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EFFECTS OF GROUNDCOVER MANAGEMENT PRACTICES IN A FRASER FIR (*ABIES FRASERI*)-COVER CROP INTERCROPPING SYSTEM ON SOIL MICROBIAL BIOMASS AND COMMUNITY CATABOLIC DIVERSITY

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Abstract: Soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N) as well as microbial community-level physiological profiling (CLPP) were investigated in an intercropping system involving Fraser fir, two leguminous (Dutch white clover and alfalfa) and a non-leguminous (perennial rye grass) cover crops. For each cover crop, two competition-management practices, banding and no banding, were evaluated. Conventionally-managed plots were used as controls. Soil microbial biomass was assessed at the 0-15, 15-30 and 30-35 cm soil depths and CLPP at the 0-15 cm soil depth. Cover cropping had limited early effects on soil organic carbon. However, significant increase of total soil nitrogen at the surface soil layer was observed. The leguminous cover crops with banding yielded higher SMB-C and SMB-N than the non legume. SMB-C and SMB-N significantly decreased with soil depth. Plots managed with bands averaged 559 mg SMB-C kg^{-1} dry soil, and plots without bands averaged 536 mg C kg^{-1} dry soil. For SMB-N, plots managed with bands averaged 83 mg N kg^{-1} dry soil, while plots without bands averaged 79 mg N kg^{-1} dry soil. Leguminous cover crops significantly improved microbial community diversity compared to the controls. Multivariate analysis showed that the microbial communities in plots with cover crops had a catabolic potential that differed from that of control, with the communities from the leguminous cover crops with bandings exhibiting the strongest dissimilarity. These results suggest that cover cropping with proper management can provide a good environment for microbial development and be an alternative approach to sustainable tree production.

Key Words: low-input system, soil fertility, sustainability, plantation, agroforestry

INTRODUCTION

In recent years, there has been increasing interest in the use of management practices that maintain soil productivity and environmental quality while improving farm profitability (Baributsa 2006). Intercropping legume and/or non-legume cover crops is a practice that has been widely investigated as a practical way to achieve sustainable production (Walsh et al. 1996). Cover crops not only reduce soil erosion but also add organic matter to the soil, conserve soil humus, improve soil aeration and structure, and improve soil nutrient status. Because of

these benefits, intercropping cover crops with cereal crops (Sainju and Singh 1997, Macdonald et al. 2005) and orchard trees (Sanchez et al. 2007) is increasingly becoming a common practice.
In low-input production systems, soil management generally involves the use of mowed, tilled or killed cover crops, animal manures, composts and the application of organic fertilizers to increase soil organic matter levels and steadily release available nutrients to the trees as the organic matter breaks down (Sanchez et al. 2007). In this process, the action of soil organisms is a major determinant of nutrient cycling rates and plant growth. Planting either cereal or legume cover crops in the interspaces of plantations increases plant residue inputs to soils (Dinesh et al. 2004) and therefore may stimulate soil microbial activity and increase mineralizable C and N (Mendes et al. 1999). It is also well documented that farming systems and management practices greatly influence microbial populations and activities in soil (Bossio et al.1998).

Soil microbial biomass and activity respond rapidly to changes in agronomic practices and other disturbances (Lundquist et al. 1999), and have been used to ascertain early changes in soil fertility due to different soil management practices (Doran and Zeiss 2000, Wang and Wang 2008). In fact, changes in microbial biomass and diversity are often used as rapid indicators of changes in soil organic matter content and soil fertility level.

The effects of growing leguminous and non-leguminous cover crops and incorporating the residues into the soil after tilling or killing them have been extensively studied for several agronomic crops. However, very little information is available concerning microbial biomass and diversity in Fraser fir (*Abies fraseri* Push [Choir]) production systems where the green manure cover crops are not only intercropped but regularly mowed, thus leaving the plant residues on the soil surface. Such information could be potentially useful to Christmas tree growers who conventionally use high amounts of commercial nitrogen fertilizers and herbicides for soil fertility management and weed control. The goal of this study was to examine the effects of various cover crop management practices on soil characteristics and microbial properties in Fraser fir production systems. We hypothesized that the overall site chemical and biological conditions; particularly soil organic carbon, total soil nitrogen, microbial biomass and microbial catabolic diversity would be increased in the cover crop managed plots compared to the conventional system.

