SPATIAL AND TEMPORAL DYNAMICS OF ARBUSCULAR MYCORRHIZAL FUNGI IN HIGH PRODUCTION CORN SYSTEMS

by

María Susana Grigera

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Agronomy

Under the Supervision of Professors Brian J. Wienhold

and Rhae A. Drijber

Lincoln, Nebraska

July, 2006

SPATIAL AND TEMPORAL DYNAMICS OF ARBUSCULAR MYCORRHIZAL FUNGI IN HIGH PRODUCTION CORN SYSTEMS

María Susana Grigera, Ph.D.

University of Nebraska, 2006.

Advisors: Brian J. Wienhold, and Rhae A. Drijber.

Soils are inherently variable in the physical and chemical properties that determine yield potential. Apparent electrical conductivity (ECa) measurements allow mapping of natural patterns of soil conditions across a field. Our first objective was to identify these soil properties that influenced ECa in an irrigated cornfield in Nebraska. The variability observed was related to soil properties that influenced soil water availability in the 0- to 90- cm: total C and clay content, total dissolved solids and depth of topsoil. Our second objective was to determine whether specific soil microbiological groups were associated with the soil properties that caused spatial variability in this field. Soil microbial populations mediate the processes of organic matter turnover and nutrient cycling. Soil microbial communities were related to diverse C pools. Bacterial and actinomycetes biomarkers covaried mainly with fine particulate organic matter (POM), while fungal and mycorrhizal biomarkers responded to coarse POM distribution. We also evaluated the distribution of soil microbial groups at a smaller scale. Row cultivation increased labile C pools and soil microbial biomass in the row position, independently of the ECa classification. Four weeks after row cultivation we observed a shift in the relative concentration of arbuscular mycorrhizal (AM) fungal

biomarkers in the row, but we found no clear differences in abundance of a specific group. The spatial variability in soil microbial groups may be accompanied by changes, over short periods of time, in AM fungi dynamics. In corn, more than 50% of the P is absorbed after tasselling. AM fungi form symbiotic relationships with most plants and play an important role in plant P nutrition. Two fields in Nebraska were selected to study the influence of available P in AM fungi during the reproductive stages of corn. The concentration of AM fungal biomarkers increased over time, and confirmed the C allocation from the plant to the symbiont during this period. Furthermore, hyphae were as efficient as roots and hyphae in reducing the P concentration from the soil matrix. It is likely that AM fungi play an important role in P uptake later in the crop season.

ACKNOWLEDGEMENTS

I had extraordinary advisors, Dr. Drijber and Dr. Wienhold. They helped me organizing a feasible research, and encourage and taught me to write in scientific English, the most challenging part of this project. I want to show gratitude to my reading committee: Dr. Ferguson and Dr. Pankhurst, because of their suggestions and thoughts.

This project was supported by a cooperative agreement between USDA-ARS-SWCRU and UNL Department of Agronomy and Horticulture. This research program was also possible because the intense collaboration and technical support of Liz Jeske, Susan Siragusa, Josh Cool, Sarah Berg, Susan Wagner, and Paul Koerner. Their hard effort and sense of humor made my work enjoyable.

Every morning during these three years I had the joy of coming to my office knowing that Pam Bushman was ready to help, to comfort, to listen, to support.

I want to thank my husband Fernando, and my kids Fernando and Julia, for their patience and for being my greatest support, and the love of my life. My parents, Marta and Miguel Grigera, who loved me and gave me the wings, the passion, and the constancy. Ana, Miguel, Fernando Grigera, Lucas, Pedro, Victoria, aunts, uncles, cousins visited me, gave me a phone call, sent an email or a letter each time my energy declined. I also want to thank my parents in law for supporting my family and me.

I am infinitely grateful to my friends/family here in Lincoln for encouraging words, and great times. And to my friends in Argentina that via email hold my hand. When we arrived to Lincoln Marylin Schepers comforted and baby-sat our kids, even if she could not communicate with them. She also helped us to put together a house from five suitcases.

I want to thank Roberto Alvarez, my former professor in Argentina. He thought me the first steps in scientific research. Drs. Fernando Garcia and Gerardo Rubio gave me the confidence to start this project, and wrote letters of recommendation for me.

My gratitude to Dr. Schepers, who trusted on me. He taught me that ‘ambitious’ is a great word, and gave me the chance to develop my wings.

Finally I want to dedicate this dissertation to my Grandma.

TABLE OF CONTENTS

Introduction

Relationship between apparent electrical conductivity and soil physicochemical properties.

Soil properties vary spatially and this variation affects plant growth and complicates management. Intensive soil sampling may provide the necessary information on soil fertility, water retention capacity and other relevant soil properties that define potential productivity, but this approach is expensive and labor demanding (Kitchen et al., 1999). Apparent soil electrical conductivity (ECa) sensors have been used for delineating soil variability. Soil ECa is affected by salinity, texture, cation exchange capacity, and moisture content (Rhoades and Corwin, 1990). Measurements of ECa are also a good estimate of topsoil thickness and may be used to diagnose potential rooting and water-related problems affecting crop production (Kitchen et al., 1999). Soil classification using ECa provides an effective basis for delineating interrelated physical, and chemical soil attributes (Johnson et al., 2001). Johnson et al. (2001) also concluded that ECa classification effectively delimited distinct zones of soil condition, making it an excellent basis for soil sampling to reflect spatial heterogeneity.

