

# Soil Microbial Community Dynamics as Influenced by Soil Properties and Landscape Position

D.B. Watts<sup>1\*</sup>, H.A. Torbert<sup>1</sup>, Y. Feng<sup>2</sup>, and S.A. Prior<sup>1</sup>

<sup>1</sup>USDA-ARS National Soil Dynamics Laboratory, Auburn, AL 36832

<sup>2</sup>Department of Agronomy and Soils, Auburn University, 36849

\*Corresponding author's email address: dwatts@ars.usda.gov

## Abstract

Factors that affect plant growth, whether it is manure addition, season, or soil-type and landscape variability may provide insight on how to better manage agricultural fields through the evaluation of soil microbial activity, biomass and community structure. Thus, an *in situ* study was conducted to evaluate microbiological properties from three different soil types and landscape positions located in close proximity of each other during the summer and winter months. The three Coastal Plain soils investigated were Bama (Sandy loam), Lynchburg (Loam) and Goldsboro (Loam). Dairy-composted manure was incorporated into *in situ* soil cores at a rate of 350 kg N ha<sup>-1</sup> and compared to unamended controls. Microbial properties were determined by microbial biomass N, dehydrogenase enzyme activity, and PLFA analysis. Dairy-composted manure addition greatly affected the microbial properties of the soil. An increase in microbial activity and immobilization of N was observed with the addition of manure, suggesting that a shift in microbial dynamics had occurred due to the changes in the available substrate. This was most evident during summer months, which suggests that warmer temperatures stimulated the microbial activities. Landscape and soil-type was also shown to affect microbial properties. The Lynchburg soil, a loam soil located in a depressed area of the field, was shown to have the highest microbial biomass and microbial activity. Canonical discriminate analysis (CDA) of the phospholipid ester-linked fatty acid (PLFA) profiles was utilized to confirm the results of microbial properties. This analysis indicated that a shift in microbial communities as indicated by PLFA profiles occurred between season, manure application, and soil landscape. Therefore, microbial properties could be a useful tool for providing insight into the long-term sustainability of the soil.

## Introduction

In recent years, there has been a renewed interest in the use of manure for agricultural row crop production, resulting from large amounts of manure being generated in confined areas. The use of manure in row crop production can be viewed as having a two-fold affect: as a means of waste disposal and building up soil fertility through the addition of organic matter. The addition of organic matter in the form of manure promotes microbial activity. Soil fertility and microbial activity go hand in hand because it is through the microbial population that mineralization (C, N, P, S) of organic material occurs (Frankenburger and Dick, 1983), which is controlled by the soil microbial community structure. Also, the topography of a landscape can influence the fertility and microbial activity of a soil resulting from water movement and distribution of nutrients carried by water. Thus, information on the affect that manure application has on microbial parameters of soils from different soil-types and landscape positions during winter and summer

months is needed to make predictions on the long-term sustainability of soil systems. The objectives of this study were to determine the effects of manure application on three different soils in close proximity to each other from different landscapes and soil textural classes on microbial parameters and community structure during two different seasons.

### **Materials and Methods**

Soil samples were collected from an ongoing precision agriculture experiment located at Auburn University's E.V. Smith Experiment Station in Macon County, Alabama (Terra et al., 2006). Soils were collected from field plots that have not received manure within the last 10 years. The three soil series evaluated (Bama, Goldsboro, and Lynchburg) were chosen because they are located in close proximity to one another, yet different in texture. The Bama series is a fine-loamy, siliceous, subactive, thermic Typic Paleudults (sandy loam - summit). Goldsboro is fine-loamy, siliceous, subactive, thermic Aquic Paleudults (loam-backslope). The Lynchburg is fine-loamy, siliceous, semi-active, thermic Aeric Paleaquults (loam-depression). The farming practice was comprised of conventional tillage, which receives inorganic fertilizer in a continuous cotton/corn rotation.