MATERIALS AND METHODS

Research site and plant materials

A field experiment was established at the Tree Research Center (TRC) (42.67ºN, 84.46ºW) on the campus of Michigan State University in East Lansing, Michigan. The local climate is mild during summer with an average temperature of 15.5 °C and cold during winter with an average temperature of -6.6 °C. Annual average precipitation is 853 mm with rainfall distributed fairly evenly throughout the year. The experiment was located in a fenced area to limit the impact of deer browsing, attracted by the leguminous cover crops. Soil at this site is classified (FAO) as a fine-loamy, mixed, active, mesic Aquic Glossudalf (Rothstein 2005). The soil type is moderately well drained, with high available water capacity and medium surface runoff.

Experimental design

The field was 32.92×51.21m (108×168 ft) in size, and the experiment was established as a randomized complete block design with three replications. Three cover crops were selected for this trial: two legumes (Dutch white clover [*Trifolium repens*] and alfalfa [*Medicago sativa*]), and one grass (perennial rye grass [*Lolium perenne*]). Two ground management types, no bands (NB) and bands (B) were assigned to each cover crop species. The band treatments consisted of intercropping the cover crop between the tree rows while maintaining a clear band of 60 cm centered on the tree row (Fig. 1-a). The no band treatments consisted of growing each cover crop in a continuous patch in the plot (Fig. 1-b). Plots managed conventionally (CONV), with no cover crop and weeds completely controlled with glyphosate (active ingredient concentration =1.1kg ha^{-1}) were included as control (Figure 1-c). Thus, the agronomic treatments were as follows: Dutch white clover with bands (DWCB), Dutch white clover with no bands (DWCNB), alfalfa with bands (ALFB), alfalfa with no bands (ALFNB), perennial rye grass with bands (PRGB) and perennial rye grass with no bands (PRGNB). Trees were established at 1.8 x 1.8 m spacing, and each plot contained 7 x 5 trees for a total of 35 trees per plot.

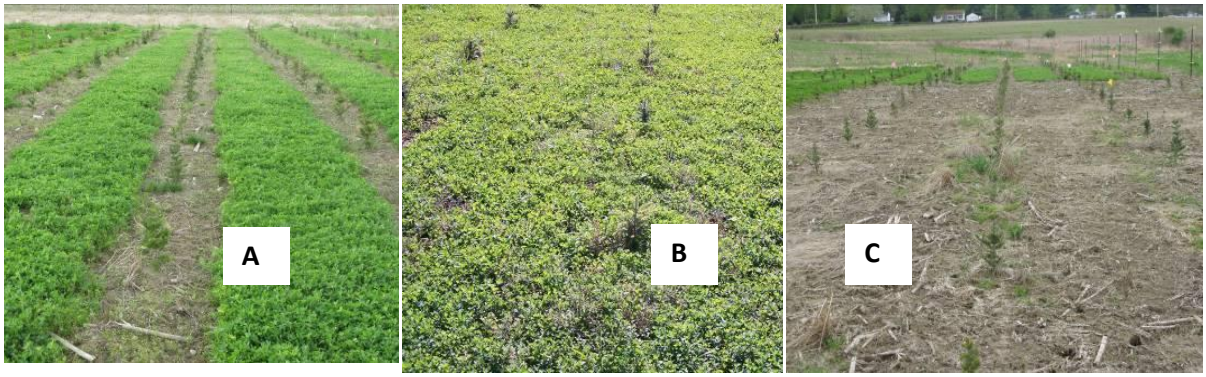

Figure 1: Banding (A), no banding (B) and conventional (C) plots in our cover crop plots

