

## Wood Chipping Almond Brush and its Effect on the Almond Rhizosphere, Soil Aggregation and Soil Nutrients

B.A. Holtz<sup>1</sup>, M.V. McKenry<sup>2</sup> and T.C. Caesar-TonThat<sup>3</sup>

<sup>1</sup>University of California, 328 Madera Avenue, Madera CA 93637, USA.

<sup>2</sup>University of California, 9240 South Riverbend Avenue, Parlier, CA 93648, USA

<sup>3</sup>USDA-Agricultural Research Service, 1500 North Central Ave., Sidney, MT 59270, USA

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### Abstract

The wood chipping of almond (*Prunus dulcis*) 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 deplete the soil of critical nutrients necessary for tree growth. An experiment was established where wood chips were mixed with soil and placed in containers, each with an almond tree, in order to quantitatively examine the effect of wood chips on soil nutrients, soil aggregation, and the rhizosphere microbial community. Control trees were planted in containers without wood chips. Tissue analysis was performed on leaf petioles to determine whether the wood chips had an effect on nutrient availability. After the 1st year, trees growing with wood chips had less N, Zn, and Mn while P was increased. After the 2nd year, trees with wood chips no longer had less N levels while P and K were significantly increased, but Zn was decreased. Soil analysis after the 1st year showed significantly higher levels of Ca, Mg, Zn, Cu, P, and K with wood chips. The % carbon, NH<sub>4</sub>-N, cation-exchange-capacity (CEC), electrical conductivity (EC), and % organic matter (OM) were increased. The soil pH and NO<sub>3</sub>-N levels were decreased. Similar results were obtained the 2nd year except that Mn and Fe levels were decreased in wood chipped soils while B and Na were increased. The CEC was no longer higher in wood chipped soils. When nematode populations were assayed after the 1st year there were less *Criconemella* and more *Bunonema*, *Doryleimida*, and free-living bacterial and fungal feeding nematodes in wood chipped soils. Similar results were obtained after the 2nd year except that root lesion was reduced in wood 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 wood chipped soils.

### INTRODUCTION

Since the passing of The Federal Clean Air Act Amendments of 1990 the San Joaquin Valley (SJV) of California has not met national ambient air quality standards for particulate matter 10 microns (PM-10) or less. The SJV Unified Air Pollution Control District restricts the burning of agricultural wastes and further restrictions are likely due to worsening air pollution. Wood chipping could provide an alternative to burning that could add valuable organic matter to almond soils. A small percentage of almond growers have been wood chipping for over 10 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 wood chipped solely to add organic matter to their soils. But many growers fear that wood chips will take valuable nutrients away from their trees (Holtz 1999). If wood chips can be shown to not interfere with harvest or take valuable nutrients from trees, then growers would be more likely to adopt wood chipping as an alternative to burning.

Research has shown that organic material can increase the humic content of soil (Sikora & 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 & DeFrank 2000). The effect of wood chips on soil nutrients and the rhizosphere microbial community was examined in a replicated experiment where the same 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 and Orchard Placement**

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 and root lesion plant pathogenic nematodes. 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 almond tree per barrel. Five trees were planted in barrels with wood chips and 5 in barrels without. The barrels were placed in an almond orchard (L. D. James Ranch, Modesto CA) 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. Mushrooms were counted in individual barrels when they appeared.

### **Leaf and Soil Sampling, Analysis**

Fifty to 75 leaves, from non-fruiting spurs were sampled per tree in July of 2000, 2001, and 2002 randomly. One kg of soil was sampled from each barrel in October of 2001 and 2002. The soil samples were split; half the sample was assayed for nematodes while the other half was analyzed for nutrients. Leaf and soil samples were analyzed by the University of California's Division of Agriculture and Natural Resources Laboratory (Davis, CA, danranlab@ucdavis.edu).

### **Nematode Assays**

Ring nematode was assayed with the sugar centrifugation method (McKenry and Roberts 1985) 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 root plant tissues (20 grams) were placed into a funnel and into a mist chamber. After 3-5 days the nematodes were removed and counted.

