Dynamics of soil fungal and bacterial biomass in a temperate climate alley cropping system

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Abstract

Soil bacterial and fungal dynamics were measured in an alley cropping system using direct microscopy techniques. The alley cropping system involved hedgerows of alder trees (Alnus rubra) and sweet corn (Zea mays) grown in the alleys. Trees were periodically coppiced and prunings were incorporated into the soil as green manure. Active fungal and bacterial biomass were greatest in tree rows and declined with distance from the trees. Active fungal biomass was greatest at the first year July sampling, ranging from 44 \( \text{mg} \, \text{g dry soil}^{-1} \) in the tree row to 22 \( \text{mg} \, \text{g dry soil}^{-1} \) in the middle of the alley. Bacterial biomass in all sampling locations peaked during May before coppicing of trees and cultivation of the alley. Bacterial and fungal biomass in the middle of the alley were similar to sweet corn monocropping plots throughout the growing season. The results suggested that the relatively low pruning biomass that can be produced and incorporated into the soil of temperate climate alley cropping systems compared to tropical alley cropping systems has little effect on microbial biomass. However, additional green manure in the form of leaf fall, additional below ground substrate from tree roots, and favorable conditions in untilled tree rows contribute to higher soil fungal and bacterial counts in and near the tree rows. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Soil fungi; Soil bacteria; Green manure; Tree prunings; Alnus

1. Introduction

Alley cropping systems represent hybrids of agricultural and natural ecosystems. In these cropping systems, annual crops are grown in alleys between hedgerows of trees. Trees are coppiced periodically and prunings are incorporated into the soil as green manure. Alley cropping systems are designed to integrate the soil enriching process of forest ecosystems into agricultural production systems (Nair, 1984; Kang et al., 1985). A primary objective is to create efficient nutrient cycles in which nutrients from prunings and other plant litter are effectively recycled into new plant biomass. The microbial biomass plays a central role in this recycling process (Paul and Clark, 1989). Microbes are responsible for the biochemical degradation of the organic litter and convert nutrients from organic to plant available, mineralized forms (Coleman and Crossley, 1996). More than 90% of all nutrients pass through the microbial biomass to higher trophic levels (Kennedy, 1995).

The composition of the microbial community influences the transformation of plant residues into soil
organic matter and plant available nutrients (Beare et al., 1993; De Ruiter et al., 1993). Therefore, separate assessment of the decomposer groups is essential to understand soil microbial dynamics. Also necessary are methods that determine whether fungi and bacteria (the two dominant decomposer groups) are functional. Failing to distinguish between active and inactive microbial biomass may lead to erroneous conclusions regarding nutrient cycling processes (Hunt et al., 1987). An assessment of active biomass is also important when considering long-term development and sustainability of an ecosystem (Klein et al., 1995).

Various methods are available to study the active portion of the soil microbial biomass. Substrate-induced respiration (SRI) measures temporal biomass changes and activity as affected by management (Horwath and Paul, 1994). However, SIR measures only the activity of microbes that respond to additions of glucose and fails to account for the whole functional soil microbial community. Enzyme activity also has been correlated with active microbial biomass. However, Kuperman and Carreiro (1997) point out that enzymes, due to their potential for persisting in the soil, reflect both the activity of microbes that grew in the past and those when sampled. Both enzyme activity and SRI fail to distinguish between the main decomposer groups. In contrast, microscopic methods offer opportunities to determine the composition of the microbial biomass and an assessment of its activity.

The composition of the microbial biomass is influenced by quantity and quality of organic matter input (Killham, 1994). Both vary considerably between tree rows and alleys of an alley cropping system. The major components of organic matter input to the soil in alleys are crop residues and tree prunings. In tree rows, leaf litter and prunings are added to the soil. Substantial organic matter input also is derived from below-ground sources such as root exudates, sloughed nodules (when nodulated N-fixing trees are grown), and decomposing roots (Brussaard et al., 1993). Different management of organic matter inputs also influences the microbial composition (Neher, 1995). Leaving plant residue on the surface (i.e., prunings and leaf litter in tree rows) selects for fungi while incorporating prunings and crop residue into the soil of the alleys will shift the balance towards bacteria (Hendrix et al., 1986; Holland and Coleman, 1987).