Fraser fir transplants (plugs+2) were obtained from a local commercial nursery, and machine planted with a Whitfield planter, into a chisel plowed and dragged field soil on May 8, 2007. Plants in border rows were used as a buffer and not included in measurements, therefore restricting data collection to the area of the remaining 15 interior trees in each plot. Seeds of common Dutch white clover, alfalfa (SS 100 brand) and perennial rye (VNS), purchased from Michigan State Seeds (Grand Ledge, Michigan) were hand-seeded, either to the entire plot or between the tree rows leaving bands along the tree rows, at a rate of 28 kg ha^{-1} for clover and alfalfa and 13 kg ha^{-1} for rye. Once the cover crops were fully established, mechanical mowing was performed every three weeks (3 inches above the ground) to control cover crop growth, minimize the competition with the trees, and add green manure to the soil. Glyphosate was sprayed twice (June 5 and July 26, 2007) during the growing season to control weed in the conventional (control) plots and the banded treatments.

Soil sampling and soil chemical characteristics

Fifteen randomly-selected soil sub-samples per plot were collected with a 2.5 cm diameter auger and composited. Soil samples were collected in mid-October 2007, corresponding to the end of

the growing season. In the cover crop plots with bands, nine (9) cores of soil were collected within the cover crop zone and six (6) cores within the bare ground zone, proportional to the area of each banding zone. The sampling depths were 0-15, 15-30, and 30-45 cm. Total C and N of soil samples were determined by combustion with an elemental analyzer (Model ECS 4010, COSTECH Analytical, Valencia, CA).

Microbial biomass analysis

Soil microbial biomass C was estimated by extracting 10 g oven-dry equivalents of field moist mineral soil samples in 0.5 M K_2SO_4 (1:4 w/v), by the chloroform fumigation-extraction method described by Brookes et al. (1985). Total dissolved C and N were determined by oxidative combustion-infrared analysis and oxidative combustion-chemiluminescence, respectively (Shimadzu Corp., Kyoto, Japan). Microbial biomass C and N were calculated as the difference between C and N in the fumigated and non-fumigated samples using 0.45 as a correction factor for SMB-C and 0.54 as a correction factor for SMB-N.

Microbial community-level physiological profile analysis

The functional diversity of the soil microbial community was measured using a BIOLOG^{TM} system. Substrate-utilization patterns of the soil microbial population were determined using BIOLOG^{TM} ECO-plates (BIOLOG^{TM}, California) by a procedure adapted from Garland and Mills (1991). The absorbance of wells at 590 nm was measured over a 10-day period, and absorbance of the tetrazolium dye reactions in each well recorded at 0, 24, 96, 120, 168, and 240 h using an automated plate reader. Microbial activity in each micro-plate, expressed as average well color development (AWCD), was calculated for each sample at each time point by dividing the sum of the optical density data by 31 (number of substrates). We used an OD of 0.25 as the threshold for a positive response (Garland 1997) to calculate richness (R), or the total number of oxidized C substrates, a Shannon-Weaver index (*H'*) of metabolic diversity and evenness of response (E), at 120 h since it was the shortest incubation time that allowed the best resolution among treatments.

Data analysis

Data were analyzed as a randomized complete block design (RCBD) using Proc Mixed in Statistical Software Package SAS version 9.1 (SAS 2002-2003). We used Fisher's Least Significant Difference Test to make pair-wise comparisons of individual treatment means. Significance for the overall treatment effects and pair-wise comparisons was accepted at $\alpha = 0.05$. The AWCD, R, H' and E data were subjected to a one-way ANOVA in SAS. The standardized OD values obtained from each of the 31 substrates for each treatment were further analyzed using multivariate techniques (principal component analysis and cluster variable analysis for similarity) to differentiate among microbial communities based on substrate utilization profiles.