The experiment was conducted using *in situ* soil cores (microplot cylinders) made of polyvinyl chloride (PVC) plastic cylinders (6.25 cm dia and 20.32 cm length). These cylinders were placed in the surface 20 cm of the soil profile. The soil cores were placed in each of the three soil types with half of the cores amended with manure the other half without manure. The appropriate amount of manure was added to the top 4 cm of the soil core in the microplot cylinders to give 350 kg N ha<sup>-1</sup> applied to a 15 cm depth. Soil cores were collected and returned to the laboratory for analysis on 0, 7, 14, 21, 49, and 70 days after manure application by randomly selecting and removing six cylinders from each plot. On each sampling day microbial biomass N was determined similar to Runion et al. (2004) using the chloroform fumigation extraction method as described by Horwath and Paul (1994) and dehydrogenase activity was determined similar to Runion et al. (2002) from a modified procedures described by Tabatabai (1982). Phospholipid fatty acid analysis was determined on field moist samples as described by Feng et al. (2003) using a modified procedure of Findlay and Dobbs (1993) and Bossio and Scow (1998).

The study was a completely randomized factorial design with three soil types amended with and without manure for the summer and winter months. Statistical analysis was performed using the GLM procedure of SAS (SAS institute, 1985), and means were separated using least significant difference (LSD) at an *a priori* 0.10 level. To access specific effects of season (winter vs. summer), soil series, and manure application on microbial community structure, canonical discriminate analysis (CDA) was performed on FAME data. CDA was analyzed using the mole percentage distribution of PLFAs with SAS software version 9.13. Canonical discriminate analysis was performed on combined PLFA data from day 70 from winter 2004 and summer 2005. All samples were analyzed for PLFA profiles using a set of 33 fatty acids that were present in most of the samples.

### **Results and Discussion**

Some of the basic soil properties of the three soil types utilized in this study are presented in Table 1 and 2. In general, the focus of this study was to access whether season and manure

addition had an impact on microbial characteristics and the microbial community as a whole when applied to different soil types and landscape positions. Season, manure application, soil type and landscape position had an effect on the microbial properties. Seasonal effect (winter season compared to summer seasons) was shown to have the greatest effect on microbial properties compared to soil type and manure application. This is similar to the results of Bardgett et al. (1999) who reported greater microbial biomass C and N and microbial activity during summer months compared to winter months. The following discussion is a more in-depth look at the specifics of how the previously mentioned management decisions affect microbial properties.

### **Dehydrogenase**

A significant increase in dehydrogenase activity was observed ( $P < 0.10$ ) on all sampling days except day 49 during the winter and day 7, 28, and 49 during the summer months (Figure 1). Although, not significant on each sampling day, an increase in dehydrogenase activity was observed with the addition of manure to the soil during the winter and summer, suggesting that changes in the size of microbial populations and respiratory activity occurred in response to the added available substrate. Season greatly impacted dehydrogenase activity. Significant differences were observed ( $P < 0.001$ ) for every sampling day except day 14. Dehydrogenase activity measured during the summer was almost double that measured during the winter months. Higher dehydrogenase enzyme activity, which is a representation of microbial activity, was probably a result of higher soil temperature, which has been shown to stimulate microbial activity. Dehydrogenase activity was also greatly affected by soil type. Significant differences were observed ( $P < 0.10$ ) on all sampling days except day 7, 14 and 49 during the winter and day 28 and 49 during the summer season. The Lynchburg soil produced higher dehydrogenase enzyme activity ( $P < 0.10$ ) on all sampling dates except day 0, 49, and 70 during the winter and day 7 during the summer months. Although no significant differences were observed between the soil X amendment effects at any sampling days, there was a trend resembling the soil effect. The Lynchburg soil with manure produced the highest microbial activity compared to the other soils. The Lynchburg soil, located in a depression area, contains the highest organic C and N content. The observed difference in microbial activity was probably attributed to nutrients accumulating in the depressed area from water movement, thus, resulting in increased organic matter. This also corresponds with the higher organic C and N, CEC values observed from the initial soil characteristics from this soil.