Soil properties are also influenced by the diverse physical and chemical properties of organic matter (OM) in soil (Baldock and Nelson, 1999). Furthermore, soil OM provides the chemical energy and nutrients for soil microorganisms. It is important to understand the influence of soil physicochemical characteristics on biological properties. Living organisms are one of the most powerful agents of geochemical changes (Stainer et al., 1970). Soil microbial communities are responsible for the cyclic turnover of carbon, nitrogen, sulfur, oxygen and phosphorous from organic constituents and influence

aggregation processes. A better understanding of the role environmental and edaphic factors have in governing microbial communities is needed.

Use of fatty acids methyl esters to characterize soil microbial biomass.

Several studies have been conducted in which fatty acid profiles proved to be sensitive in detecting soil microbial community changes and provided more detail than the classic fumigation-incubation and fumigation-extraction techniques for quantification soil microbial biomass. Phospholipid fatty acids (PLFAs) have been used to study differences in community composition resulting from differences in organic C input under organic and conventional agroecosystems (Bossio et al., 1998). Bossio et al. (1998) also found that soil type was the most important determinant of microbial communities due to the influence of clay content and aeration. Schutter and Dick (2000) found that ester linked fatty acid (EL-FAMEs) profiles distinguish soils differing in texture and total organic C content. Drijber et al. (2000) used both PLFAs and EL-FAMEs to fingerprint soil microbial communities in a dryland cropping system in western Nebraska and found that FAME C16:1*cis*11, cited as a biomarker for arbuscular mycorrhizal (AM) fungi, was greater in sod plots than in cropped soil, and were lower in no-till soils than plowed soils. These results demonstrated that mycorrhizae are sensitive to tillage.

Fatty acids have been utilized to measure changes in microbial composition over time. Quincke (2002) studied soil microbial community responses to supplemental N and P starters during early season corn growth. He found that soil microbial biomass increased during the vegetative stages of plant growth. Not only did the quantity of microbial biomass increase, but a shift in EL-FAME biomarkers indicated changes in the composition of that biomass. These changes appeared to be driven by localized changes

in the rhizosphere environment caused by plant processes. Spedding et al. (2004) evaluated changes in soil microbial biomass C, N and PLFAs in response to tillage, residue management, and sampling time throughout the maize-growing season. Changes in soil conditions that occurred over time promoted increases in microbial biomass and changes in microbial communities in the soil. Seasonal variations in microbial community composition were greater than those associated with either tillage or residue management regime. On-farm experiments conducted in Nebraska (Drijber, 2002) showed separation of EL-FAMEs characterized microbial communities due to variation in plant density, and fertilizer management. Her study also documented an increase in AM fungi biomarker concentration during vegetative and reproductive growth stages of corn.

Spatial and temporal dynamics of arbuscular mycorrhizal fungi in corn.

Arbuscular mycorrhizal fungi are ubiquitous throughout the soils of the world and form symbiotic relationships with roots of most terrestrial plants (Smith and Read, 1997). The AM fungi forming this association are zygomycetous belonging to the genera *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerosystis* and *Scutellospora* (Suresh and Bagyaraj, 2001). Arbuscular mycorrhizae have been established as the transporter of various immobile elements like P, Zn, and Cu from the surrounding soil to the host plant. Among the beneficial effects of mycorrhizal fungi are: increase water uptake, reduction of stress due to high salt concentrations, and antagonistic interactions with root pathogenic fungi (Li et al.,1991).

Mycorrhizal biomass decreased as the availability of P increased in the soil (De Miranda and Harris 1994). Spore germination and hyphal growth of mycorrhizal fungi is

known to be heavily dependent on the availability of soil P (De Miranda and Harris 1994). It was observed in *Trifolium subterraneum* that increasing soil P availability resulted in a decrease of fatty acids signature of AM fungi in the soil and in the roots (Olsson et al., 1997). In addition, low input agricultural systems were shown to be more dependent on AM fungi than conventionally managed systems, affecting the AM fungus population, mycorrhiza formation, and nutrient utilization in corn (Galvez et al., 2001).

Various forms of P present in soil have very low solubility and the concentration of P in soluble forms is extremely low (Singh and Adholeya, 2001). Phosphate transport across the root is usually faster than diffusive transport in soil. This lowers the concentration of phosphate in the soil solution immediately surrounding the root. This zone is called the phosphate depletion zone, which depending on the diffusion coefficient and the duration of active uptake may extend out from the root surface to a distance of 2 mm (Jungk, 1987). An individual root hair that is much shorter than the depletion zone radius or that is less than that distance from surrounding hairs should have less impact on P uptake than those that are longer or more sparsely distributed on the root (Clarkson, 1985).

