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SUMMARY – The wood chipping of almond prunings in California, instead of burning, can reduce air pollution and return organic matter to soils. The success of wood chipping depends on whether the chips do not deplete critical nutrients necessary for tree growth. An experiment was established where soil was mixed with or without wood chips and placed in containers, each with an almond tree. There were more free-living nematodes in the chipped soils when compared to non-chipped soils. More basidiomycetes were counted in wood chipped soils and detected at higher levels with ELISA. Larger soil aggregates were found in wood chipped soils. Undisturbed wood chipped soils had more soil aggregates than disturbed soils. After the first year trees growing with wood chips had less shoot growth, but by the second year trees with wood chips had more shoot growth. Soil analysis after two years showed higher levels of calcium, magnesium, sodium, boron, zinc, copper, carbon, phosphorus, potassium, ammonium, and % organic matter in wood chipped soils. There was less manganese, iron, and nitrate in the wood chipped soils and the pH was reduced. Tissue analysis was performed on leaf petioles. After the first year trees growing with wood chips had less nitrogen, zinc, and manganese, while phosphorus was increased. After the second season trees with wood chips no longer had less nutrient levels while phosphorus was still increased. Water infiltration was significantly greater in wood chipped soils all three years.

Key words: *Prunus dulcis*, free-living nematodes, soil aggregating basidiomycetes, petiole and soil nutrients.

RESUME *– "Les copeaux de bois provenant de la taille de l'amandier et leurs effets sur les nutriments du sol et du pétiole, sur les agrégats du sol, l'infiltration de l'eau et les populations de nématodes et de basidiomycètes". Les copeaux de bois de la taille de l'amandier en Californie, au lieu d'être brûlés, peuvent réduire la pollution atmosphérique et retourner la matière organique aux sols. Le succès de la fragmentation du bois en copeaux dépend si les morceaux n'épuisent pas les nutriments critiques nécessaires pour la croissance de l'arbre. Une expérience a été menée où le sol a été mélangé ou non à des copeaux de bois et placé dans des récipients, chacun avec un amandier. Il y avait plus de nématodes libres dans les sols avec copeaux comparés aux sols sans copeaux. Un nombre supérieur de basidiomycètes ont été comptés dans les sols avec copeaux et des niveaux plus élevés ont été détectés avec ELISA. De plus grands agrégats de sol ont été trouvés dans les sols avec copeaux. Les sols avec copeaux de bois non remués ont eu plus d'agrégats de sol que les sols remués. Après la première année les arbres grandissant avec les copeaux de bois ont eu moins de croissance des pousses, mais lors de la deuxième année les arbres avec les copeaux de bois ont eu plus de croissance des pousses. L'analyse du sol après deux ans a montré des niveaux plus élevés de calcium, de magnésium, de sodium, de bore, de zinc, de cuivre, de carbone, de phosphore, de potassium, d'ammonium, et de % de matière organique dans les sols avec copeaux de bois. Il y avait moins de manganèse, fer, et nitrate dans les sols avec copeaux de bois, et le pH a été réduit. L'analyse du tissu a été exécutée sur les pétioles des feuilles. Après la première année les arbres croissant avec les copeaux de bois ont eu moins d'azote, de zinc, et de manganèse, alors que le phosphore avait augmenté. Après la deuxième saison les arbres avec les copeaux de bois n'avaient plus de niveaux inférieurs en éléments nutritifs tandis que le phosphore était encore augmenté. La pénétration de l'eau était sensiblement plus grande dans les sols avec copeaux de bois pendant chacune des trois années.*

Mots-clés : Prunus dulcis*, nématodes libres, agrégation du sol, basidiomycètes, pétiole, nutriments du sol.*

Introduction

The San Joaquin Valley (SJV) Unified Air Pollution Control District restricts the burning of agricultural wastes and further restrictions are likely due to worsening air pollution. Since the passing of The Federal Clean Air Act Amendments of 1990 the SJV 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 can add valuable organic matter to SJV soils typically low in organic matter. A small percentage of almond growers have been chipping or shredding their prunings for over 14 years; some because they are farming on the agricultural-urban interface where brush burning is prohibited because of its close proximity to housing. Other almond 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 their trees (Holtz, 1999). 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.