### **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 10 years. Soil samples were collected from 2 treatment sites: 1) where the soil had been left undisturbed, and 2) where the 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, 3 replications per treatment, from a depth of 20 cm using a step-down soil probe and divided into increments of 0-to-5, 5-to-10, and 10-to-15 cm. The 3 replications 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 (>2mm, 0.84mm, 0.42mm, and 0.25mm-mesh).

### **Enzyme Linked Immunosorbent Assay (ELISA)**

Soil aggregating basidiomycetes were determined in soil aggregate size fractions using Enzyme Linked Immunosorbent Assay (ELISA) (Caesar-TonThat et al. 2001). Soil samples (500 mg/ml) were prepared by homo-genization of samples in a mortar and pestle in carbonate buffer (20 mM NaHCO<sub>3</sub>, 28 mM Na<sub>2</sub>CO<sub>3</sub>, 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 3 washings with 0.01M Phosphate buffer saline-Tween 20, 0.138 M NaCl, 2.7 mM KCl, pH 7.4 (PBST, Sigma, St Louis), 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× 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) added to each well. After 3 further PBST washings, the substrate, consisting of a solution of 3,3', 5,5' tetramethylbenzidine (0.4 g/l) (Pierce, Rockford, Illinois) 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**

Leaf petiole analysis showed that trees growing 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 with wood chips all 3 years. Potassium (K) increased in trees growing with wood chips in 2001 and 2002 significantly. Calcium (Ca) levels increased significantly in trees growing with wood chips in 2002. Zinc (Zn) levels decreased significantly for trees growing in wood chips all 3 years. Manganese (Mn) levels also significantly decreased for trees growing with wood chips in 2000 and 2002. Boron levels increased for trees growing in wood chips in 2002 significantly. Sodium (Na) and Magnesium (Mg) levels were unaffected by the addition of wood chips.

### **Soil Analysis**

Soil analysis showed that the addition of wood chips significantly increased soil electrical conductivity (EC), Ca, Mg, Zn, Cu, P, K, ammonia (NH<sub>4</sub>-N), carbon total (C-Tot %), and organic matter (OM %) in 2000 and 2001 (Table 2). Boron and Na levels were significantly higher in wood chipped soils in only 2001, while the cation-exchange-capacity (CEC) was only higher in wood chipped soils in 2000. Soil analysis showed that the addition of wood chips significantly lowered soil pH and nitrate levels (NO<sub>3</sub>-N). Manganese (Mn) and Iron (Fe) levels with wood chips were lowered in 2001.

### **Nematode Assays**

The effect of wood chips in soil on plant parasitic and free-living nematode populations were examined. In 2000, *Criconemella* (ring) was reduced while *Bunonema* and *Dorylaimida* were significantly increased in wood chipped soils (Fig. 1). In both 2000 and 2001 free-living bacterial and fungal feeding nematodes increased in wood chipped soils significantly (Fig. 2). In 2001, ring nematode was lower while root lesion was reduced in wood chipped soils significantly (Fig. 3).

## Soil Aggregation

Results of the comparison between the amount and size (>2mm, 2-0.8mm, 0.8-0.4mm, and 0.4-0.2mm) of soil aggregates in undisturbed and disturbed almond orchard soils with chip residues at 4 different soil depths (0-3 cm, 3-8 cm, 8-13 cm, and 13-18 cm) are shown in Fig. 4. There was significantly more soil aggregates >2 mm in all the layers of undisturbed soils with chips than disturbed soils with chips. For example, in layer 1 (0-5 cm) 63.2 % of >2 mm soil aggregates were in undisturbed soils compared to 36.43 % in disturbed soils, in layer 2 (5-10 cm) 69.10 % compared to 16.80 %, in layer 3 (10-15 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 addition, it was noted that in all soil layers, undisturbed soils with chips contain a significant greater amount of >2 mm aggregates when compared to the other size fractions whereas there was no significant difference between the different aggregate size fractions in disturbed soils with chips.

## Elisa

The same size fractionated soil samples from undisturbed and disturbed sites with wood chip residues were analyzed using ELISA to detect and quantify populations of specific soil aggregating basidiomycete fungi (Fig. 5). Results showed a greater response to antibodies in soils from undisturbed sites when compared to disturbed sites in the 4 soil layers. In the surface soil layer, the amount of soil aggregating fungi were significantly greater in the undisturbed aggregates compared to the disturbed soils, mostly in the >2 mm and 2-0.84 mm aggregate size fractions. In the other deeper layers, no difference in populations of these fungi was detected.