### **Soil microbial biomass N**

Similar to dehydrogenase activity, microbial biomass N also increased following the application of dairy compost (Figure 2). Significant differences were observed ( $P < 0.10$ ) on all sampling days except day 7 and 70 during the winter and day 49 during the summer. Although not significant on every sampling day, microbial biomass was higher in manure compared to no manure treatments. It is well known that changes in microbial biomass concentrations observed in the soil correspond to changes in the availability of decomposable substrate. The addition of manure provided the microbes with readily available C and N. This is consistent with the finding of Bohme et al. (2005) who reported that microbial biomass was greater in soil following the application of farmyard manure. The same trend was also shown for soil X season effect. During the summer more microbial biomass N was observed compared to the winter months at all sampling dates. This corresponds to dehydrogenase activity, suggesting that as microbial activity increased, more N was immobilized into microbial cells. A comparison of soil type shows that

significant differences were observed on every sampling date for the winter and summer season ( $P < 0.10$ ). In the Lynchburg soil, which contained the highest initial soil organic C and N content, microbes were more efficient in immobilizing the N, suggesting that land-use and topography of a landscape could cause changes in soil C and N cycling rates and accumulation of organic matter (Chen and Stark, 2000). The microbial biomass was the lowest in the Goldsboro soil. This indicates that less N was being immobilized into the microbial cells. The reduced microbial biomass N occurring in the Goldsboro soil could be attributed to more nitrification occurring and less immobilization. This also corresponds with the low C:N ratio that was observed in the soil, thus suggesting that although the Goldsboro soil had a higher clay content, microbial biomass N was more closely related to the C, N, and C:N ratio of the soil. Also, the textural differences in these were not great enough to affect the microbial biomass N.

### **Soil microbial community structure**

In this study, PLFAs analysis identified 48 fatty acids. However, of these, only 33 were present in most samples used in data analysis. CDA was carried out by comparing the summer and winter seasons to identify differences between the dairy compost additions and soil series. The first 3 canonical discriminant variants (CDV) accounted for a total of 84% of the total variance. The first CDV, which accounts for 48% of the variance, discriminated the with and without composted manure treatments, the second accounted for 25% of the variance, and discriminated the seasonal effect, and the third accounted for 11% of the variance, discriminated the soil type effect (Figure 3&4). PLFAs 16:1 $\omega$ 5c, 18:3 $\omega$ 6c, 18:1 $\omega$ 7c, cy19:0, 20:4 $\omega$ 6, 9,12 were identified by CDA as influential bio markers for the CV1 and 16:1 $\omega$ 7c / i15:0 2OH, 18:1 $\omega$ 7c, 18:0, 18:3 $\omega$ 6c for CV2, respectively (Table 3). The PLFAs i17:0, a18:0/18:2 $\omega$ 6, 9c, 16:1 2OH, cy17:0, and 17:0 10 methyl were influential biomarkers for CV3. The metabolic association of the fatty acids previously mentioned are described by Frostegard et al., 1993; Zelles, 1997; Fierer et. al, 2003; Feng et al, 2003. The PLFA 16:1 $\omega$ 5c is associated with monounsaturated fatty acids, which have been shown to increase with manure addition. Also 16:1 $\omega$ 5c, 18:1 $\omega$ 7c and cy19:0 are Gram-negative bacteria and which are associated with an increased readily-available substrate. On the other end of the spectrum 18:3 $\omega$ 6c and 20:4 $\omega$ 6, 9,12 are associated with fungi and were shown to decrease with the addition of available substrate. The PLFA identified for the second CV 16:1 $\omega$ 7c, 18:1 $\omega$ 7c accounted for most of the discrimination. Fatty acid 16:1 $\omega$ 7c is associated with monounsaturated fatty acids and 18:1 $\omega$ 7c is associated with gram-negative bacteria, both of which increased with the addition of manure. The biomarker 18:0 is a non-specific fatty acid, which is found in all organisms. The signature fatty acid biomarker a15:0 is associated with gram-positive bacteria and 18:3 $\omega$ 6c is associated with fungi. The increase in soil temperature probably affected the PLFA concentrations, thereby causing a shift in lipid composition between seasons. The PLFAs identified for the third CV i17:0 is a gram-positive bacteria, 18:0 gram-negative bacteria, 16:1 2OH non-specific bacteria, cy17:0 and 17:0 10 methyl were all found in more abundance in the Lynchburg and Goldsboro soil, which are both loam soils. These fatty acids played an integral role in discriminating the loam two soils from the sandy loam soil suggesting that lipid composition changed due to texture and available substrate.

