 4º C on a rotary shaker set at 200 rpm to disrupt all the microstructures (modified Ranjard and Richaume, 2001). Soil suspensions were allowed to settle for 2 min then diluted 10 times with buffer before transferring to plastic bags consisting of 2 compartments separated by a filter membrane (280 µm pore size) (Intersciences Laboratories, Inc. Weymouth, MA, USA). Bags were agitated vigorously for 1 min using a blender (Stomacher 80, Seward Medical, London, UK). This allows filtering of the bacteria through the membrane and separates them from large soil particles. Bacterial suspensions were further diluted 100 times with buffer then plated on 0.3% tryptic soy bean agar (TSBA) medium in triplicate using a spiral platter (Don Whitley Scientific Limited, West Yorkshire, UK). Plates were incubated for 24 to 48 h at 28^{o}C. Five bacterial colonies delivered at the end of the spiral

(the most dilute portion of the spiral) were collected from each spiral plate. Such colonies were deemed to be predominant among bacteria isolated this way. Fifteen isolates representing the predominant bacteria were collected for each of the three replicate samples from each site and a total of 45 colonies represent the predominant bacteria for each site. The colonies were further purified onto 3% TSBA. Gram test, test for catalase and oxidase and morphology test were performed for each bacterial isolate. Isolates were stored long term at -80^{o}C in Luria-Bertani medium amended with 15% glycerol or for the short term in saline phosphate buffer (NaCl, 1.55 mM; K_2HPO_4, 15.40 mM; Na_2HPO_4, 27.00 mM; pH 7.2) at 4^{o}C.

Identification by BIOLOG^{TM} GN and GP microplate system.
Biolog^{TM} GN and GP microplates (Biolog, Inc., Hayward, CA, USA), each containing 95 individual carbon substrates (polymers, carbohydrates, carboxylic acids, amino acids, amines/amides, and miscellaneous compounds), plus a negative control, were designed to determine the nutritional versatility of a broad range of GN and GP based on biochemical tests that allow the determination of their taxonomic identity. Two days before the inoculation of Biolog^{TM} plates), the isolates were streaked on 3% TSBA from frozen cultures. Then colonies were transferred to Biolog^{TM} universal growth medium and incubated for 24 h at 28ºC. On the day of inoculation, a cotton swab was used to transfer bacteria to glass tubes (20 x 150 mm) with 19 ml of GN-GP inoculating fluid (Biolog, Hayward, CA, USA) and adjusted to a bacterial concentration of 52% turbidity for the GN and 20% turbidity for the GP using a turbidomiter (Biolog, Hayward, CA, USA). Thioglycolate solution was added to GP suspension to a final concentration of 6% solution. Within 10 min, the Biolog GN and GP were inoculated with 150 µl of the adjusted bacteria suspension in each well. The GN isolates were incubated at 28^{o}C and the GP at 35^{o}C. Color development was read once after 20 to 24 h of incubation for the GN and read twice for the GP, after 4 to 6 h and after 20 to 24 h of incubation. The development of color was read using a 590-nm wavelength filter in a microplate reader (EMax Precision Microplate reader, Molecular Devices Hayward, CA, USA). Identification was considered conclusive when the similarity index (SIM) was $\geq$ 0.500 (Biolog^{TM} GN and GP Aerobic Bacteria Databank, Release 6.01).

Nematode and basidiomycete sampling - Ring nematode was assayed with the sugar centrifugation method where 1-2 kg of soil is placed into a pan with water and mixed. Nematodes were suspended in water and decanted. A 1-molar solution of sugar plus separan was added to a cylinder and stirred. After 1 minute the nematode-soil separation was passed through a 400-mesh screen. With a small quantity of water, the nematodes were washed from the screen into a counting dish. Nematodes per 1 kg of soil (250 cc) were reported. Root lesion and free-living nematodes were extracted by a combined sieve-mist extraction method where the final screenings from a 500-mesh sieve containing 20 grams of root plant tissues were placed into a funnel and then into a mist chamber. After 3-5 days the nematodes were removed and counted.