RESULTS AND DISCUSSION

Soil organic carbon and nitrogen

Although the control had the lowest mean soil total C concentration, it was not significantly different ($P > 0.05$) from any of the other treatment means (Table1). No specific trend was observed between treatments at the 15-30 cm and 30-45cm depths. There was a significant decrease in soil total carbon with soil depth ($P<0.001$), certainly due to decreases in plant-derived in deeper soil layers. Total soil N was significantly higher in soils under cover crop treatments compared to conventional control plots at the 0-15 cm depth. Similar to organic carbon, there was no statistical difference of organic N in deeper soil core specimens. The C:N ratio values followed the same trend, generally decreasing significantly with soil depth ($P<0.001$), without any statistical difference among treatments at each depth ($P>0.05$).

Table 1: Soil total carbon, total nitrogen and C:N ratio as influenced by groundcover management

Groundcover management	Total C (%)			Total N (%)			C:N ratio		
	0-15cm	*15-30cm*	*30-45cm*	*0-15cm*	*15-30cm*	*30-45cm*	*0-15cm*	*15-30cm*	*30-45cm*
ALFB	2.07 [a]	1.51 [a]	0.58 [a]	0.18 [b]	0.14 [a]	0.06 [a]	12.26 [a]	10.92 [a]	9.96 [a]
ALFNB	2.23 [a]	1.62 [a]	0.71 [a]	0.19 [b]	0.14 [a]	0.07 [a]	12.06 [a]	11.44 [a]	10.52 [a]
CONV	1.99 [a]	1.58 [a]	0.56 [a]	0.16 [a]	0.14 [a]	0.05 [a]	12.49 [a]	11.29 [a]	11.50 [a]
DWCB	2.14 [a]	1.56 [a]	0.62 [a]	0.19 [b]	0.14 [a]	0.06 [a]	11.57 [a]	11.16 [a]	10.68 [a]
DWCNB	2.38 [a]	1.65 [a]	0.61 [a]	0.20 [b]	0.15 [a]	0.06 [a]	11.93 [a]	11.24 [a]	10.00 [a]
PRGB	2.11 [a]	1.66 [a]	0.69 [a]	0.18 [b]	0.15 [a]	0.06 [a]	11.98 [a]	11.01 [a]	10.90 [a]
PRGNB	2.27 [a]	1.49 [a]	0.59 [a]	0.18 [b]	0.13 [a]	0.05 [a]	12.44 [a]	11.32 [a]	11.77 [a]

[†]Treatments are: Conventionally managed (CONV), Dutch white clover with banding (DWCB), Dutch white clover with no banding (DWCNB), alfalfa with banding (ALFB), alfalfa with no banding (ALFNB), perennial rye grass with banding (PRGB), and perennial rye grass with no banding (PRGNB). [*] *Treatments with the same letter at each soil depth are not statistically different.*

It has been reported that long term cover cropping markedly influence soil organic C and N levels (Dinesh et al. 2004, Sanchez et al. 2007). We did not observe significant changes in soil total soil C as affected by our groundcover management, which is not unexpected due to the very short nature of the study. However, our groundcover management practices had an early effect on soil total N, probably due to greater N input from the cover crop residues.

Soil microbial biomass

Soil microbial biomass C and N as well as C:N ratio measured at 0-15, 15-30 and 30-45 cm depths of soil are shown in Fig. 2 (a, b &c). Groundcover treatments significantly affected soil microbial biomass (SMB-C and SMB-N) at the 0-15 cm depth ($P<0.001$). However, no statistical difference was observed among groundcover treatments for both SBM-C and SMB-N at the 15-30 cm and 30-45 cm depths (Fig. 2-a; 2-b). At the 0-15cm depth, ALFB and ALFNB averaged 49% and 35% higher SMB-C compared to CONV controls (Fig. 2-a). Similarly, SMB-N in ALFB and ALFNB plots was 80% and 53% higher than in CONV treatments (Fig. 2-b). SMB-C and SMB-N were also significantly higher in all Dutch white clover and perennial rye grass plots (banded and non-banded) compared to the CONV plots. SMB-C was 57% and 49% higher, and

