The effect of slash/mulch and alleycropping bean production systems on soil microbiota in the tropics

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Abstract

Mulch applied to tropical soil may increase soil health both through stimulation of microbiota beneficial to plant nutrient uptake and the suppression of plant disease. To test this hypothesis, we compared beans in three cropping systems: (1) mulched with secondary vegetation (slash/mulch), (2) mulched with foliage from alleycropped nitrogen-fixing trees, Caliandra calothyrsus, Gliricidia sepium and Inga edulis (alleycrop/mulch), and (3) unmulched, for their effect on positive microbial interactions (nodulation with Rhizobium and arbuscular mycorrhiza (AM)), and pathogenic associations (foliar and root diseases) in long-term field experiments in Costa Rica. In some dry years slash/mulched beans nodulated significantly more than the unmulched, though in wet years the nodule biomass was not significantly different between treatments. Beans grown in alleycropping systems mulched with foliage from Calliandra and Inga had lower nodule biomass than beans in slash/mulched and Gliricidia mulched plots at 3 and 5 weeks, probably due to high nitrogen levels from the decomposing vegetation of the tree mulch. Roots of bean plants were 95–98% colonized by AM fungi and there were no differences between the slash/mulched beans and the unmulched treatments. Slash/mulch had no effect on the foliar diseases angular leaf spot and web blight, due to their naturally low incidence in that year; slash/mulched decreased the severity of anthracnose (caused by Colletotrichum lindemuthianum). Slash/mulch decreased severity of a Fusarium-type root rot, but increased a Rhizoctonia-type. In the laboratory, microbial activity as determined by measurements of CO2 respiration was greater in the slash/mulched than the unmulched system due to the high respiration of the mulch material. Our hypothesis that the use of mulch would favor mutualistic symbionts was not supported, which may be due to weather and nutrient interference. However, the hypothesis that mulch would reduce disease incidence was supported for certain diseases. A comparison of the microbial community with those of nearby natural systems can help provide a standard for the assessment of microbial health in agroecosystems and allow us to avoid the circularity of defining microbial communities as ‘healthy’ because they are found in cropping systems whose plants appear without disease. © 2000 Published by Elsevier Science B.V.

Keywords: Soil health; Plant disease; Alleycrop; Mulch; Mycorrhiza; Nodulation; Costa Rica

1. Introduction

Soil health has been broadly defined as “the continued capacity of the soil to function as a vital living system, within ecosystem and land use boundaries, to sustain biological productivity, promote the quality of...
air and water environments and maintain plant, animal and human health” (Doran and Safley, 1997). A healthy soil is one that can provide the service of organic matter breakdown and nutrient release such that plant and animal biomass are supported. The microbiota are critical to the wide array of functions in the soil contributing to soil health including: (1) decomposition of organic matter and (with microfauna) provide nutrients to sustain biological productivity of plants and animals, (2) participation in symbioses with plants facilitating nutrient uptake and (3) suppression of pathogens through competition or antagonism, thereby sustaining plant and animal health. Microbiota are also the foundation of the detritus food web, which because of efficient transfer between trophic levels, supports lengthy food webs of organisms which are the basis for integrated soil function (Coleman and Crossley, 1996).

Microbes constitute about one quarter of all living biomass on earth (Jong, 1989) and during the early stages of the earth’s history, the major nutrient cycles have existed with microorganisms and one-celled organisms alone. Our present-day nutrient cycles, regardless of the inclusion of animals and plants, are not profoundly different from their pre-historic antecedents (Knoll and Awrawik, 1983). In other words, microbes are key facilitators in the major nutrient cycles. Energy from the decomposition of carbon compounds such as polysaccharides, cellulose, and lignin fuels heterotrophic microbes that are responsible for nutrient transformations, such as asymbiotic nitrogen fixation, protein and amino acid decomposition, mineralization and immobilization of nitrogen (N) and phosphorus and mineral transformations (Roper and Orphel-Keller, 1997). Microorganisms are responsible for significant nutrient transformations involving both macro and micronutrients: N, P, K, S, Fe, K, Ca, Mg, Mn, Al, Zn, Se (Alexander, 1997). These microbially-driven processes influence nutrient availability and ultimately soil health and quality. Since they are such a critical component of soil and ecosystem function, these microbial groups, if sensitive to disturbance, may serve as important indicators of ecosystem function (Turco et al., 1994).