Research has shown that organic material can increase the humic content of soil (Sikora and Stott, 1996), the nutrient holding capacity of soils (Gaskell *et al*., 2000, Hartz *et al*., 2000), and the cation-exchange-capacity (Fox *et al*., 1990), which is a measure of the ability of soil to hold nutrients. Soil organic matter has also been shown to increase the water holding capacity of soil, the pH buffering capacity, the microbial diversity of soils (Scow *et al*., 1994), and to even reduce plant parasitic nematode populations (Leary and DeFrank, 2000). Saprophytic lignin-decomposing basidiomycetes have been shown to produce large quantities of extracellular materials that bind soil particles into aggregates (Caesar-TonThat and Cochran, 2000). The effect of wood chips on soil and petiole nutrients, soil aggregation, water infiltration, and nematode and basidiomycete populations was initiated in a replicated experiment where soil was amended with and without wood chips (Holtz and McKenry, 2001). If results were available to growers that show enhanced nutrient value due to wood chipping, it would speed adoption of the practice and help reduce air pollution in the SJV. There are over 250,000 hectares of almonds in California, and burning is still the primary method of brush disposal.

Materials and methods

Wood chipping, tree placement, and water infiltration

Almond prunings were chipped with a brush bandit wood chipper (Bandit Industries, Remus, MI). The wood chips were mixed with Tujunga loamy sand high in ring (*Macroposthonia* spp.) and root lesion (*Paratylenchus* spp.) plant pathogenic nematode populations taken from almond orchard soil (L.D. James Ranch, Modesto, CA). The wood chips were mixed with soil at approximately 1/3 part wood chips to 2/3 parts soil, and placed in 133 liter barrels (Monsanto, St. Louis, MO), with a single 1-year old bare root 'Nonpareil' almond tree per barrel. Five trees each were planted in barrels with and without wood chips. The barrels were placed in an almond orchard in a replicated manner, consisting of five single-tree replicates per treatment. The barrels prevented the mixing of roots, wood chips, and microbial communities and allowed placement of a replicated trial in a small area. Trees were not fertilized but were irrigated twice weekly with a drip irrigation system from ground water. The time in seconds for 18.9 liters (5 gallons) of water to infiltrate soil amended with and without wood chips was measured on four occasions.

Leaf, shoot growth, and soil sampling

Fifty - 75 leaves were collected randomly from non-fruiting spurs from each tree in July of 2000, 2001, and 2002. One kg of soil was removed from just under the surface of each barrel in October of 2000, 2001, and 2002. Half of each sample was assayed for nematodes while the other half was analyzed for nutrients. Leaf and soil samples were analyzed for mineral content by the University of California's Division of Agriculture and Natural Resources (DANR) Laboratory (Davis, CA). Current season shoot growth was measured (cm) in May of 2000, 2001 and 2002. The five longest shoots per tree were selected and measured.

Nematode and basidiomycete sampling

Ring nematode was assayed with the sugar centrifugation method (McKenry and Roberts, 1985) where 1-2 kg of soil are 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. Basidiomycetes were counted in plots (mushrooms/barrel) when they appeared, usually after January and February winter rainfall.

Orchard soil sampling and separation of soil aggregates

In January 2001, Tujunga loamy sand soils were sampled from a 30 year-old almond orchard where prunings were chipped and left on the orchard floor annually for 14 years. Soil samples were collected from 2 treatment sites: 1) where the orchard floor soil had been left undisturbed, and 2) where the orchard floor soil was disturbed prior to harvest (August 2000) with a rotary-tiller (Maschio, Padova, Italy) to a depth of 12-15 cm. Soil samples were collected from three areas in each site. At each site three soil cores were taken to a depth of 18 cm using a step-down soil probe and divided into increments of 0-to-3, 3-to-8, and 8-to-13 cm and 13-to-18 cm. The three samples at each depth were mixed to form a composite sample. Samples were collected using a stratified sampling scheme so that within-row and between-row areas of the plots comprised the proper proportion of the composite sample (Caesar-TonThat *et al.*, 2000). Soils were dried in a forced-air oven at 50°C and then passed through a series of sieves (>2 mm, 0.84 mm, 0.42 mm, and 0.25 mm-mesh).