SMB-N was 80% and 76% higher in DWCB and DWCNB than in CONV. For PRGB treatments, SMB-C was 15% and SMB-N was 33% higher than in CONV while PRGNB yielded 19% and 40% SMB-C and SMB-N, respectively, higher than in CONV. Results obtained indicate that creating bands did not significantly affect SMB-C or SMB-N in both legume and non legume cover crop treatments. SMB-C and SMB-N in plots managed with bands averaged 558.8 mg C kg^{-1} dry soil and 83.2 mg N kg^{-1} dry soil while plots without bands averaged 535.8 mg C kg^{-1} dry soil and 79.3 mg N kg^{-1} dry soil, respectively.

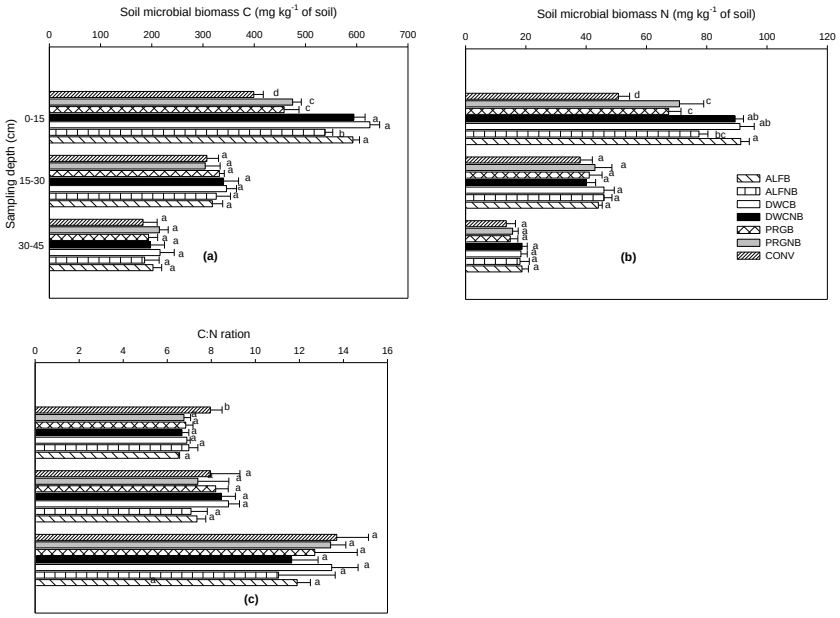

Figure 2: Soil microbial biomass carbon (a), microbial biomass nitrogen (b) and microbial biomass C:N ratio (c) as influenced by groundcover management. †Treatments are: Conventionally managed (CONV), Dutch white clover with banding (DWCB), Dutch white clover with no banding (DWCNB), alfalfa with banding (ALFB), alfalfa with no banding (ALFNB), perennial rye grass with banding (PRGB), and perennial rye grass with no banding (PRGNB). * *Treatments with the same letter at each soil depth are not statistically different at P>0.05. NS: treatment means are not significant.*

Soil microbial biomass C: N ratio is often used to describe the structure and state of the microbial biomass (Moore et al. 2000). Bacteria and fungi generally comprise 90% of the total soil microbial biomass (Six et al. 2006) and the substrate quality is known to have a major influence on fungal: bacterial ratios, with low quality substrates (high C: N ratio) favoring fungi and high quality (low C: N ratio) substrates favoring bacteria (Bossuyt et al. 2001). High soil microbial biomass C: N ratio generally indicates higher fungi with respect to bacteria populations in this soil (Moore et al. 2000).