The use of microflora as potential indicators of soil health is not new. Waksman, in his 1927 textbook *Principles of Soil Microbiology*, considers several microbial criteria as indicators of soil fertility, including nitrogen fixing capacity, nitrifying capacity, numbers of microbes, cellulose decomposition rate and carbon dioxide (CO2) evolution. Nevertheless, as an indicator of soil health microbes have been little used because of their small size and the difficulty in culturing them, and most importantly the lack of knowledge of the uncultured species. At present, uncultured species are nine times greater than those able to be reproduced on media (Roper and Orphel-Keller, 1997). Recently various techniques have been used to characterize microbial community structure or function, e.g. fatty acid methyl ester (FAME) and rRNA fingerprinting to determine types of organisms present and BIOLOG plates for substrate utilization (Heuer and Smalla, 1997). However, currently these techniques present difficulties in addressing the question of soil health because they are able to resolve the species level and because of the vast gap in knowledge concerning these organisms and their ecological roles.

There is increasing evidence of changes in certain microbial populations with cultural management and agrochemical application (Roper and Orphel-Keller, 1997). Studies assessing the effect of agrochemicals on microbes have roughly identified three sensitivity groups: low, medium and high (Domsch et al., 1983). Along with other microbial indicators these sensitivity groups may contribute to identifying a healthy soil or at least identify which groups may have lower than optimum activity when converting from high agrochemical use to low input agroecosystems. High sensitivity groups include nitrifying bacteria, nitrogen-fixing bacteria and actinomycetes responsible for some steps in organic matter decomposition (Domsch et al., 1983). Additionally if certain critical groups, e.g. ammonia oxidizers and basidiomycete fungi that decompose lignin, have a low physiological tolerance to certain environmental disturbances, these might also serve as indicators of soil health under certain conditions (Andrén et al., 1999). Molecular techniques are being developed to assess their diversity (Gollwitzer, 1999).

Disease suppression is also an important function of the soil microbial component in contributing to soil health. From a soil manager’s perspective the absence of disease in a particular crop plant may define a healthy soil (Hornby and Bateman, 1997). Although this clearly involves a set of defined value judgements, the absence of disease, whether it be in a crop plant
or generally throughout an ecosystem, is an important aspect of ‘maintaining biological productivity’ in general or ‘plant, animal and human health’ specifically.

Likewise plant symbioses with microbes especially under low external input situations (low agrochemical use) are critical for plant health and productivity. Arbuscular mycorrhiza (AM) fungi, with their broad host range have been instrumental not only in plant nutrient uptake but also soil aggregation. Water stable aggregates increase with mycorrhizally-secreted protein, glomalin (Wright and Upadhyaya, 1998). Therefore, high colonization of roots with AM fungi might indicate optimum levels of water stable soil aggregation important in providing space for soil atmosphere and water infiltration. Nitrogen-fixing bacteria are essential for legumes where no nitrogenous fertilizers are applied. In addition, the lack of this part of the microbial complement may indicate other missing elements in the system.

Since cropping systems can affect soil biota (and thereby affect soil health), we studied three systems to see how Costa Rican farmers could best manage their soil. The slash/mulch system is pre-Hispanic in origin but, unlike many traditional systems, still relevant to Costa Rican bean production. After about 2 years of fallow, bean seed is broadcast into the second growth vegetation, which is cut down to form a mulch through which the bean seeds emerge. Bean acreage in the slash/mulch system has not changed much over the last 20 years and still accounts for some 30–40% of Costa Rican bean production, 60% of which is sold off the farm (Rosemeyer, 1995). Demand for higher production has caused shortened fallows and beans are produced every year. Consequently the composition of the secondary vegetation changes as dicotyledonous plants are replaced with monocots that are less productive for slash/mulch beans. Alleycropped planting of leguminous trees that are coppiced yearly for mulch might circumvent this problem by their continuous production of high-quality foliage.

In a series of experiments spanning more than a decade, we have explored agronomic and nutrient cycling aspects of the slash/mulch, alleycrop/mulch and unmulched systems (Rosemeyer and Gliessman, 1992; Rosemeyer, 1994; Kettler, 1997a,b). Soil health is one component of our assessment and here we test the hypotheses that slash/mulch and alleycrop/mulch systems provide greater soil health via three contributions of the microbiota: facilitation of symbioses (nodulation and AM of beans), disease suppression and increased microbial respiration.