Statistical analysis
Principal components analysis (PCA) was performed using the covariance matrix on Biolog^{TM} data to characterize bacterial communities from the two different treatments. The JMP statistical software package (version 5.1.2, 2004 SAS Institute Inc. Cary, NC) was used for the analysis.

RESULTS

Wood chipping orchard trial

Pruning wet and dry weights were determined in 2003 and 2004. The pruning wet weights for 12 randomly selected almond trees were determined. An average of 1,247 and 502 pounds per acre of wet weight prunings were pruned in the orchard trial in 2003 and 2004, respectively. After sub samples of prunings were dried down, pruning weights averaged 791 and 316 pounds of dried prunings per acre for 2003 and 2004, respectively. The grower considered the pruning performed in 2003 and 2004 to be "light" and speculated that on a "heavy" pruning year 3-5 times as much prunings could be removed.

Wood debris in the orchard at harvest

After the nuts had been shaken to the ground at harvest, dried, and wind rowed, the amount of wood debris were determined per 22 feet of wind-row by hand sorting and weighing. The wood chipped or shredded treatments averaged 0.136 kg of wood debris in 22 feet of wind-row and were significantly greater than the non wood chipped treatments that averaged only 0.073 kg of wood debris (figure 1).

Sub samples from the harvest carts after pick up were removed from both the wood chipped and non wood chipped treatments and sent to the USDA for a Federal State Inspection Certificate. Ten-pound bulk inshell almond samples harvested from both the wood chipped and non-wood chipped treatments were analyzed with respect to foreign material consisting of wood debris. Ten pound bulk inshell almond samples from the wood chipped treatments averaged 1.499 % wood debris and were significantly greater than non wood chipped treatments that averaged only 0.799 % wood debris (figure 2).

Isolation of bacteria

Oakdale

Layer 1-The effect of wood chipping on soil ecology is seen when considering some patterns in microbial community structure. The effects of chipping reached to the subspecies level of some bacteria. For example, *Pseudomonas fluorescens* biotype F and G strains from the rhizospheres of almonds growing in chipped soils were distinctive based on analysis of 95 tests for their utilization of carbohydrate, polymers, organic acids and other compounds. At the Oakdale site, an entire group of enteric bacteria that included *Raoultella* spp. (a high degrader of phytase; the almond fruit has a fairly high level of P among fresh foods) were present in the rhizospheres of almonds in chipped soils and absent in almond rhizospheres from nonchipped soil (figure 3). Other species, such as *Flavobacerium* spp. were only found in nonchipped soils. Some species such as *Stenotrophomonas* were found in both chipped and nonchipped situations. Finally, the range of phenotypes of various *Pseudomonas* spp. was wider in the almond rhizosphere of nonchipped versus chipped soils. Thus, the effects of chipping, even within the same orchard were a mix of both subtle and pronounced effects on the associated almond rhizosphere bacterial communities. Studies have begun in our lab on the possible functions of several of the predominant species.

Layer 2-Nearly all Gram-negative species found in layer 2 at the Oakdale site, chipped or nonchipped were *Pseudomonas* spp., while Gram-positive species found in layer two whether chipped or nonchipped, were *Arthrobacter* spp., *Microbacterium* spp. or *Tsukamurella inchonensis*.