Enzyme Linked Immunosorbent Assay (ELISA)

Presence of soil aggregating basidiomycetes was determined for each soil aggregate size fraction using Enzyme Linked Immunosorbent Assay (ELISA) (Caesar-TonThat *et al.*, 2001). Dry soil samples (500 mg/ml) were prepared by homogenization of samples in a mortar and pestle in carbonate buffer (20 mM $NaHCO_3$, 28 mM Na_2CO_3, pH 9.6), and a dilution series (1.17 to 75 mg/ml) was prepared in this buffer. Homogenates were centrifuged for 10 min (14,000 *g*) after which 100 µl of the supernatant was loaded in flat bottom microtiter plate wells (Immulon 4HBX, Dynex Technologies Inc., Chantilly, VA) followed by incubation overnight at 55°C. After three washings with 0.01M phosphate buffer saline-Tween 20, 0.138 M NaCl, 2.7 mM KCl, pH 7.4 (PBST, Sigma, St Louis, MO), 100 µl of a 1/10,000 dilution of the third boost rabbit serum was added to each well. Microtiter plates were incubated for 90 min at 22°C on an orbital shaker, washed 3 times with PBST, and incubated for 60 min at 22°C with a 1/13,000 dilution of horseradish peroxidase-conjugated goat anti-rabbit polyspecific immunoglobulins (Sigma, St Louis, MO) added to each well. After three further PBST washings, the substrate, consisting of a solution of 3,3', 5,5' tetramethylbenzidine (0.4 g/l) (Pierce, Rockford, IL) and 0.02% hydrogen peroxide, was added. The reaction was stopped after 30 min with 2.5 M sulfuric acid. Absorbance was read at dual wavelength of 450 nm/655 nm using a BioRad 550 microplate reader, controlled by a computer using the Plate Reader Manage program (BioRad, Hercules, CA). All incubation steps were performed at room temperature. All samples were processed in triplicate.

Results and discussion

Leaf petiole analysis and shoot growth

Leaf petiole analysis showed that trees growing in soil amended with wood chips had significantly less nitrogen (N) in 2000, reduced levels in 2001, and higher levels in 2002 (Table 1). Phosphorus (P) increased significantly in trees growing in soil amended with wood chips all three years. Potassium (K) was significantly increased in trees grown with wood chips in both 2001 and 2002. Calcium (Ca) and boron (B) levels increased significantly in trees grown with wood chips in 2002. Zinc (Zn) and manganese (Mn) levels decreased significantly in trees grown in soil amended with wood chips. Sodium (Na) and magnesium (Mg) levels were unaffected by the addition of wood chips. Trees growing in soil amended with wood chips had significantly less current season shoot growth in 2000 and 2001 when compared to trees growing in soil without wood chips, but by 2002 and 2003 the growing in soil with wood chips had significantly more shoot growth (Fig. 1).

Table 1. Leaf petioles were sampled in July in 2000, 2001 and 2002 from trees growing in soil amended with and without (no-chips) wood chips and analyzed for the following nutrients

	2000		2001		2002	
	Wood chips	No-chips	Wood chips	No-chips	Wood chips	No-chips
% N	1.55 a†	2.21 b	1.38 a	1.58 a	1.92 a	1.6 a
% P	0.33 a	0.18 b	0.96 a	0.31 b	0.66 a	0.43 b
% K	2.69 a	2.67 a	2.47 a	2.01 b	1.92 a	1.62 b
% Na	0.02 a	0.02 a	0.02 a	0.01 a	0.02 a	0.02 a
% CA	1.63 a	1.62 a	2.69 a	2.48 a	3.04 a	2.76 b
% Mg	0.5 a	0.39 a	0.78 a	0.86 a	0.7 a	0.74 a
Zn ppm	41 a	88 b	53 a	63.23 b	10.0 a	6.5 b
Mn ppm	163 a	245.66 b	93.75 a	90.75 a	18.4 a	48.4 b
B ppm	51 a	50.66 a	47.5 a	43.5 a	45.6 a	37 b
Fe ppm	196 a	183 a	323.5 a	292.5 a	---††	---
Cu ppm	11.66 a	9.33 a	16.75 a	17 a	---	---