2. Materials and methods

2.1. Site

Studies were conducted at Finca Loma Linda, near the town of Cañas Gordas, in the province of Puntarenas, southern Costa Rica at an elevation of 1200 m on an Andisol soil type. In this pre-montane rain forest life zone (Holdridge, 1971), the precipitation averages 3600–4000 mm per year. It rains daily during the wet season April through November and once every 2 weeks during the dry season December through March.

2.2. Experiments

The experiments and their response variables are summarized in Table 1.

2.2.1. Experiment 1: slash/mulch versus unmulched beans the first year after fallow

In 1986 and 1988, both after 1.75 years of fallow, the slash/mulched and unmulched bean production systems were compared. In 1986, plot size was 5 m × 5 m in a randomized complete block design of five blocks. Plots in 1988 were 3 m × 3 m with six repetitions. In 1986, land was disked with a tractor before planting and in 1988 vegetation removal and land preparation was done by hand. Planting in the unmulched systems was done by hand with a dibble stick to a depth of 3 cm in holes 30 cm × 30 cm apart and thinned to three plants per hole. To obtain comparable plant spacing in the slash/mulch plots the slashed vegetation was temporarily removed before the seed was broadcast and then replaced to permit comparison with the unmulched (traditionally seed is broadcast and then vegetation is cut afterward to form a mulch).

A traditional, semi-vining Type II (López et al., 1985) variety of black bean, widely used in both systems and locally known in the area as ‘Chimbo Negro’, was selected based on local acceptance and level of nodulation in comparison to four
Table 1
Experiments performed and measured response variables

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Year response variables measured</th>
<th>Field or lab experiment</th>
<th>Response variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1: slash/mulch vs. unmulched in the first year after the fallow</td>
<td>1986, 1988</td>
<td>Field</td>
<td>Yield, nodulation at 7 weeks and mycorrhizae</td>
</tr>
<tr>
<td>Experiment 2: slash/mulch with fertilizer vs. unmulched, four consecutive years after the fallow</td>
<td>1994</td>
<td>Field</td>
<td>Yield, angular leaf spot, web blight, Fusarium-type root rot and Rhizoctonia-type root rot</td>
</tr>
<tr>
<td>Experiment 3: slash/mulch vs. alleycrop/mulch vs. unmulched in five consecutive years after the fallow</td>
<td>1997</td>
<td>Field</td>
<td>Yield, nodulation at 3, 5 and 7 weeks and Fusarium-type root rot</td>
</tr>
<tr>
<td>Experiment 4: slash/mulch vs. unmulched</td>
<td>1995</td>
<td>Lab</td>
<td>Microbial respiration (CO₂ evolution)</td>
</tr>
</tbody>
</table>

other locally-used varieties (Rosemeyer and Gliessman, 1992). Seeding rate was 340,000–400,000 (60–65 kg/ha), and varied depending on the year but did not vary between treatments. No pesticides were applied and unmulched plots were manually weeded. Bean seed was harvested at maturity (about 90 days) by hand excluding 0.5 m borders to eliminate border effects and seeds dried at 65°C for 48 h and weighed. Yield, nodulation and mycorrhizae at 7 weeks will be reported from this experiment (Table 1).

2.3. Plant sampling for nodulation and mycorrhizae

Plants were sampled for vegetative biomass at the R-3 stage (about 50 days after planting), when the first pod is 2.5 cm in length (Lebaron, 1974) and nodulation has been reported to be the highest (Nash and Schulman, 1976; Westerman and Kolar, 1978). For each plot in 1986, three to six sampling sites containing three plants were sampled. In 1988, two to three sites of 30 cm diameter containing three plants were sampled. Sampling was done on a per area basis to eliminate error associated with the inability to remove the entire root system of the plant and the entire soil volume was removed and examined for nodules and roots. Nodules were dried at 65°C for 48 h and reported in mg dry biomass per plant. Roots preserved in formaldehyde, acetic acid and ethyl alcohol (FAA) were cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol according to the method of Phillips and Hayman (1970). Percent of roots colonized by mycorrhizae was assessed by the proportion of 1000 intersections that were mycorrhizal using the grid-intersect method (Giovannetti and Mosse, 1980). Arbuscular mycorrhizal fungi can be distinguished from plant pathogens by the lack of tissue disorganization where they colonize.