Communities at Sites DH nonchipped and SI, chipped

Layer 1-At a pair sites, one chipped, the other unchipped, among Gram-positive species there was a total dominance of Gram-positive coryneform bacteria, including *Arthrobacter ilicis*: a pathogen of American holly, *Ilex opaca*, USA and *Curtobacterium pusillum*, associated with grasses. Many of the other Gram-positive bacteria at theses sites are animal- associated. At the DH nonchipped site, there were only two Gram-negative species found in rhizospheres, whereas at the companion chipped SI site, *Pantoea* spp. and *Pseudomonas fluorescens* and *putida* were present in the greatest frequency. Two predominant isolates from almond rhizospheres of chipped soils were *Pseudomonas syringae* pv *cunnighamiae* and *Pseudomonas syringae* pv *papulans*, both, if the identification is confirmed, would be plant pathogens. Two other species of note predominating from almond rhizospheres in the chipped site were *Pseudomonas marginalis*, typically a pectin degrading species and a pathogen of lettuce and related species and *Burkholderia caryophylli*, a pathogen of carnation and closely related species.
Layer 2-Similar to the Oakdale site, nearly all the species identified as predominant from layer 2 were *Arthrobacter* and *Microbacterium* species. Both of these species are considered highly durable and persistent in soil.

FS and HH

Layer 1-There were striking differences in community composition between the chipped FS site and the companion nonchipped HH site. The species exclusive to the nonchipped site were *Pantoea agglomerans* and *Pseudomonas citronellolis* (figure 4), the latter known to be active in degrading certain recalcitrant hydrocarbons. In addition, in nonchipped almond rhizosphere soil, the low-level pathogen causing corky root, *Pseudomonas corrugata* was pervasive, whereas in the chipped soil, only one isolate of *P. corrugata* was identified. Conversely, both *Pseudomonas putida* and *P. fluorescens* were highly frequent as predominant bacterial species in chipped soils and comparatively infrequent among predominant species in the nonchipped almond rhizosphere soil. In chipped soils at the FS site as with the SI chipped soils, in addition to *P. corrugata* mentioned above, plant pathogens were found among these predominant isolates (each however relatively low level pathogens): *Acidovorax avenae ss avenae* and *P. marginalis*. In addition in almond rhizosphere soil of the nonchipped HH site, *Burkholderia glumae*, a pathogen was identified.

Layer 2-Interestingly, the community of Gram-positive species in layer two (chipped or nonchipped) closely resembled those found at the other two sites, being evenly divided among *Arthrobacter* spp., *Microbacterium* spp. and *Tsukamurella inchonensis*, while the Gram-negative communities resembled those of the Oakdale site but with a higher frequency of *Pseudomonas citronellolis*.

Nematode

The effect of wood chips in soil on plant parasitic and free-living nematode populations were examined. In 2000, shortly after the addition of wood chips to soil, *Macroposthonia* (ring nematode) populations were significantly reduced while *Bunonema* and

Dorylaimida populations were significantly increased in wood chip amended soils (Table 3). But from 2001-2004 *Macroposthonia*, *Bunonema* and *Dorylaimida* populations appeared unaffected by the wood chips (Tables 1 & 2). From 2000-2004 *Paratylenchus* (root lesion) species were significantly reduced in wood chip amended soils. From 2000-2003 free-living nematode populations (Bacterial and fungal feeding nematodes) were significantly increased in wood chip amended soils. Throughout the study there were more fungal feeding nematodes in the wood chipped soils when compared to the non wood chipped soils. *Trichodorus* and *Monochida* species appeared unaffected by the addition of wood chips to soil.

CONCLUSIONS

Wood chipping or shredding of almond prunings and leaving them on orchard soils resulted in significantly more wood debris in the wind rows at harvest and in harvest carts with nuts after pick up. Leaf analysis showed no effect of the wood chips on tree nutrient status in the orchard trial. There were more free-living bacterial and fungal feeding nematodes in the wood chipped soils when compared to non-chipped soils. Rhizosphere inhabiting bacteria from wood chipped soils were found to be distinctive from bacteria from non wood chipped soils based on analysis of 95 tests for their utilization of carbohydrate, polymers, organic acids, and other compounds. The addition of wood chips to almond orchard soils enhanced the soils water infiltration rate when compared to soils that were not amended with wood chips. Significantly more soil aggregating basidiomycete fungi were counted in soils amended with wood chips when compared to soils that were not amended with wood chips.