Across all treatments, SMB-C and SMB-N decreased with soil depth for all cover crop treatments (Fig 2-a and 2-b), and legume cover crops treatments generally yielded the highest microbial biomass C compared to grass cover crop and the conventional treatments. However, soil microbial biomass C: N ratio significantly ($P<0.05$) increased with soil depth (Fig. 2-c), ranging from 6.5-7.1; 7.1-8.8 and 11.1-13.7 at the 0-15, 15-30 and 30-45 cm soil depths, respectively. This trend is the opposite of that observed for soil total C:N and could indicate a

shift in community composition, perhaps from a bacterial-dominated community on the top soil layer to a fungal-dominated community in deeper soil layers.

Community-level physiological profile of microbial communities

The color response in a given well is related to the number of microorganisms (functional diversity) which are able to use the substrate within the well as a sole carbon source, and are therefore used to assess microbial community structure in a given ecosystem (Garland and Mills 1991). Average well color development (AWCD) recorded as optical density (OD) and the number of well response expressed as the catabolic diversity from all groundcover management treatment followed the same pattern (sigmoidal curve) throughout the incubation period (Fig.3-a and 3-b) and the rate of increase varied with different treatments. However, both the AWCD and the catabolic diversity of communities from control plots were lower than the cover crop managed plots. These results suggest that microbial communities from the cover crop plots had higher substrate utilization rate than the control plots. Perennial rye grass plots treated with bands showed significantly higher overall AWCD values throughout the incubation period compared to perennial rye grass plots with unbanded treatments. However, the AWCD recorded from the two legume cover crop treatments, irrespective of the management type, were not statistically different. The number of well responses (catabolic richness) followed the same pattern as AWCD throughout incubation (Fig.3-b).

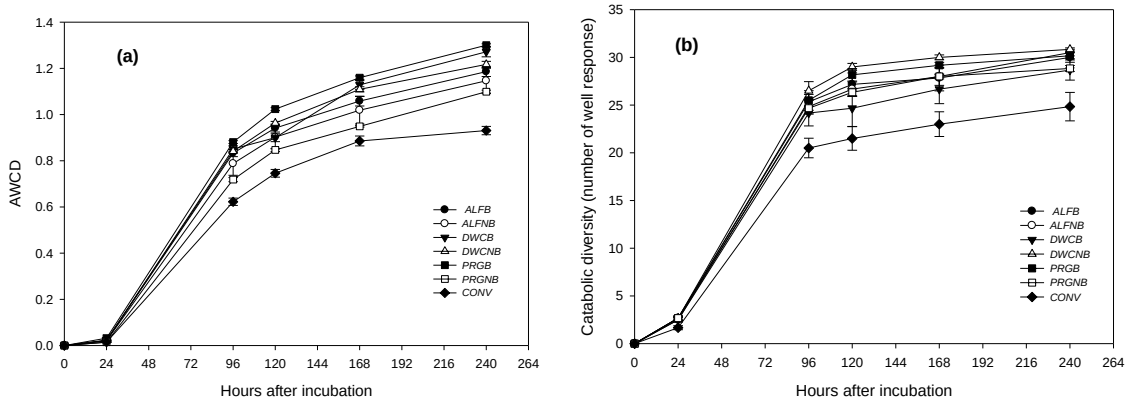

Figure 3: (a) Average well color development (AWCD) and **(b)** average catabolic diversity obtained from BIOLOG™ ecoplate incubation of all groundcover treatments in a Fraser fir plantation. †Treatments are: Conventionally managed (CONV), Dutch white clover with banding (DWCB), Dutch white clover with no banding (DWCNB), alfalfa with banding (ALFB), alfalfa with no banding (ALFNB), perennial rye grass with banding (PRGB), and perennial rye grass with no banding (PRGNB).

In all the soil samples from the different groundcover treatments, only a few wells showed no color response after 96 h of incubation. Microbial community richness was significantly lower in the conventional plots than in all cover crop treatments ($P<0.01$). Significant differences among treatments ($P<0.01$) were found in catabolic richness, Shannon diversity and evenness (Fig. 4-a, b and c). All cover crop treatments, both with bands and no bands, had significantly higher ($P<0.001$) microbial species catabolic richness levels than the conventional treatments (Fig. 4-a). However, there were no significant difference in microbial catabolic richness among cover crop treatments ($P>0.05$).