2.3.1. Experiment 2: slash/mulch versus unmulched in consecutive years after the fallow

Experiment 2 was designed to test the effect of long-term fertilizer applications to the slash/mulch and unmulched systems on bean yield when the slash/mulch system is performed every year (reported here is data from the fifth year of the experiment, 1994). In 1990, a randomized complete block experiment of six blocks with 4 m x 4 m plots was initiated after 1.75 years of fallow. The bean variety and the sowing procedure were as described for 1988 in Experiment 1.

Fertilizer (10(N)–30(P)–10(K)) was broadcast applied to the slash/mulch system at the following levels: 0, 108, 216 and 325 kg/ha. This is equivalent to 0, 14.1, 28.3 and 43.5 kg elemental P/ha, respectively. The unmulched treatments were fertilized at 0 and 325 kg 10–30–10/ha (43.5 kg elemental P/ha), which was divided and placed about 2 cm from the seeds 2 cm deep at time of planting. Not all fertilizer levels were applied since this was the Costa Rican Ministry of Agriculture recommendation for unmulched production under this soil type and was included as a ‘control’. In addition, two treatments with single vegetation types (and the lowest level of fertilizer) were instituted by cutting the vegetation from off the site and placing on the plots by: Melanthera aspera, a weed considered favorable for slash/mulch, and Mucuna pruriens, a leguminous green manure/cover crop that is a high biomass producer and increasingly used in
Central America. The unmulched plots were weeded by hand at 30 days. No other cultural treatments were applied and no severe pests or disease outbreaks occurred, except for rabbits in certain unmulched plots, the yields from which were not included in the statistical analysis. After each bean harvest, the plots were left fallow for 9 months until the next cropping season.

Yield, nodulation (as described in Experiment 1), angular leaf spot, anthracnose, web bight, Fusarium-type root rot and Rhizoctonia-type root rot and yield will be reported from the fifth year of this experiment (1994) (Table 1).

2.4. Sampling for diseases in Experiment 2

In 1994, nine plants per plot at the R-3 stage were scored for the following diseases as follows:

Angular leaf spot (*Phaeoisariopsis griseola*): Using standardized visual observation scale of Centro Internacional de Agricultura Tropical (CIAT) (van Schoonhoven and Pastor-Corrales, 1987) foliage was scored from 1 (lowest) to 9 (highest) disease severity. This scale is from (1) with no visible symptoms to (9) where 25% of the leaves or pods are covered with sporulating lesions.

Anthracnosis (*Colletotrichum lindemuthianum*): Severity of foliar symptoms was assessed by visual observations using a modification of CIAT’s scale (1) no infection to (9), where 100% of the leaves are infected (van Schoonhoven and Pastor-Corrales, 1987). Since CIAT’s standard method is based primarily on pod lesions and pods at the R-3 stage were only about 3 cm in length, leaf infection was scored as follows: (1) no infection, (2) presence on one or two leaves, (3) secondary veins of the plant are affected, (5) 50% of the leaves are affected with foliar symptoms, (7) 75% leaves affected and (9) 100% of the leaves infected by the pathogen. The identity of the pathogen was confirmed by the Department of Plant Pathology at the University of Costa Rica (UCR).

Web-blight (*Thanatephorus cucumeris* sexual stage, *Rhizoctonia solani* assexual stage confirmed at UCR): Severity of foliar symptoms was assessed by visual observations using CIAT’s scale (1) no infection to (9), where 100% of the leaves are infected (van Schoonhoven and Pastor-Corrales, 1987, Fig. 4, pp. 36–37).

Root rot (*Fusarium*-type symptoms): This root rot caused reddish longitudinally-streaked lesions and was assessed by damage to the root system as follows: (1) no symptoms, (3) light discoloration and between 0 and 10% of the hypocotyl and root tissue with lesions, (5) lesions on 25% of the tissue but it remains firm with little decay, (7) lesions on 50% of tissue and roots suffer considerable decay and reduction with fungal structures visible and (9) 75% of the root system with advanced decay and extensive fungal growth (Abawi, 1994). Identity of the pathogen was confirmed as *Fusarium* sp. by the UCR.