The objective of our study was to determine the influence of alley cropping on the dynamics of soil bacteria and fungi. Because of the green manure additions, we expected to find greater microbial biomass in alley cropping systems compared to monocrop systems. We also expected to find variations in microbial biomass and composition in different locations of the alley cropping system due to differences in organic matter input and management. We hypothesized that if trees provide microbial substrate in the form of additional green manure and labile, nitrogen rich compounds from root litter, then microbial activity will be higher in soil close to the trees compared to soil away from the trees. In addition, we hypothesized that differences in organic matter input and management will shift the microbial composition toward bacteria in the alleys and fungi in the tree row.

2. Materials and methods

2.1. Study site and field operations

The study was conducted at the Oregon State University Horticultural Research Farm in Corvallis on a Chehalis clay loam soil. Low precipitation during the growing season requires regular irrigation of row crops, which in our study was supplied by overhead sprinklers. In April 1991, one-year-old red alder (Alnus rubra) seedlings were planted in plots measuring 9 by 4.5 m (Fig. 1). From 1991 to 1995, sweet corn (Zea mays var. “Jubilee”) was planted in alley cropping plots and monocropping plots. The experimental design involved a randomized block design, replicated in three blocks.

Corn seedbed preparation in the alleys involved chisel plowing and rotary hoeing. Each year, all plots were fertilized with 170 kg N, 130 kg P, 100 kg K ha$^{-1}$. Nitrogen was split into two applications. The first application was broadcast at planting in ammonium form; the second was side dressed as urea, 6 weeks after planting. No pesticides were applied. Weeds were controlled by hand and mechanical cultivation. After corn harvest, corn stalks were mowed with a flail mower and left on the soil surface until next year’s seedbed preparation.

Trees were cut 30 cm above ground level and prunings were shredded with a tree shredder shortly before corn was seeded during the first two weeks of
June (i.e., first coppicing). Shredded prunings were incorporated into the top 10 cm of the soil with a rotary hoe during seedbed preparation. A second coppicing was performed six weeks after planting when the corn reached a height of 30 cm. These prunings were not shredded and left in the tree row on the soil surface.

2.2. Soil sampling and analysis

Soil microbial biomass in the alley cropping system were measured six times during the experiment: before seedbed preparation in May 1994 and 1995, in the middle of the growing season in July 1994 and 1995, and after the growing season in October 1993 and 1995. Three replicate 2 cm diameter, 5 cm deep soil cores were collected at three distances from the trees: in the tree row; 50 cm from the trees (inside alley); and middle alley samples (M) were collected at a distance of 1.5 m from trees.

Soil samples were collected before tree planting in May 1991 to establish baseline values of soil chemical properties. Four years later, in May 1995, soil in the alley cropping and monocropping plots was sampled from three soil depths: 0–15, 15–30, and 30–45 cm. In alley cropping systems, soil was collected 50 cm (inside alley) and 150 cm from the trees (middle alley). Each sample represented composites of 15 thoroughly mixed sub-samples. Samples were air dried for 72 h at 80°C. Soil samples were analyzed for soil organic matter (SOM) (Nelson and Sommers, 1982) and total Kjeldahl nitrogen (Isaac and Johnson, 1976).

3. Results

3.1. Fungal biomass

Active fungal and active bacterial biomass were determined by measuring the length and width of fluorescein diacetat (FDA) stained hyphae and counting the number of FDA-stained bacteria, respectively, using epi-fluorescent microscopy. Total fungal biomass was determined using DIC microscopy by measuring the length and width of all hyphae in a known volume of soil (Ingham and Klein, 1984). Total bacterial biomass was determined by counting the numbers and measuring diameters of all bacteria in soil suspension stained with FITC and filtered on Nuclepore black-stained filters (Babiuk and Paul, 1970). Microbial biomass was determined by converting biovolume to biomass using standard factors recommended by Van Veen and Paul (1979).