ACKNOWLEDGEMENTS

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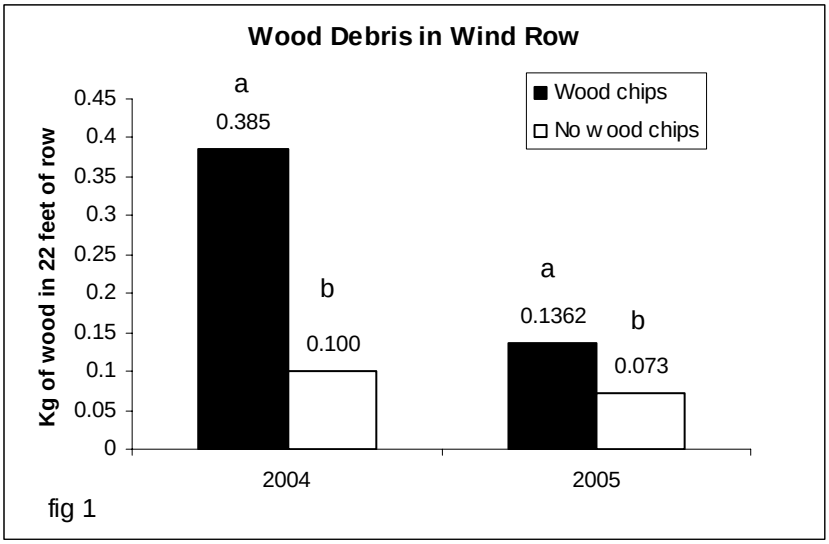

figure 1. The amount of wood debris per 22 feet of wind-row at harvest. Paired columns with different letters were statistically different when compared in a Student's T-test (P # 0.05).

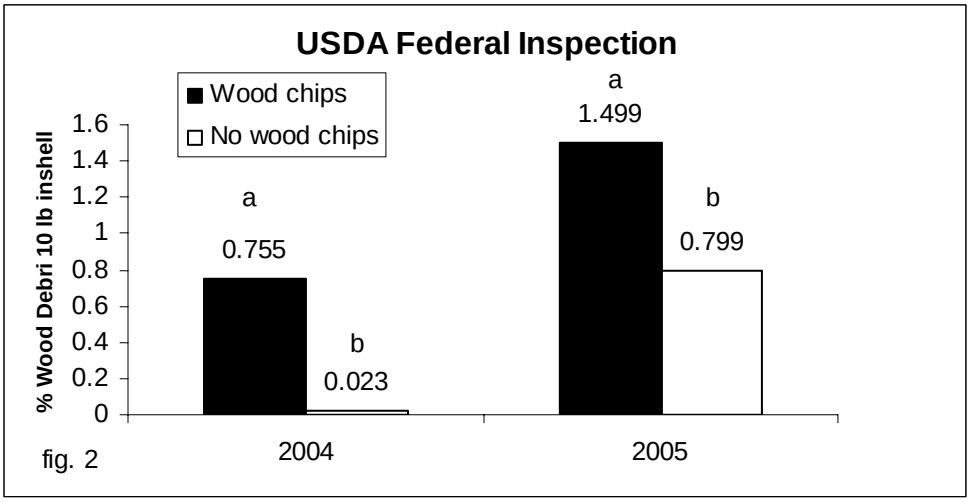

figure 2. Ten-pound bulk inshell almond samples from wood chipped and non-wood chipped treatments were analyzed by the USDA and given Federal State Inspection Certificate with respect to foreign material consisting of wood debris. Paired columns

with different letters were statistically different when compared in a Student's T-test (P # 0.05).