Root rot (*Rhizoctonia*-type symptoms): This root rot causes reddish-brown sunken lesions whose severity was assessed using visual scoring using CIAT’s standardized method (1 lowest to 9 highest) (van Schoonhoven and Pastor-Corrales, 1987). Identity of pathogen was confirmed as *Rhizoctonia* sp. by the UCR.

2.4.1. Experiment 3: slash/mulch versus alleycrop/mulch versus unmulched in consecutive years after the fallow

In 1992, an alleycropping experiment was planted in a randomized complete block design with five blocks in plots 5 m × 11 m in size. The six treatments were as follows:

1. unmulched, no fertilizer,
2. unmulched, with 325 kg 10–30–10 fertilizer/ha,
3. slash/mulch with secondary vegetation as described above; slash/mulch enriched with foliage from leguminous alleycropped trees grown on the plots and applied separately (designated as alleycrop/mulch) from the following species:
4. *Calliandra calothyrsus*,
5. *Gliricidia sepium*, and
6. *Inga edulis*.

The trees were planted in 1992, 1.5 years before they were cut for mulch. The three rows of trees per plot were planted 4 m apart and 50 cm within rows. Every year the trees were slashed at 1 m from the ground at the time of bean planting providing foliage and branches to the secondary vegetation forming the mulch. Seed variety and bean seeding densities were as above; however, the seeds were broadcast into the standing vegetation before cutting as in the traditional slash/mulch system. Details of the experiment can be found in Kettler (1997a). The following response vari-
ables will be reports from Experiment 3 in the 1997 field season after 5 years of bean production with alleycrop/mulch application: yield; nodulation at 3, 5 and 7 weeks; and *Fusarium*-type root rot severity. In order to precisely quantify the extent of the root rot, the length of the *Fusarium*-type lesion on the hypocotyl, as well as the hypocotyl itself, was measured. Other diseases were not at a high enough incidence to assess.

2.5. **Microbial respiration**

The respiration of freshly cut mulch, litter and soil respiration of the slash/mulch system was assessed by determining CO\(_2\) evolution. CO\(_2\) evolution was allowed to proceed over 20 min for mulch and 60 min for litter and soil using the method of Beare et al. (1990).

2.6. **Statistical analysis of the data**

Plots in shade or damaged by rabbits were removed from the analysis. Statistical treatment of the data involved testing homogeneity of variances for non-significance, two-way analysis of variance (ANOVA) with Duncan’s multiple range test to separate means, \(a=0.05\). Where only two means were compared in a pre-planned comparison, a paired two-tailed Student’s \(t\)-test was used. In Experiment 2, a pre-planned orthogonal contrast compared parameters of the unmulched and slash/mulched systems with each other. In Experiment 3, pre-planned orthogonal contrasts compared parameters of the unmulched, slash/mulched and alleycrop mulch systems with each other: unmulched versus slash/mulch and alleycrop/mulch, slash/mulch versus alleycrop/mulch, *Gliricidia* mulch versus *Calliandra* and *Inga*, slash/mulch and *Gliricidia* versus *Calliandra* and *Inga*.

### Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Nodule biomass at 7 weeks (mg per plant)</th>
<th>Arbuscular mycorrhizae at 7 weeks (% root colonization)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unmulched</td>
<td>Slash/mulch</td>
</tr>
<tr>
<td>1986</td>
<td>8 a</td>
<td>13 a</td>
</tr>
<tr>
<td>1988</td>
<td>15 a</td>
<td>15 a</td>
</tr>
<tr>
<td>1992</td>
<td>7 b</td>
<td>29 a</td>
</tr>
</tbody>
</table>

*Numbers followed by the same letter are not significantly different according to Duncan’s multiple range test, \(a=0.05\). The orthogonal contrast tested is unmulched vs. slash/mulch.*

3. **Results**

3.1. **Nodulation and mycorrhizae**

In Experiments 1 and 2, the biomass of nodules per bean plant was greater on unfertilized bean roots in the slash/mulch plots than on bean roots in the unfertilized, unmulched in 2 of the 3 years (Table 2). However, in only 1 of those 2 years was the difference was significant, due to the variability in nodulation.