†Paired columns within the same year with different letters were statistically different when compared in a Student's T-test (P<0.05).
††---- data was unavailable.

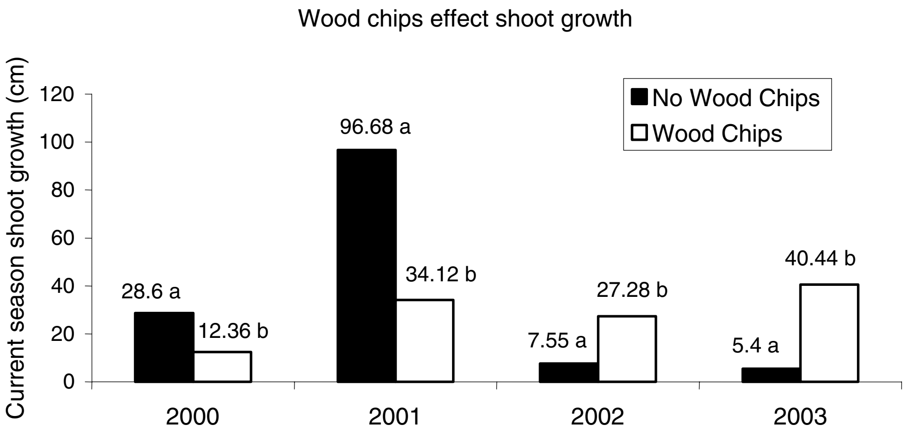

Fig. 1. Ten trees were planted in soil amended with wood chips and another ten trees in soil without wood chips. The five longest shoots per tree were selected and measured. Paired columns within the same year with different letters were statistically different when compared in a Student's T-test (P<0.05).

Soil analysis and water infiltration rate

Soil analysis showed that the addition of wood chips significantly increased soil electrical conductivity (EC), Ca, Mg, Na, Cl, Zn, copper (Cu), P, K, ammonia (NH_4-N), carbon total (C-Tot%), and percent organic matter (OM %) (Table 2). Boron (B) levels were significantly higher in soil amended with wood chips in 2001. The cation-exchange-capacity (CEC) was higher in wood chip amended soil in only 2000. The addition of wood chips significantly lowered soil pH all three years. Nitrate levels (NO_3-N) were significantly lowered by the addition of wood chips in 2000 and 2001, but by 2002 nitrate levels in wood chipped soils were actually higher than in non-wood chip amended soils. Manganese (Mn) and iron (Fe) levels were initially lowered by the addition of wood chips in 2001, but by 2002 their levels with were significantly increased (Table 2). The rate at which 18.9 liters (5 gallons) of water infiltrated soil amended with and without wood chips was measured on four occasions. In soils amended with wood chips we observed significantly faster water infiltration times when compared to soils not amended with wood chips (Fig. 2).

Table 2. Soil samples were taken in October 2000, 2001, and 2002 from trees growing in soil with and without (no-chips) wood chips and analyzed for the following nutrients