### **Conclusions**

Microbial parameters evaluated in the study suggest that season, addition of manure, and changes in the topography of a landscape can greatly affect soil microbial community structure.

The addition of dairy compost manure resulted in a diverging microbial community structure probably by increasing soluble C in soil. Season also increased the microbial parameter resulting in increased metabolic activity during the summer compared to the winter. Soil landscape positions that have resulted in a buildup of organic matter were observed to enhance and alter the microbial community. The significant changes in microbial parameters were evident by observing increases in microbial biomass N, dehydrogenase (microbial activity), total PLFAs, as well as changes in microbial community structure. Canonical discriminate analysis clearly discriminated PLFA profiles by season, manure addition, and soil type and landscape, thus confirming that changes in microbial community structure diverged, resulting from the agronomic management practices evaluated. Thus, consideration of microbial parameters should be taken into account when developing management practices in order to maximize the use of plant nutrients contained in manure without negatively affecting the environment.

### **Acknowledgements**

The authors acknowledge Barry G. Dorman, and Sheryl A. Morey (USDA-ARS National Soil Dynamics Laboratory) for technical assistance.

### **References**

- Bardgett R.D., R.D. Lovell, P.J. Hobbs, and S.C. Jarvis. 1999. Dynamics of below-ground microbial communities in temperate grasslands: influence of management intensity. *Soil Biol. Biochem.* 31: 1021-1030.
- Bohme, L., U. Langer, and Bohme. 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. *Agric. Ecosyst. Environ.* 109: 141-152.
- Bossio, D.A., and K.M. Scow 1998. Impacts of carbon and flooding on soil microbial communities: phospholipids fatty acid profiles and substrate utilization patterns. *Microbial Ecology* 35: 265-278.
- Chen J., and J.M Stark 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. *Soil Biol. Biochem.* 32: 47-57.