Different types and quantities of mulch vegetation affect bean nodulation. In Experiment 3, the nodulation of bean under the alleycrop/mulch of *Calliandra*, *Gliricidia* and *Inga* was significantly less than that of the fertilized, unmulched treatment at all three sampling times (Fig. 1). When comparing different alleycrop/mulches in orthogonal contrasts, nodulation is depressed in beans grown under *Calliandra* and *Inga* compared to *Gliricidia* and slash/mulch at both 3 and 5 weeks. In Experiment 1, percent colonization of mycorrhizae ranged from 94.6 to 97.7% and was not significantly different in the mulched versus unmulched treatments (Table 2).

3.2. **Plant diseases as a function of soil health**

Angular leaf spot incidence was low and not significantly different between treatments in Experiment 2 (\(F=0.84, P=0.62\)). Web blight disease incidence was low and not significantly different between treatments, except that *Mucuna* mulch resulted in significantly greater web blight than the slash/mulch beans (\(t\)-test, \(P=0.0172\)). Anthracnose affected leaves in the unmulched to a greater extent than slash/mulched treatments (see in pre-planned orthogonal contrast in Table 3), though not all mulch species affected dis-
Fig. 1. Bean nodule biomass in the unmulched without fertilizer (UM-0 and UM-325, respectively) slash/mulched (SM) and alleycropped systems at 3, 5 and 7 weeks after planting, Experiment 3, 1997. Bars with the same letter are not significantly different within the same sampling time according to Duncan’s multiple range test, α=0.05. Data from Woike and Rosemeyer (unpublished).

Table 3
Effect of system and fertilization on anthracnose, root rot with *Fusarium*-type symptoms and root rot with *Rhizoctonia*-type symptoms using relative scoring values (see text), Experiment 2, 1994–1995a

<table>
<thead>
<tr>
<th>System</th>
<th>10–30–10 fertilizer applied (kg/ha)</th>
<th>Yield in 1994 (kg/ha)</th>
<th>Anthracnose (<em>Colletotrichum</em>)</th>
<th>Root rot (<em>Fusarium</em>)</th>
<th>Root rot (<em>Rhizoctonia</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slash/mulch</td>
<td>0</td>
<td>430 c</td>
<td>3.6 bc</td>
<td>2.9 a</td>
<td>1.8 ab</td>
</tr>
<tr>
<td>Slash/mulch</td>
<td>108</td>
<td>1109 a</td>
<td>2.8 cd</td>
<td>3.3 a</td>
<td>1.9 ab</td>
</tr>
<tr>
<td>Slash/mulch</td>
<td>216</td>
<td>1326 a</td>
<td>2.8 cd</td>
<td>2.4 a</td>
<td>2.1 ab</td>
</tr>
<tr>
<td>Slash/mulch</td>
<td>325</td>
<td>1254 a</td>
<td>1.5 d</td>
<td>3.1 a</td>
<td>2.1 ab</td>
</tr>
<tr>
<td><em>Melanthera</em> mulch</td>
<td>108</td>
<td>730 b</td>
<td>4.1 bc</td>
<td>2.5 a</td>
<td>2.1 ab</td>
</tr>
<tr>
<td><em>Mucuna</em> mulch</td>
<td>108</td>
<td>736 b</td>
<td>2.2 d</td>
<td>3.1 a</td>
<td>2.4 a</td>
</tr>
<tr>
<td>Unmulched</td>
<td>0</td>
<td>173 d</td>
<td>5.6 a</td>
<td>4.1 a</td>
<td>1.2 c</td>
</tr>
<tr>
<td>Unmulched</td>
<td>325</td>
<td>807 b</td>
<td>4.9 ab</td>
<td>3.7 a</td>
<td>1.6 abc</td>
</tr>
</tbody>
</table>

| $F_{treatment}$ | 13.84***                         | 5.94***               | 1.41                           | 3.23*                 |
| $F_{orthogonal contrast}$ | 26.49***                        | 45.6***               | 7.40**                         | 6.70**                |

* Numbers followed by the same letter are not significantly different according to Duncan’s multiple range test, α=0.05. Orthogonal class comparisons compared parameters of the slash/mulch and unmulched with each other.

* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

ease incidence similarly. Mulch of only *Melanthera* produced significantly greater anthracnosis on bean foliage than mulch of *Mucuna* or the slash/mulch system with 325 kg 10–30–10 fertilizer applied/ha (Table 3). Fertilization increased disease incidence. *Fusarium* root rot disease incidence and severity was lower in slash/mulch and alleycropped systems than in unmulched systems (orthogonal contrast, $F=8.9, P=0.007$) (Fig. 2 and Table 3). However, damage due to *Rhizoctonia* root rot was higher in the slash/mulch and alleycrop system and with fertilizer there also appears to be an increase (Table 3). The effect of this pathogen on yield was not directly measured though observed yields were greater under the slash/mulch or alleycrop/mulch systems than the unmulched.
Fig. 2. Yield and hypocotyl lesion length due to *Fusarium*-type root rot of bean in the unmulched without fertilizer (UM-0 and UM-325, respectively) slash/mulched (SM) and alleycropped systems 7 weeks after planting, Experiment 3, 1997. Bars with the same letter are not significantly different according to Duncan’s multiple range test, $\alpha=0.05$.

3.3. Microbial respiration

The decomposing slash/mulch vegetation demonstrated five times greater microbial respiration than the litter or the soil (Fig. 3).

Fig. 3. Respiration of slash/mulch vegetation immediately after cutting, litter (decomposed mulch from soil surface) and soil performed in the laboratory. Bars with the same letter are not significantly different according to Duncan’s multiple range test, $\alpha=0.05$.

4. Discussion

4.1. Nodulation and mycorrhizae

The effect of mulch on beneficial plant-microbe associations, such as nodulation and mycorrhizae, has been hypothesized (Rosemeyer and Gliessman, 1992) and previous evidence of increased nodulation under mulch reported (Masefield, 1957). The hypothesis that bean grown in a slash/mulched system will nodulate more was supported in some dry years where mean nodulation was twice that of the unmulched and the data was less variable. Phosphorus fertilizer application to the unmulched plots also increased nodulation and the effect of P has been found by other researchers (Isreal, 1987). Different types and quantities of mulch vegetation affect bean nodulation, e.g. nodulation is depressed in beans grown under *Calliandra* and *Inga* compared to *Gliricidia* and slash/mulch at both 3 and 5 weeks. This is possibly because of the high quantities of N released from the decomposing vegetation in these two treatments (a hypothesis which is presently being tested). High quantities of applied N are known to depress nodulation (Sprent and Minchin, 1983). Though not significant, with more sampling we may see the depressive effect decrease over time in *Calliandra* but not *Inga*, which correlates with *Calliandra*’s faster rate of decomposition (Kettler, 1997b). Since
mycorrhizal fungus colonization rates were near 100% of available root, the positive effect of mulch application may not be detectable. Mulches of Inga result in the highest bean yield, which supports the idea that the system is healthy even if there is lower levels of symbioses due to nutrient depression. In summary, the results show that these symbioses may be depressed by high nutrient levels, either exogenous, in the case of inorganic fertilizers, or endogenous, in the case of high quantities or types of mulch. For these reasons as well as differences in weather conditions, our hypothesis that mulch favors mycorrhizae and nodulation as contributors to soil health was not well-supported, but may be important under specific environmental conditions.

4.2. Plant diseases as a function of soil health

Foliar diseases of angular leaf spot and web blight were not favored by either system, probably because disease pressure was low. However, Mucuna mulch on beans resulted in significantly greater web blight than the slash/mulch beans perhaps because Mucuna itself may be a host of Rhizoctonia, though lesions of this fungus were not diagnosed on Mucuna in our fields. Web blight incidence was surprisingly low considering the high root rot incidence (see below). Previous studies have shown that the physical barrier of the mulch prevents the splashing of soil-borne sclerotia and thick walled hyphae (Galindo et al., 1983). Additionally, microbial activity of the mulch might suppress or inhibit the raindrop-splashed inoculum from reaching the leaves.

Anthracnose affected leaves in the unmulched to a greater extent than slash/mulched treatments, though not all mulch species affected disease incidence similarly. Mulch of only Melanthera produced significantly greater anthracnosis on bean foliage than mulch of Mucuna or the slash/mulch system where 325 kg 10–30–10 fertilizer was applied/ha, and fertilization increased disease incidence. Spores (conidia) are disseminated by seed, wind and splashing rain from plant residues (Pastor-Corrales and Tu, 1989). Since the mulch vegetation is not of bean plant residues, it may act like a physical barrier to the soil. Anthracnose is the most serious foliar disease of beans in the world (Pastor-Corrales and Tu, 1989) and the most serious foliar disease of beans at mid-elevation tropics where this study is situated.