	2000		2001		2002	
	Wood chips	No-chips	Wood chips	No-chips	Wood chips	No-chips
pH	6.5 a[†]	7.2 b	6.7 a	7.5 b	6.88 a	7.38 b
EC (mmhos/cm)	0.5 a	0.3 b	0.5 a	0.3 b	0.48 a	0.29 b
Ca (meq/l)	2.8 a	1.2 b	2.8 a	1.4 b	3.20 a	1.47 b
Mg (meq/l)	1.6 a	0.8 b	1.6 a	1 b	1.97 a	0.98 b
Na (meq/l)	0.90 a	1.00 a	1.5 a	1.1 b	0.93 a	0.62 b
Cl (meq/l)	0.50 a	0.50 a	0.60 a	0.60 a	1.15 a	0.48 b
B ppm	0.50 a	0.60 a	0.8 a	0.5 b	----[††]	----
Zn ppm	12.2 a	4.7 b	5.7 a	3.2 b	6.80 a	3.58 b
Mn ppm	34.30 a	34.70 a	8.7 a	25.4 b	7.98 a	3.37 b
Fe ppm	176.40 a	122.00 a	18.6 a	67.5 b	16.88 a	10.17 b
Cu ppm	8.4 a	3.8 b	4.1 a	2.4 b	----	----
C-Tot%	6.6 a	0.4 b	1.0 a	0.4 b	1.09 a	0.40 b
NH_4-N ppm	10.7 a	3.1 b	6.8 a	2.7 b	8.78 a	2.75 b
NO_3-N ppm	0.7 a	2.2 b	0.1 a	0.6 b	0.65 a	0.32 a
Bray P ppm	56.9 a	46.3 b	46.9 a	24.2 b	----	----
X-K ppm	114.4 a	49 b	94.2 a	55.8 b	54.17 a	36.50 b
CEC meq/100g	9.0 a	5.9 b	3.90 a	3.40 a	----	----
OM%	6.4 a	0.5 b	1.2 a	0.4 b	----	----

[†]Paired columns within the same year with different letters were statistically different when compared in a Student's T-test (P<0.05).
[††]---- data was unavailable.

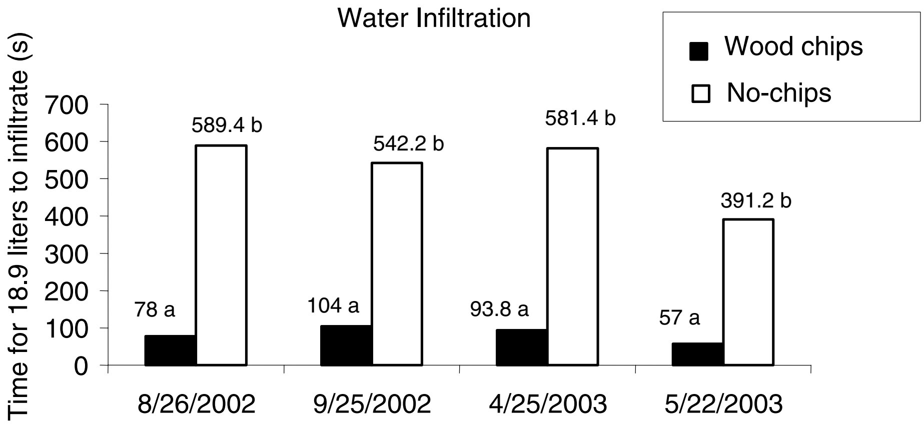

Fig. 2. The time in seconds (s) for 18.9 liters (5 gallons) of water to infiltrate soil amended with and without wood chips was measured on four occasions. Paired columns on the same day with different letters were statistically different when compared in a Student's T-test (P < 0.05).

Nematode and basidiomycete sampling

The effect of wood chips in soil on plant parasitic and free-living nematode populations were examined. In 2000, *Macroposthonia* (ring) populations were significantly reduced while *Bunonema* and *Dorylaimida* and free-living nematode populations were significantly increased in wood chip amended soils (Table 3). In 2001 and 2002 *Macroposthonia* (ring) populations were lower while *Paratylenchus* (root lesion) species were significantly reduced in wood chip amended soils. Free-living bacterial and fungal feeding nematodes were significantly increased in wood chip amended soils (Table 3). In 2001 and 2002 *Bunonema, Trichodorus, Dorylaimida*, and *Monochida* species appeared unaffected by the addition of wood chips to soil. The effect of wood chips in soil on basidiomycete populations was also examined. Basidiomycetes were only found in soils amended with wood chips, averaging 5.8 mushrooms per barrel. Basidiomycetes were never found in plots without wood chips.