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approximately 12-fold in the tree row and fourfold in the alley. In 1995, seasonal trends differed between sampling locations. Fungal biomass increased in the tree row between May and July and decreased in October. In the alley, we observed a decrease from May through July and October. Seasonal differences in the two alley locations were insignificant, except for the decrease from July to October in the middle of the alley.

Seasonal changes of total fungal biomass mirrored those in active fungal biomass, both peaking in July. In 1995, total fungal biomass in the tree row remained high from July through October but decreased significantly in the alley. Comparisons between the same dates of the two sampling periods show that fungal biomass was similar in July 1994 and 1995, but was higher in May and October of the second sampling period.

3.2. Bacterial biomass

Mean active bacterial biomass was highest in the tree rows, except in May 1995 (Fig. 3(a)). Differences between tree rows and alleys were statistically significant (p=0.05) only in the last two sampling dates. Seasonal trends of active bacterial biomass were inconsistent across sampling locations and years. For example, in 1994, we found no significant changes from May to July. In 1995, however, we observed seasonal variations that differed between sampling locations. Active fungal biomass in the tree row increased from May to July and decreased in October. In the alley, on the other hand, the biomass decreased from May through July and October.

In 1995, we observed similar total bacterial biomass in all sampling locations (Fig. 3(b)). The variations on earlier sampling dates did not follow a particular pattern. Seasonal trends of total bacterial biomass were different from those of fungal biomass. Bacterial biomass was greatest in May and decreased through July and October during both sampling periods while total fungal biomass was usually highest in July. We also observed a year to year difference in bacterial biomass. Biomass in 1994 was significantly higher than that in 1995.

3.3. Ratio of fungi to bacteria

We observed significant differences in the ratio of total fungi to bacteria between the sampling locations on two sampling dates (Fig. 4). In July 1994 and October 1995, this ratio was more than three times larger in the tree row than in the alley. Seasonal trends in the ratio of fungal to bacterial biomass generally...
were characterized by an increase from May to July followed by a decrease in October. However, it remained high in the tree row during October 1995 while it dropped significantly in the alley.

The ratio of total fungi to bacteria in the tree row and on the inside of the alley was higher in 1995 compared to the same months in 1993 and 1994. However, in the middle of the alley, the values in October 1993 and 1995 were similar and had decreased to below one.

3.4. Comparison between alley cropping and monocropping systems

We found higher active and total fungal, and active bacterial biomass in the tree row of the alley cropping system compared to the monocropping system. However, when we compared the soil in the monocropping system with the soil in the alley cropping area that received the shredded prunings but was treated the same otherwise (i.e., sampling location “middle alley”), bacterial and fungal biomass was similar throughout the growing season.

We also compared soil chemical properties in the cropping area of both cropping systems. Differences were observed in the top 15 cm of the soil but not in the soil layers below (Table 1). We observed higher organic matter content and Kjedahl-N in the top soil of the alley cropping locations. However, organic matter content in the monocropping and all locations of the alley cropping system were below the baseline values of samples taken in 1991, before the first season of alley cropping (Table 2).

4. Discussion

We attribute higher active fungal biomass in and near the tree row to increased substrate availability. In the middle of the alley, prunings of the first coppicing constituted the only green manure input. In contrast, in
the tree row and the inside of the alley, green manure originated from prunings of two coppicings and leaf fall at the end of the growing season. The additional green manure was not incorporated but stayed on the soil surface near the trees. More than other decomposers, fungi are able to use plant residues that are deposited on the soil surface because of their ability to grow across air filled space (Killham, 1994; Neher, 1995). The increased fungal biomass in the tree row and the inside alley during the July and October samplings reflects the use of the additional organic material as a fungal substrate. Surface tree litter near the trees may have contributed indirectly to the increased microbial activity because it provided substrate for the soil fauna. We observed that tree leaves appeared to be a favorite food source for earthworms (*Lumbricus terrestris*) on our study site. Several weeks after the trees had shed their leaves, most tree litter had been pulled into earthworm channels (data not shown). Similar to Hauser (1992), we observed significantly more earthworm casts in the vicinity of the hedgerows where earthworms provide physical habitat and nutrient-rich substrate for soil bacteria and fungi.