## CONCLUSIONS

The addition of wood chipped prunings to soil reduced plant and soil nitrogen (NO<sub>3</sub>-N) levels after the 1<sup>st</sup> year. But by the 2<sup>nd</sup> year many nutrients were significantly increased. Wood chips increased soil organic matter in both years. In addition, wood chips appear to be reducing ring and root lesion nematodes while increasing free-living nematodes. More basidiomycete fungi were counted in wood chipped soils and detected at higher levels with ELISA. Larger soil aggregates were found in wood chipped and undisturbed soils.

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## **Tables**

Table 1. Leaf petioles were sampled in July in 2000, 2001, and 2002 from trees growing with wood chips and without (no-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	1.58	1.92	1.6
% P	0.33 a	0.18 b	0.96 a	0.31 b	0.66 a	0.43 b
% K	2.69	2.67	2.47 a	2.01 b	1.92 a	1.62 b
% Na	0.02	0.02	0.02	0.01	0.02	0.02
% CA	1.63	1.62	2.69	2.48	3.04 a	2.76 b
% Mg	0.5	0.39	0.78	0.86	0.7	0.74
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	90.75	18.4 a	48.4 b
B ppm	51	50.66	47.5	43.5	45.6 a	37 b
Fe ppm	196	183	323.5	292.5		
Cu ppm	11.66	9.33	16.75	17		

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

	2000		2001	
	Wood chips	No-chips	Wood chips	No-chips
pH	6.5 a	7.2 b	6.7 a	7.5 b
EC	0.5 a	0.3 b	0.5 a	0.3 b
Ca	2.8 a	1.2 b	2.8 a	1.4 b
Mg	1.6 a	0.8 b	1.6 a	1 b
Na	0.90	1.00	1.5 a	1.1 b
Cl	0.50	0.50	0.60	0.60
B ppm	0.50	0.60	0.8 a	0.5 b
Zn ppm	12.2 a	4.7 b	5.7 a	3.2 b
Mn ppm	34.30	34.70	8.7 a	25.4 b
Fe ppm	176.40	122.00	18.6 a	67.5 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
NH <sub>4</sub> -N ppm	10.7 a	3.1 b	6.8 a	2.7 b
NO <sub>3</sub> -N ppm	0.7 a	2.2 b	0.1 a	0.6 b
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
CEC meq/100g	9.0 a	5.9 b	3.9	3.4
OM %	6.4 a	0.5 b	1.2 a	0.4 b
CEC meq/100g	9.0 a	5.9 b	3.90	3.40
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).

## Figures

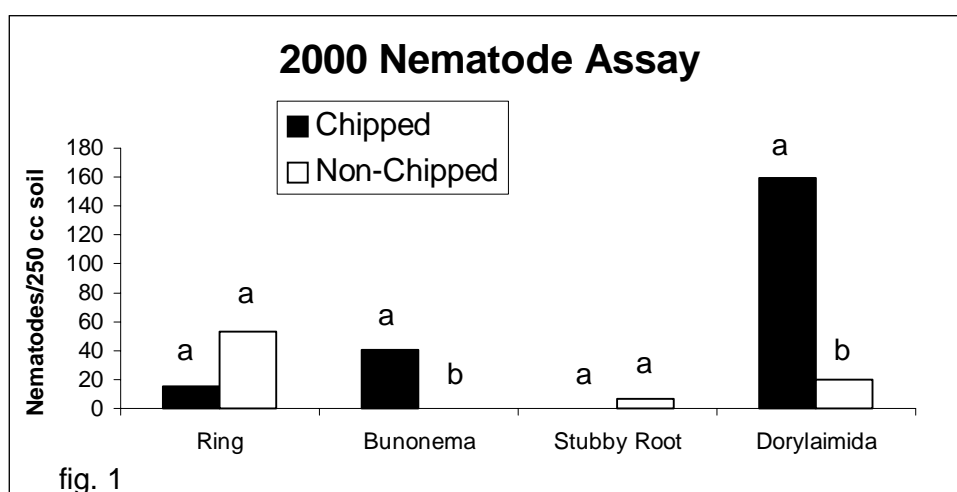


Fig. 1. Effect of wood chips ion soil on nematode populations in 2000, Paired columns with the same letters are not statistically different, T-test (P < 0.05).