- Feng Y., A.C. Motta, D.W. Reeves, C.H. Burmester, E. van Santen, and J.A. Osborne. 2003. Soil microbial communities under conventional-till and no-till continuous cotton systems. *Soil Biol. Biochem.* 35: 1693-1703.
- Fierer N., J.P. Schimel, and P.A. Holden. 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biol. Biochem.* 35: 167-176.
- Findlay, R.H., and F.C. Dobbs. 1993. Quantitative description of microbial communities using lipid analysis, p 777. In P.F. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole (eds), *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton.
- Frankenberger Jr., W.T., and W.A. Dick 1993. Relationship between enzyme activities and microbial growth and activity indices in soil. *Soil Sci. Soc. Am. J.* 47: 945-951.
- Frostegard, A., E. Baath, and A. Tunlid. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biol. Biochem.* 25: 723-730.
- Horwath, W.R., and E.A. Paul. 1994. Microbial biomass pp. 753-773. In R.W. Weaver, J.S. Angle, and P.S. Bottomley (eds.) *Methods of Soil Analysis. Part 2 Microbiological and Biochemical Properties*. SSSA Book Series No. 5, Soil Science Society America Inc., Madison, WI.
- Runion, G.B., Prior, S.A., Reeves, D.W., Rogers, H.H., Reicosky, D.C., Peacock, A.D., White, D.C. 2004. Microbial responses to wheel-traffic in conventional and no-tillage systems. *Commun Soil Sci. Plant Anal.* 35: 2891-2903.
- Tabatabai, M.A., 1982. Soil enzymes; Dehydrogenases. Pp937-940. In A.L. Page, R.H. Miller and D.R. Kenney (eds) *Methods of Soil Analysis. Part 2 Chemical and Microbiological Properties*. 2<sup>nd</sup> Edition, Agronomy Monograph 9. ASA and SSSA, Madison, WI.
- Terra J.A., J.N. Shaw, D.W. Reeves, R.L. Raper, E. van Santen, E.B. Schwab, and P.L. Mask. 2006. Soil management and landscape variability affects field-scale cotton productivity. *Soil Sci. Soc. Am. J.* 70:98-107.
- Zelles, I., Q.Y. Bai, R.X., Ma R. Rackwitz, K. Winter, and F. Beese. 1994. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. *Soil Biol. Biochem.* 24: 317-323.



Table 1. Characteristics of soil properties used in the in situ field study reported on a dry wt basis.

Soil Series	pH	CEC cmol kg <sup>-1</sup>	Total C -----g kg <sup>-1</sup> -----	Total N -----	C:N Ratio
<b>Spring 2004</b>					
Bama	6.31	5.84	4.42	0.48	9.21
Lynchburg	6.1	5.46	5.57	0.51	10.92
Goldsboro	6.24	6.09	3.77	0.41	9.2
<b>Summer 2005</b>					
Bama	6.26	5.7	3.77	0.39	9.67
Lynchburg	6.25	7.79	6.12	0.58	10.56
Goldsboro	6.86	5.12	4.02	0.54	7.41

Table 2. Soil physical characteristics of soils used in this study

	BD g cm <sup>-3</sup>	Sand -----	Silt ----- % -----	Clay
Bama	1.68	66.25	21.25	12.50
Lynchburg	1.64	46.25	41.25	12.50
Goldsboro	1.61	33.75	48.75	17.50



Table 3. PLFAs of the first five scores accounting for the variance of the first three canonical axes

Fatty acid	Score	Score	Specificity as a biomarker
<b><u>Canonical variable 1</u></b>			
16:1? 5c		0.82	Bacteria (Gram-positive and Gram-negative)
18:3? 6c		-0.43	Fungi
18:1? 7c		0.42	Aerobic bacteria, Gram-negative
cy19:0		0.40	Anaerobes, Gram-negative bacteria
20:4? 6,9,12		0.39	Fungi
<b><u>Canonical variable 2</u></b>			
16:1? 7c/15:0 2OH		-0.70	Nonspecific
18:1? 7c		-0.53	Aerobic bacteria, Gram-negative
18:0		0.50	Biomass all organisms
a15:0		-0.38	Gram positive bacteria
18:3? 6c		0.37	Fungi
<b><u>Canonical variable 3</u></b>			
i17:0		0.38	Gram-positive bacteria
a18:0/18:2? 6,9c		-0.42	Gram-positive/ Fungi
16:1 20H		0.37	Nonspecific
cy 17:0		0.37	Gram-negative
17:0 10 methyl		0.36	Actinomycetes