No significant differences in microbial biomass between locations were observed at the May sampling dates. Various management factors contributed to this observation. For example, each year, 2 t ha\(^{-1}\) of corn residue was left shredded on the soil surface of the alley during the winter fallow. This residue provided ample substrate for microbes in the alley, effectively eliminating any differences in substrate availability between locations in the field at the May sampling date. Cultivation in the alley (but not the tree row) after the May sampling most likely amplified differences in

Table 1
Soil organic matter (SOM) and soil nitrogen (Kjedahl-N) in alley cropping and monocropping sampling locations

<table>
<thead>
<tr>
<th>Year</th>
<th>Soil depth (cm)</th>
<th>Sampling location</th>
<th>% SOM</th>
<th>% Soil nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>0–15</td>
<td>Inside alley</td>
<td>2.41a</td>
<td>0.150a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle alley</td>
<td>2.37a</td>
<td>0.142ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monocrop</td>
<td>2.25b</td>
<td>0.137b</td>
</tr>
<tr>
<td></td>
<td>15–30</td>
<td>Inside alley</td>
<td>2.15b</td>
<td>0.132a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle alley</td>
<td>2.13b</td>
<td>0.132a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monocrop</td>
<td>2.05b</td>
<td>0.129a</td>
</tr>
<tr>
<td></td>
<td>30–45</td>
<td>Inside alley</td>
<td>2.22a</td>
<td>0.141a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle alley</td>
<td>2.07a</td>
<td>0.132a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monocrop</td>
<td>2.04a</td>
<td>0.132a</td>
</tr>
<tr>
<td>1991</td>
<td>0–15</td>
<td>All</td>
<td>2.57</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>15–30</td>
<td></td>
<td>2.52</td>
<td>0.134</td>
</tr>
<tr>
<td></td>
<td>30–45</td>
<td></td>
<td>2.54</td>
<td>0.136</td>
</tr>
</tbody>
</table>

1991 measurements represent baseline values before alley cropping was practiced. 1995 measurement were taken in May, before the fourth year of sweet corn alley cropping. “Inside alley” and “middle alley” indicate sampling locations 50 cm and 150 m from trees, respectively. Values are means of three measurements each composed of 15 sub-samples. Different letters denote statistically significant differences between sampling locations within the indicated soil depth at a \(p\)-value of 0.05.

Table 2
Above-ground organic dry matter (DM), carbon (C) and nitrogen (N) added to the soil in the alley cropping system (values are means of three samples)

<table>
<thead>
<tr>
<th>Year</th>
<th>Above-ground organic matter input</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prunings 1st coppice</td>
</tr>
<tr>
<td></td>
<td>DM (t/ha)</td>
</tr>
<tr>
<td>1993</td>
<td>0.74</td>
</tr>
<tr>
<td>1994</td>
<td>0.85</td>
</tr>
<tr>
<td>1995</td>
<td>0.95</td>
</tr>
</tbody>
</table>
microbial biomass at later dates. Numerous studies showed that cultivation is detrimental especially to the growth of fungi because fungi strands are easily broken during break-up of soil particles and recover only slowly from tillage disturbance (Hendrix et al., 1986; Holland and Coleman, 1987; Andrén et al., 1990; Kennedy, 1995). A third management factor that most likely amplified differences at the later sampling dates was coppicing (carried out after the May sampling dates). Coppicing of alley cropping trees increases the speed of fine root turnover (Fernandes, 1991) and the exudation of nitrogen rich root compounds (Sanginga et al., 1990). In a separate alley cropping study in adjacent field plots, we found that *Alnus sinuta* trees labeled with $^{15}$N released a substantial amount of nitrogen from roots into the soil. Soil microbe mediated transfer of $^{15}$N to corn plants growing at a distance of 0.63 cm occurred within six weeks after coppicing (Seiter et al., 1995). Therefore, additional substrate was provided to the fungi and bacteria in the tree row and inside alley location.