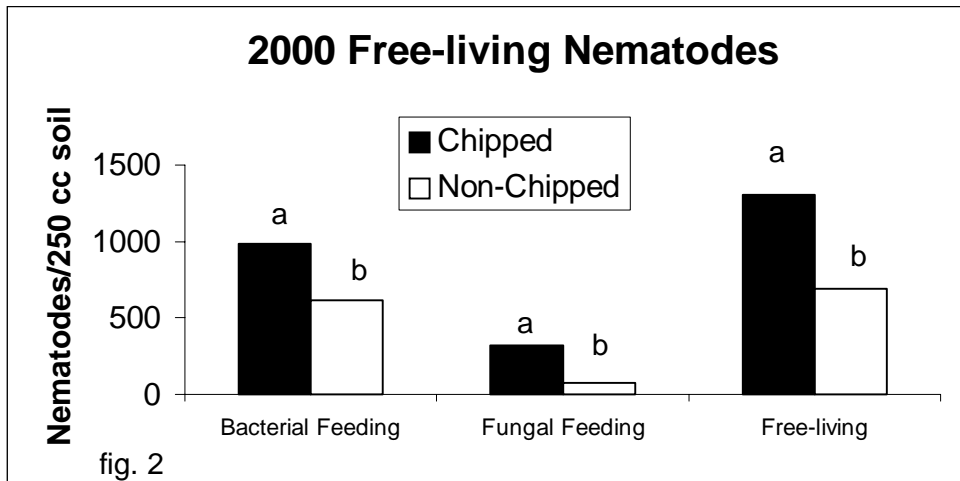


Fig. 2. The effect of wood chips in soil on free-living nematode populations in 2000, Paired columns with the same letters are not statistically different, T-test ( $P < 0.05$ ).

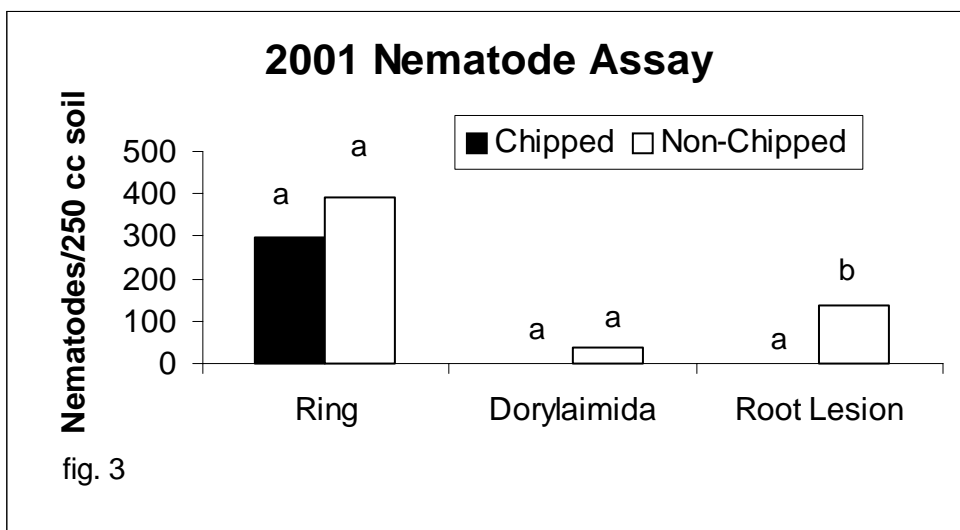


Fig. 3. The effect of wood chips in soil on nematode populations in 2001. Paired columns with the same letters are not statistically different, T-test ( $P < 0.05$ ).