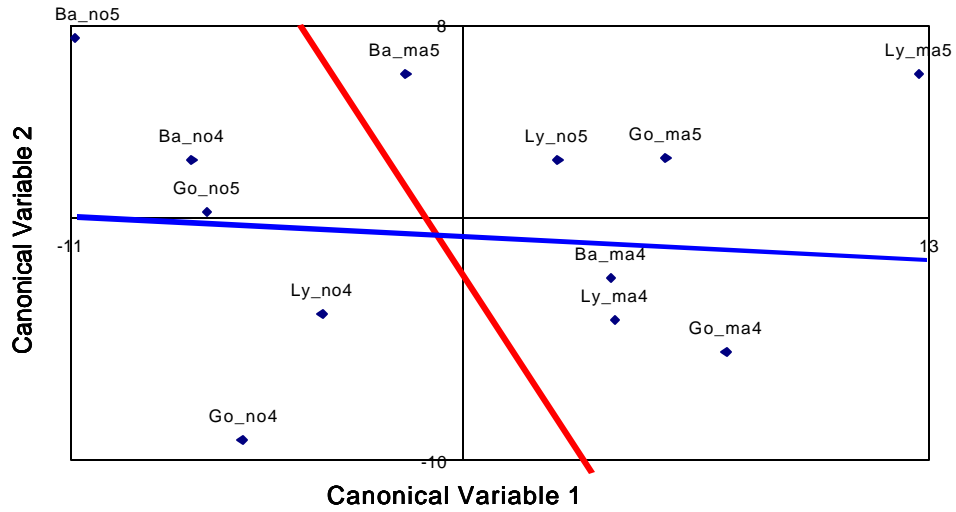


Figure 3. Canonical discriminant analysis (CDA) of phospholipid fatty acid profiles of the canonical variables (CV). Plot of ordination of CV1 against CV2 during the summer (05) and winter (04) months for the Bama (Ba), Lynchburg (Ly) and Goldsboro (Go) soil with (ma) and without dairy compost manure (no).

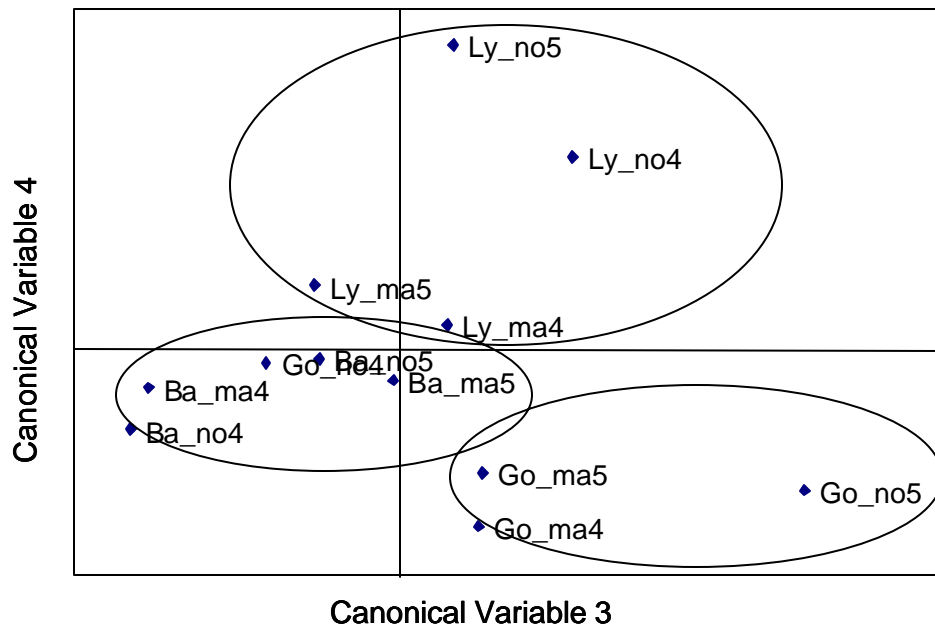


Figure 4. Canonical discriminant analysis (CDA) of phospholipid fatty acid profiles of the canonical variables (CV). Plot of ordination of CV3 against CV4 during the summer (05) and winter (04) months for the Bama (Ba), Lynchburg (Ly) and Goldsboro (Go) soil with (ma) and without dairy compost manure (no).