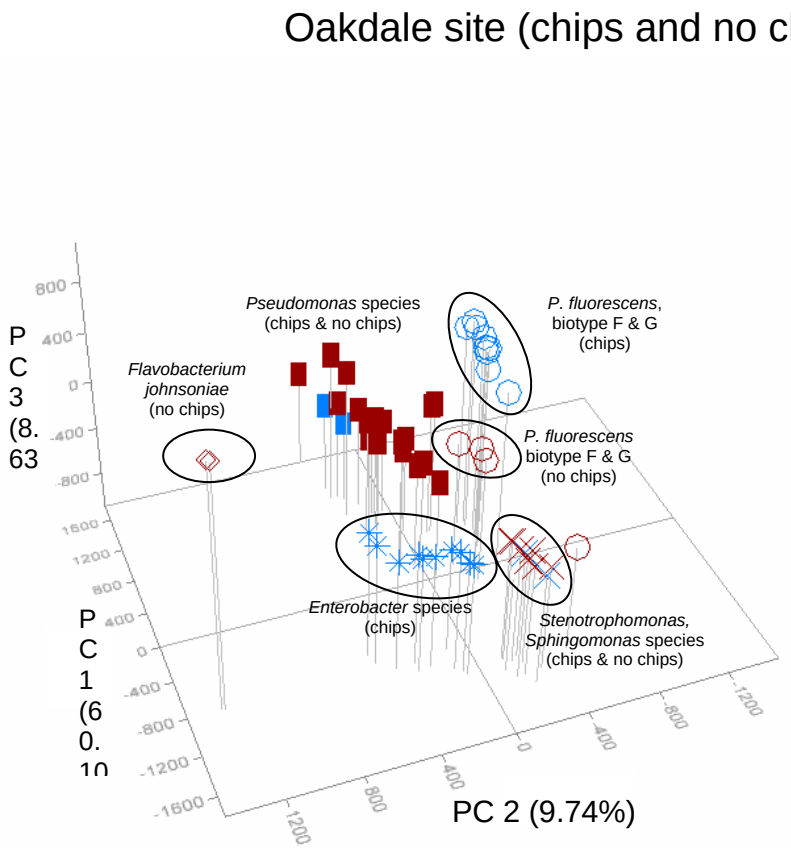

figure 3. Plot of principle components of community of predominant bacteria from chipped and unchipped portions of the Oakdale almond orchard site. Some species were predominant from chipped soil and not from unchipped soil (*Flavobacterium johnsoniae*) and vice versa (Enteric species). *Pseudomonas* species displayed a greater degree of diversity in nonchipped soil than chipped soil while other groups such as *Stenotrophomonas* and *Sphingomonas* species were found among predominant species in both chipped and nonchipped almond rhizosphere soils. Chipping enriched for a community of *Pseudomonas fluorescens* biotype F and G distinct from the community of the same subspecies from chipped soil.