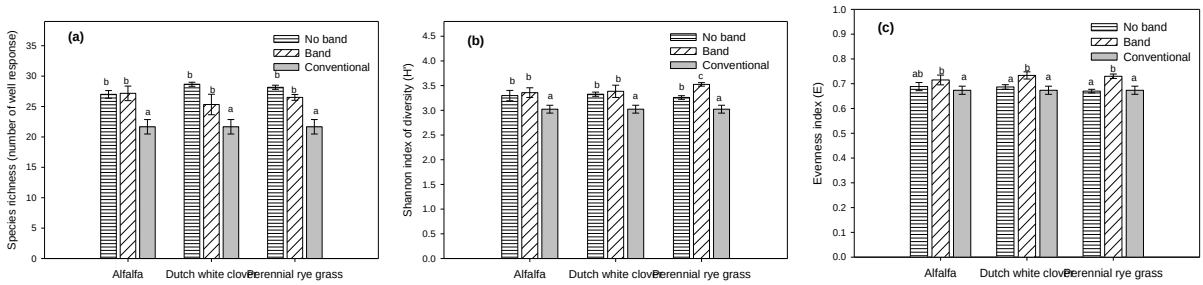

Figure 4: (***a***) Species richness (S), (***b***) Shannon index of diversity (H') and (***c***) Evenness index (E) as influence by different groundcover management. †Treatments are: Conventionally managed (CONV), Dutch white clover with banding (DWCB), Dutch white clover with no banding (DWCNB), alfalfa with banding (ALFB), alfalfa with no banding (ALFNB), perennial rye grass with banding (PRGB), and perennial rye grass with no banding (PRGNB). * *Treatments with the same letter at each soil depth are not statistically different.*

Plots managed with both legume cover crops, either with or without bands, had significantly higher Shannon-Weaver index means than the control. Similarly, the H' value was significantly higher in the banded non-legume cover plots than the control plots. Conversely, no statistical difference was found (P>0.05) between plots managed with perennial rye grass without banding and the conventional plots. There was a statistically significant difference in H' between PRGB and PRGNB, suggesting that rye grass with banding management could develop a more diverse microbial catabolic diversity than rye without banding and control treatments, while for both legume cover crops, there was no significant difference in Shannon-Weaver index level between banding and no banding treatments. Using CLPP and genetic community structure, Wu et *al.* (2008) found that different agricultural practices in a given soil type greatly affect soil bacterial diversity and community structures. Mäder et al. (2002) found a significantly higher value for H′ in organic systems compared to conventional farming systems.

In general, microbial species catabolic response was found to be significantly different among the various groundcover management practices (P=0.01). Microbial species evenness index was the highest in DWCB and PRGB plots. The plots managed with DWCB and PRGB showed significant differences in microbial species evenness index compared to all other groundcover treatments, including conventional.

In order to determine the extent of differentiation between the conventional and the cover crop treatments with regard to carbon source utilization, the OD data from the various treatments were subjected to multivariate analysis (principal component and similarity distance cluster analyses). The trends observed on soil microbial biomass and CLPP data were supported by results from the multivariate analysis. Contrasting patterns were apparent between the cover crop treatments and the controls as a result of the different groundcover management practices (Fig 4-a&b).