Table 3. 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 b	298 a	392 a	545 a	399.6 a
Bunonema spp.	40.8 a	0 b	0 a	0 a	0 a	0 a
Trichodorus spp.	0 a	6.6 a	0 a	0 a	0 a	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 a	0.4 a	0 a	0 a	55 a	59.4 a
Paratylenchus spp.	0 a	1.8 a	0 a	138.8 b	24.4 a	255 b
Free-living spp.	1307.2 a	690.4 b	1703.4 a	246 b	1006.4 a	437.4 b
Bacterial feeding spp.	987.2 a	612 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).

Soil aggregation

There were significantly more soil aggregates >2 mm in all the layers (0-3 cm, 3-8 cm, 8-13 cm, and 13-18 cm) of undisturbed soils amended with wood chips than in disturbed soils also amended with wood chips (Fig. 3). In layer 1 (0-3 cm), 63.2% of >2 mm soil aggregates were in undisturbed soils compared to 36.43% in disturbed soils. In layer 2 (3-8 cm), 69.10% compared to 16.80%, and in layer 3 (8-13 cm), 80.87% compared to 30.10%. In layer 1, the size fractions smaller than 2 mm aggregates were higher in soils from the disturbed compared to the undisturbed site, except for the 0.8-2 mm size-fraction, which is significantly higher in the undisturbed soils. In all soil layers, undisturbed soils amended with wood chips contained significantly greater amount of >2 mm aggregates when compared to the other size fractions. In contrast there was no significant differences among the aggregate size fractions in disturbed soils amended with wood chips.

ELISA

The same size fractionated soil samples from undisturbed and disturbed sites amended with wood chips were analyzed using ELISA to detect and quantify populations of specific soil aggregating basidiomycete fungi (Fig. 4). Results showed a greater response to antibodies in soils from undisturbed sites when compared to disturbed sites in the four soil layers. In the surface soil layer, the amount of soil-aggregating fungi was significantly greater in the undisturbed soils when compared to the disturbed soils, and a greater response to the antibodies was observed in the >2 mm and 2-0.84 mm aggregate size fractions. In the other deeper layers, no differences in populations of these fungi were detected.

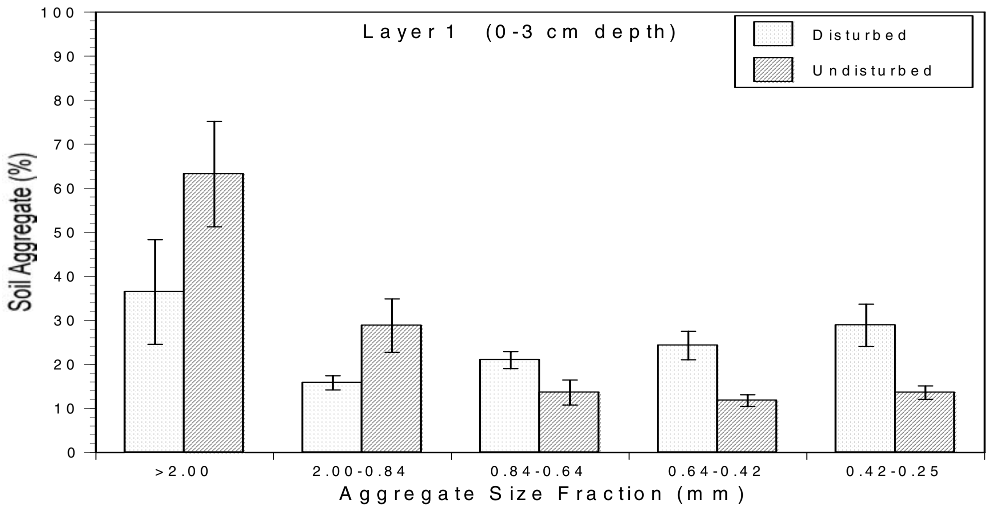

Fig. 3. Soils were sampled from disturbed (with a rotary-tiller) and undisturbed plots from and almond orchard where wood chipped prunings were added seasonally for 14 years. Soils were obtained from four debts with layer 1 (0-3 cm) shown here. Soils were dried in a forced-air oven at 50°C and then passed through a series of sieves (>2 mm, 0.84 mm, 0.42 mm, and 0.25 mm-mesh) in order to collect soil aggregates.