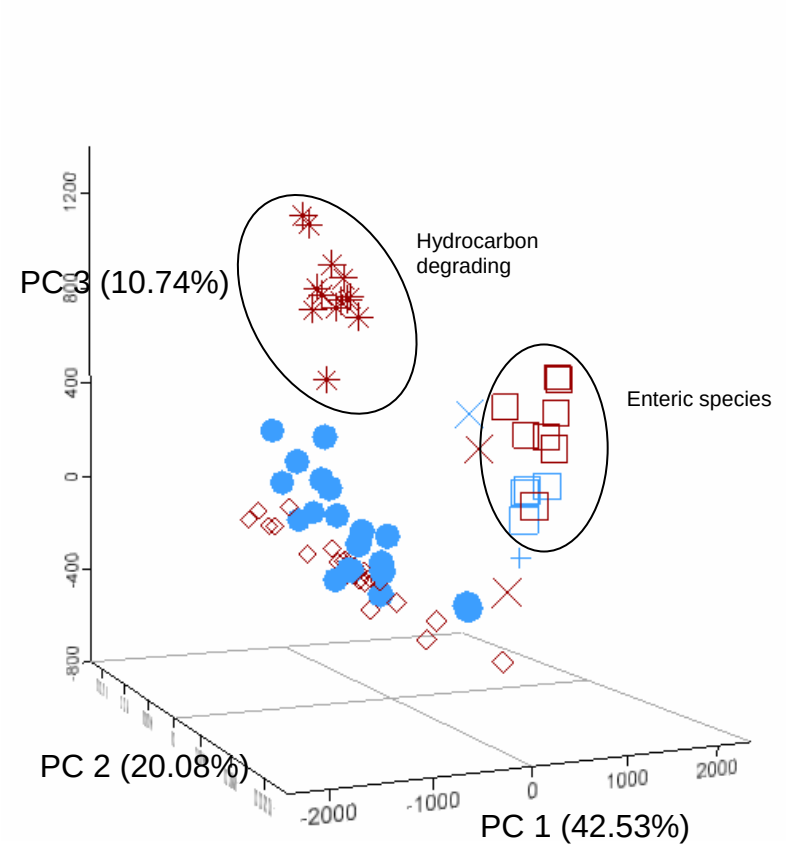

figure 4. A group of *Pseudomonas citronellolis* predominated among Gram-negative species in nonchipped soils at the HH site. This species contains isolates that are capable of biodegradation of recalcitrant hydrocarbons. The respective communities of enteric bacteria were only slightly different between chipped and nonchippped almond rhizosphere soils, whereas the respective communities of nonenteric species from chipped (blue circles) and nonchipped soils (diamonds) were generally more distinct.

Table 1. One kg soil samples were taken in 2000, 2001, and 2002 from soil amended with and without wood chips and assayed for the following nematodes

	2000		2001		2002	
	Wood chips	No chips	Wood chips	No chips	Wood chips	No chips
Macroposthonia spp.	15.4 a*	53.0 b	298.0 a	392.0 a	545.0 a	399.6 a
Bunonema spp.	40.8 a	0.0 b	0.0 a	0.0 a	0.0 a	0.0 a
Trichodorus spp.	0.0 a	6.6 a	0.0 a	0.0 a	0.0 a	0.0 a
Dorylaimida spp.	159.4 a	19.6 b	2.2 a	36.2 a	47.4 a	38.6 a
Monochida spp.	0.0 a	0.4 a	0.0 a	0.0 a	55.0 a	59.4 a
Paratylenchus spp.	0.0 a	1.8 a	0.0 a	138.8 b	24.4 a	255 b
Free-living spp.	1307.2 a	690.4 b	1703.4 a	246.0 b	1006.4 a	437.4 b
Bacterial feeding spp.	987.2 a	612.0 b	1371.3 a	223.3 b	872.128 a	394.1 b
Fungal feeding spp.	320 a	78.4 b	332.1 a	22.7 b	134.272 a	43.4 b

*Paired columns within the same year with different letters were statistically different when compared in a Student's T-test (P # 0.05)

Table 2. One kg soil samples were taken in 2003 and 2004 from soil amended with and without wood chips and assayed for the following nematodes

	2003		2004	
	Wood chips	No chips	Wood chips	No chips
Macroposthonia spp.	298.0 a	392.0 a	545.0 a	399.6 a
Bunonema spp.	0.0 a	0.0 a	0.0 a	0.0 a
Trichodorus spp.	0.0 a	0.0 a	0.0 a	0.0 a
Dorylaimida spp.	2.2 a	36.2 a	47.4 a	38.6 a
Monochida spp.	0.0 a	0.0 a	55.0 a	59.4 a
Paratylenchus spp.	0.0 a	138.8 b	24.4 a	255 b
Free-living spp.	1703.4 a	246.0 b	1006.4 a	437.4 b
Bacterial feeding spp.	1371.3 a	223.3 b	872.128 a	394.1 b
Fungal feeding spp.	332.1 a	22.7 b	134.272 a	43.4 b

*Paired columns within the same year with different letters were statistically different when compared in a Student's T-test (P # 0.05)