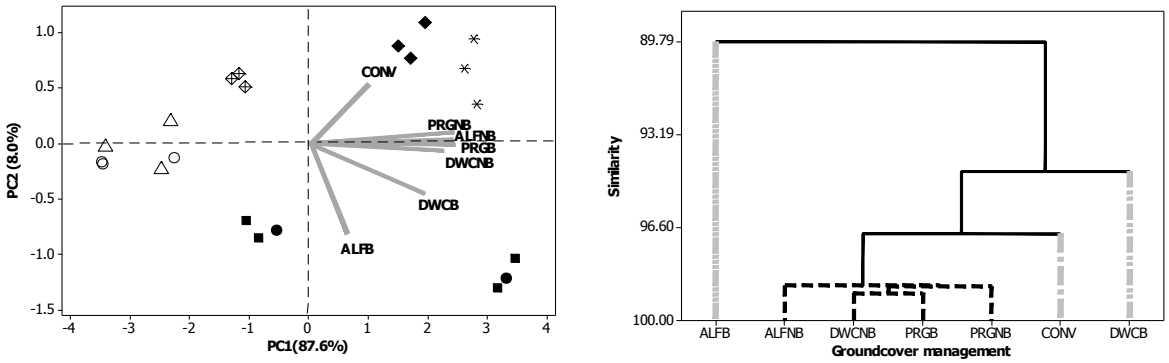

Figure 4: (a) PCA and **(b)** Cluster analysis performed on BIOLOG™ of soil extracts from different groundcover management treatments. †Treatments are: Conventionally managed (CONV), Dutch white clover with banding (DWCB), Dutch white clover with no banding (DWCNB), alfalfa with banding (ALFB), alfalfa with no banding (ALFNB), perennial rye grass with banding (PRGB), and perennial rye grass with no banding (PRGNB).

The separation of groundcover treatments in PC space can be related to differences in carbon source utilization by examining the correlation of the original variables to the PCs. The principal component analysis showed that the first principal component had high coordinate values (Eigen value) of 6.30 which explained 90.0% of the total variance in the data. The second principal component had variance 0.08 and accounted for 7.9% of the data variability. Together, the first two components of this PCA accounted for 97.9% of the variation in the data, with good discrimination ($P<0.05$). The plots managed with the two legume cover crops with banding exhibited dissimilar patterns of substrate utilization.

Consistent with the soil microbial biomass data, CLPP profiles also showed that groundcover management with cover crops stimulated the development of a diverse microbial population to a larger extent than did CONV management. It is possible that low organic matter and nitrogen concentration as well as low plant root exudates in CONV management could be responsible for the low catabolic diversity of microbial population found on the CONV. This was consistent with previous observations that management practice and host plants influence bacterial community structures (Bossio et *al.* 1998, Buckley and Schmidt 2001).

CONCLUSIONS

This study investigated the effects of various groundcover management practices on soil microbial biomass in a Fraser fir tree production system. Overall, the results demonstrated the potential for various groundcover management practices to increase soil microbial biomass and soil organic matter in the top soil layer (0-15cm). However, we observed no significant effects of the groundcover management practices on soil moisture content, soil pH and soil organic C at this specific depth. Moreover, no significant differences were found on the effect of our groundcover treatments on all the various parameters in the deeper soil layers (15-30 and 30-45 cm). As expected, soil moisture, soil organic carbon and nitrogen significantly decreased with soil depth.

Analysis of the soil microbial biomass carbon and nitrogen data indicated that leguminous cover crops (alfalfa and Dutch white clover) significantly increased soil microbial biomass C and N at

the soil surface layer (0-15 cm depth). Perennial rye, a non leguminous cover crop, also increased soil microbial biomass C and N, but at a much lower level compared to the legume cover crops. Cover cropping, management practices (banding and no banding) and cover crop species all affected soil bacterial diversity and communities at different levels. A significant shift in the structure of soil bacterial communities was associated with groundcover management practices.

The study also indicated that groundcover management with leguminous cover crops was more efficient at improving soil microbial biomass quotients than non-leguminous cover crops due to the quality and quantity of the green manure mowed and returned into the system. These preliminary results indicate that inclusion of leguminous or non-leguminous cover crops into Fraser fir production systems can lead to healthier soil by improving soil organic matter compared to conventional practices. The microbial community diversity as well as nitrogen fluxes, and their effect on tree growth are being investigated to confirm these trends and determine the overall impact of these management practices on Fraser fir production.

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