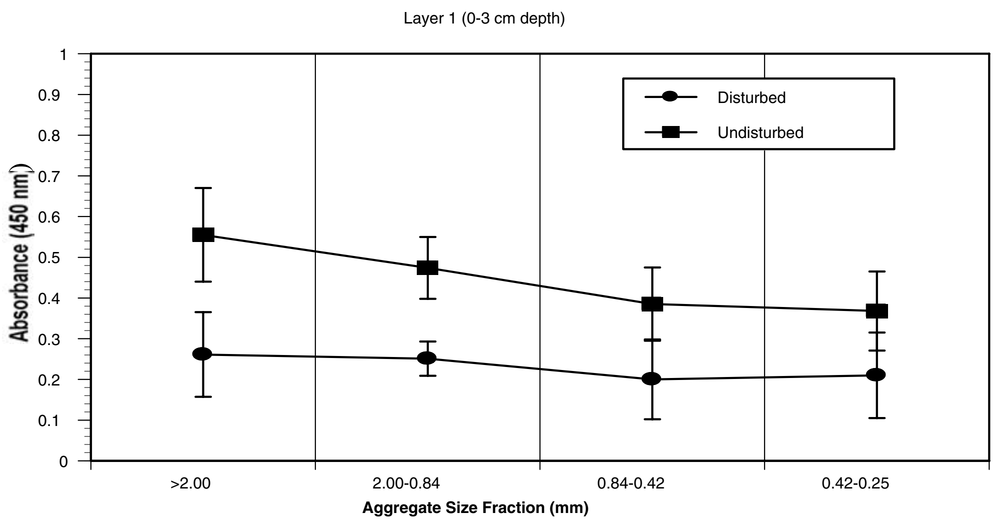

Fig. 4. Presence of soil aggregating basidiomycetes was determined for each soil aggregate size fraction using ELISA by quantifying the response to antibodies in soil. The same size fractionated soil samples from undisturbed and disturbed sites amended with wood chips were analyzed using ELISA to detect and quantify populations of specific soil aggregating basidiomycete fungi. Absorbance was read at dual wavelength of 450 nm.

Conclusions

The addition of wood chipped prunings to soil planted with almond trees initially reduced leaf petiole and soil nitrate levels in the first year, but by the second year leaf petiole levels were no longer significantly less, and by the third year both leaf petiole and soil nitrate levels were higher in leaves and soils from trees grown in wood chip amended soils. Most nutrients by the third year were significantly higher in soil amended with wood chips or leaf petioles from trees grown in wood chip amended soils. This data was supported by greater current season shoot growth from trees grown in wood chip amended soils in the third and fourth seasons, providing evidence that even though wood chips initially tied up nitrogen when first added to the soil, as they decomposed the wood chips eventually returned more nutrients to soils amended with wood chips when compared to soils not amended with wood chips.

The addition of wood chips significantly lowered soil pH all three years, while soil carbon and soil organic matter was increased. Water infiltrated wood chip amended soil much more quickly when compared to soils without wood chips, presumably due to greater pore spaces due to the additional organic matter. The wood chips also appear to be reducing ring and root lesion pathogenic nematodes while increasing free-living bacterial and fungal feeding (non-pathogenic) nematodes. More basidiomycete fungi were counted in soils amended with wood chips, and soil-aggregating basiciomycetes were detected at higher levels with ELISA. Larger soil aggregates were found in wood chipped and undisturbed soils. The addition of wood chipped almond prunings to soils appear to be enhancing soil nutrient levels, basidiomycete wood rotting and soil aggregating fungi, and free-living nematode populations while providing almonds growers with a more sustainable method of brush removal.

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