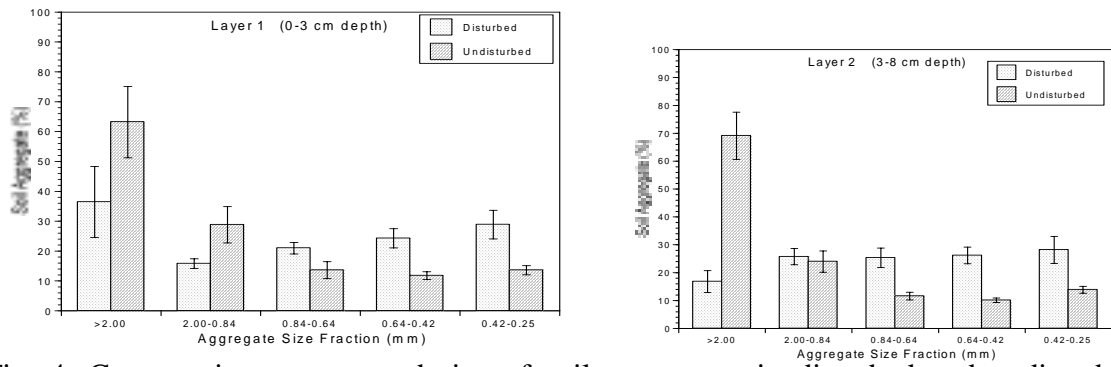


Fig. 4. Comparative amount and size of soil aggregates in disturbed and undisturbed almond orchard soils at different soil depths.

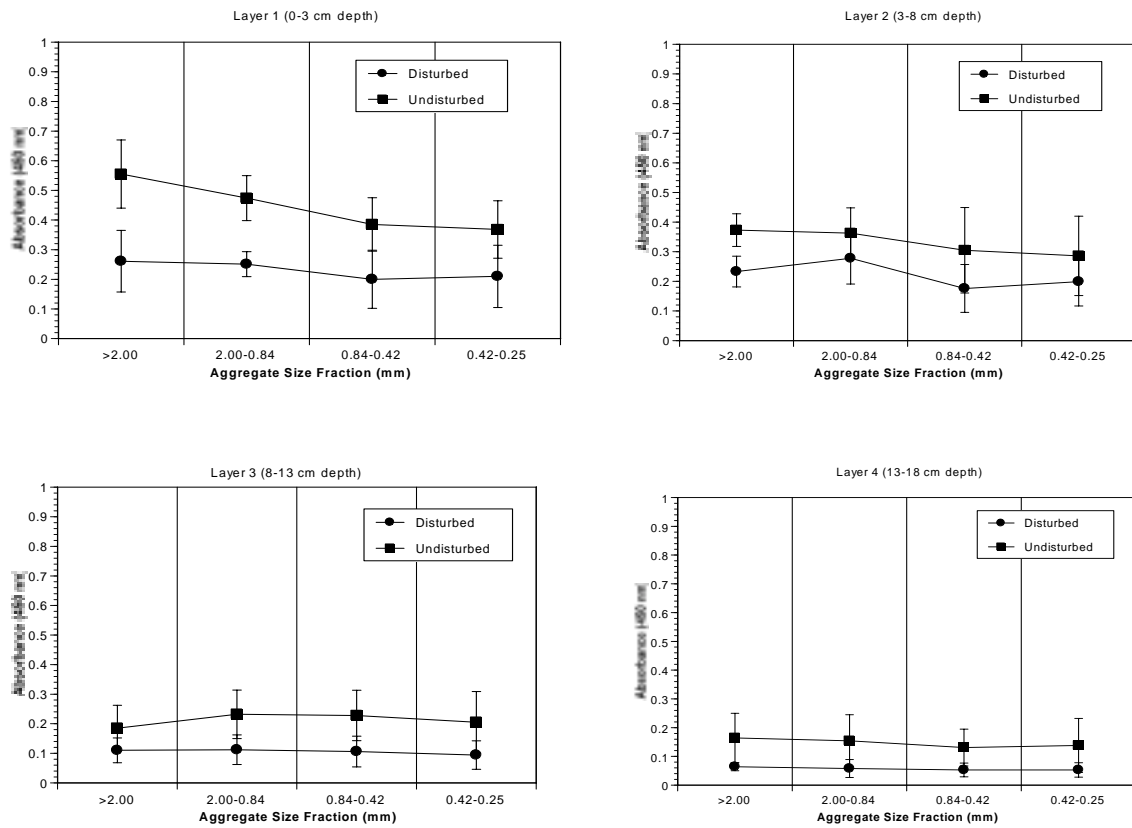


Fig. 5. Population size of soil-aggregating basidiomycete fungi in fractionated soils from disturbed and undisturbed orchard sites.