Lack of moisture caused low fungal and bacterial biomass in October of the first year. Because of the warm growing season, the corn was harvested early (September 8) and less than 12 mm of rainfall fell between the time when irrigation was turned off and microbial biomass sampling on October 15 (climate data not shown). In 1995, the harvest occurred later (September 19) extending the irrigation into mid-September. In addition, more rain fell prior to the soil sampling, creating favorable soil conditions and a less dramatic drop of bacterial biomass. Under the present climate conditions, a peak of microbial growth usually occurs in spring, explaining the high values of bacterial biomass in the May samplings. During this time, rapid plant growth of the native alley vegetation (i.e. weeds during winter fallow) and trees is accompanied by high root activity, which provides the necessary substrate for the microbial development (Lynch and Painting, 1980; Patra et al., 1995). Bacterial biomass remained high from May to July. The high July values in the alleys may actually present a second peak during which bacteria, after being reduced during seedbed preparation, responded to favorable conditions provided by rapid corn root growth coupled with ample soil moisture from irrigation.

Soil bacterial and fungal biomass in the middle of the alley were similar to soil in monocropping systems suggesting that a yearly organic matter input of less than 1 t ha$^{-1}$ t to the soil of the alley was too small to significantly affect fungal and bacterial biomass. Significant changes of microbial biomass were found in alley cropping systems in tropical regions where pruning yield was much greater than that in our study. For example, Van der Mersch et al. (1993) found consistently higher microbial biomass in alley cropping systems than in a monocropping control after adding 21.7 t ha$^{-1}$ of *Leucaena leucocephala* and 15.23 t ha$^{-1}$ of *Senna siamea* prunings in one year. Similarly, Kachaka et al. (1993) found significantly higher microbial biomass C in soil that was amended with alley cropping prunings compared to controls. In other studies with high pruning inputs, however, results were less conclusive. For example, Haggar et al. (1993) added 7.9 t ha$^{-1}$ of *Erythrina* and 11.4 t ha$^{-1}$ yr$^{-1}$ of *Gliricidia* prunings in an alley cropping system in Costa Rica. They observed that microbial N was 30% higher in alley crop treatments than the monocrop only at 105 days after the application of prunings but not at earlier or later sampling dates. Mazzarino et al. (1993) found no effect of pruning additions on microbial C and N in one season unless carbon inputs in the form of prunings and crop residues were more than 400% higher than in a control.

5. Conclusion

Management of soil microbes plays a key role in the effort to synchronize green manure nutrient release and crop nutrient uptake. This effort remains challenging because soil microbial dynamics are the result of many interacting factors. Our study showed that quality and quantity of plant residue interact with management activities such as coppicing and tillage. Below-ground organic matter sources also appeared to play a significant role in microbial dynamics. Other interactions were evident in seasonal changes when, for example, climate conditions favored microbial growth but management activities limited substrate availability. Closer spaced sampling intervals would have provided a better insight into these interactions since microbial biomass and composition react quickly to changing conditions.
Differences in the response of fungi and bacteria to the alley cropping treatment demonstrated the need to distinguish between these two decomposer groups. Neher (1995) suggested that inclusion of perennial crops in agricultural systems shifts the soil foodweb more toward a natural ecosystem characterized by a higher fungi to bacterial ratio. In the uncropped area of the alley cropping system, we were able to confirm this hypothesis. However, since trends in the cropped area were inconsistent, we concluded from our study that the amount of incorporated prunings is critical. Alley cropping systems are not shifted toward a fungal dominated ecosystem when pruning biomass is small. The effects of larger green manure quantities on multiple trophic food web levels need to be examined to draw further reaching conclusions.

References