Fusarium root rot disease incidence and severity was lower in slash/mulch and alleycropped systems than in unmulched systems. Fusarium chlamydospores in soil or infected plant residue, are stimulated to germinate by nearby bean seed or root exudates (Abawi, 1989). Since the bean seed germinates in the mulch (which does not usually include bean plant residue) and a range of 77–89% of the root is on the mulch at 7 weeks in these treatments (Woike and Rosemeyer, unpublished), the bean plant in this system may essentially be avoiding the source of inoculum by proliferating in the decomposing mulch layer. It may be more appropriate to think about a ‘system health’ as opposed to ‘soil health’, since these plants exhibit less disease symptoms because most of their roots are not located in the soil!

However, damage due to Rhizoctonia root rot was higher in the slash/mulch and alleycrop system and increased with fertilizer application. The effect of this pathogen on yield was not directly measured though observed yields were greater under the slash/mulch or alleycrop/mulch systems than the unmulched. Manning et al. (1967) found that deep planting of beans in soil favors Rhizoctonia infection, so hypocotyl elongation may expose a greater length of tissue to the pathogen. Hypocotyl length and thus contact with mulch was significantly greater in the slash/mulch or alleycrop/mulch than in the unmulched bean plants. In general, increased incidence of Rhizoctonia has been associated with no-till beans (Abawi and Pastor-Corrales, 1990) and no-till systems in general (Abawi and Thurston, 1994; Pankhurst, 1994), probably due to greater contact between the bean hypocotyl and infected residues from previous crops and weeds. Since Rhizoctonia solani is worldwide and found in uncultivated soils, weeds and native vegetation may be involved (Baker and Martinson, 1970).

We should also note the effects of certain types of vegetation on both mutualistic and pathogenic effects of microflora. Mulch of various types affected disease incidence differently, e.g. Melanthera mulch resulted in significantly more anthracnose than Mucuna or mixed species mulch. Inga and Calliandra depressed nodulation more than Gliricidia and the slash/mulch. Although there are specific differences that may distinguish one type of mulch from another, mulch and alleycropping systems commonly suppress plant...
pathogens. In this sense, we can say that mulching contributes to soil health.

The hypothesis that mulch systems prevent disease is generally supported, but not in with all diseases. Some authors consider disease incidence and severity to be possibly of questionable value as indicators of soil health (Hornby and Bateman, 1997), because of the varying effect of what might be considered healthy soil practices on diseases, e.g. in what would be considered a ‘healthy’ management in our experiments some diseases are increased (Rhizoctonia). However, what farmer would not consider the absence of plant disease as an indicator of soil health? Perhaps the definitive practical bioindicator of soil health is the absence of soil pathogens and perhaps certain root pathogens, excluding those widespread and found in uncultivated soil. Considering the general lack of knowledge about what constitutes as healthy microbiota, using plant health as proxy may be useful.

4.3. Microbial respiration

High microbial activity associated with plant decomposition may provide a barrier of actively metabolizing microbes and associated soil fauna which may be antagonistic to the spores of a fungal plant pathogen. The mulch barrier may be a protagonist of plant health not only because it is a physical barrier to plant pathogens and it is an important site of decomposition and nutrient cycling, but because it may also be a source of general microbial activity which can prevent the establishment of any one pathogenic microorganism.

5. Conclusion

Waksman (1927) wrote that microbial parameters are not good indicators of soil fertility (soil health) because they vary with climate, soil type, crop and soil management. When looking to find a formula that indicates soil health across many environments, this may be correct, but when comparing management strategies in one ecoclimatic zone, it may be precisely because of the sensitivity of microflora to crop and soil management that microflora can indicate soil health or better yet, system health.

In an absolute sense if we want to know if a management system is functioning at its optimum ‘healthy’ level, it can be instructive to use the ecological standard provided by a dynamic, natural system under a similar nearby environmental condition. The natural system may be useful for comparative purposes and as a standard of soil health, allowing us to avoid the circularity of defining a system as healthy and then examining it characteristics. Although some natural systems would not be considered healthy due to natural or anthropogenic disturbance, many are, and exploring their belowground workings may help us to understand the microbial contribution to soil health, and to develop a flexible set of indicators that can be applied to evaluate management strategies for soil and agroecosystem health.

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