Arbuscular mycorrhizal fungi colonization increases the concentration of P in the plants. This may be due to: increased physical exploration of the soil (Tinker, 1978); increased movement of P into AM fungi hyphae (Bolan et al., 1987); modification of the root environment (Hoffland et al., 1989); increased storage of absorbed P (Chilvers and Harley, 1980); and efficient transfer of P to plant roots (Gianinazzi-Pearson and Gianinazzi, 1988). In corn, the amount of dry matter in roots remains almost constant after R1 (Ritchie et al., 1997). The below ground dry matter/above ground dry matter

ratio is maximum at V10 (61%) and decreases with time, being 34 to 25% 7 days before R1, 17 to 20% at R1 and 10 to 13% at R5 (Plenet, 1995).

Understanding the composition of ecosystems and identifying the regulations that operate over spatial and temporal scales, are useful to identify the patterns of plant-fungal interactions (Allen et al., 2003). Therefore, the general objectives of this work were: (i) to identify the soil properties that influenced ECa, (ii) to determine the relationship between these soil properties and specific soil microbiological communities, and (iii) to evaluate AM fungi dynamics during the reproductive stages of high productivity corn in soils with different P availability.

The objectives addressed in Chapter I were to identify the soil physical and chemical properties influencing ECa in a field at Shelton and to determine whether variation in these properties influenced the composition of soil microbial communities. Chapter II addresses the hypothesis that row cultivation redistributes crop residues creating row and furrow environments that differ in C availability and associated soil microbiological communities. The objectives of Chapter III were: to study the influence of available P and roots on extramatrical mycorrhizal fungal growth, to provide evidence for C allocation to AM fungi during the reproductive stages of high productivity corn, and to link AM fungal growth dynamics with changes in soil P availability. The objective of Chapter IV was to study C partitioning in AM fungi in soil samples during the reproductive growth stage of corn.

References

Allen, M.F., Swenson, W., Querejeta, J.I., Egerton-Warbuton, L.M., Treseder, K.K., 2003. Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. Annu. Rev. Phytopathol. 41:271-303.

Baldock, J.A., and Nelson, P.N., 1999. Soil organic matter. p. D3-D18. In M.E. Summer et al. (Eds.) Handbook of soil science. CRC Press, Boca Raton, FL.

Bolan, N.S., Robson, A.D. and Barrow N.J., 1987. Effect of phosphorous application and relation to phosphorus uptake. Plant Soil 104:294-298.

Bossio, D.A., Scow, K.M., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipids fatty acid profiles. Microb. Ecol. 36:1-12.

Chilvers, G.A. and Harley, J.L., 1980. Visualization of phosphate accumulation in beech mycorrhizas. New Phytol. 4:319-326.

Clarkson, D.T., 1985. Factors affecting mineral nutrient acquisition by plants. Annual Rev. Plant Phys. 36:77-115.

de Miranda, J.C.C and Harris, P.J., 1994. Effects of soil phosphorous on spore germination and hyphal growth of arbuscular mycorrhizal fungi. New Phytol. 128:103-108.

Drijber, R.A., 2002. Microbial signatures for crop production systems. Proc. of the OECD workshop on innovative soil-plant systems for sustaintable agricultural practices, Izmir. J.M. Lynch, J.S. Schepers and I ünver (Eds.) Tübitak, Turkey. pp 132-146.

Drijber, R.A., Doran J.W., Parkhurst, A.M. and Lyon D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol.Biochem. 32:1419-1430.

Galvez, L., Douds, Jr. D.D., Drinkwater, L.E., Wagoner, P., 2001. Effect of tillage and faming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. Plant Soil 228:299-308.

Gianinazzi-Pearson, V. and Gianinazzi, S., 1988. Morphological interaction and functional compatibility between symbionts in vesicular arbuscular mycorrhizas. V. Is

H^+-ATPase a component of ATP-hydrolyzing enzyme activities in plant-fungus interface? New Phytol. 117:61-76.

Hoffland, E., Findenegg, G.R. and Nelemans J.A., 1989. Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to P starvation. Plant Soil 113:161-166.

Johnson, C.K., Doran, J.W., Duke, H.R., Wienhold, B.J., Eskridge, K.M., and Shanahan, J.F., 2001. Field-scale electrical conductivity mapping for delineating soil condition. Soil Sci. Soc. Am. J. 65:1829-1837.

Jungk, A., 1987. Soil-root interactions in the rhizosphere affecting plant availability of phosphorus. J. Plant Nutr. 10:1197-1204.

Kitchen, N.R., Sudduth, K.A., and Drummond, S.T., 1999. Soil electrical conductivity as a crop productivity measure for claypan soils. J. Prod. Agric. 12:607-617.

Li, X-L., George, E. and Marschner, H., 1991. Extension of the phosphorous depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant Soil. 136:41-48.

Olsson, P.A., Bååth, E., Jakobsen, I., 1997. Phosphorous effects on mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by fatty acids signatures. Appl. Environm. Microbiol. 63:3531-3538.

Plenet, D., 1995. Fonctionnement des cultures de maïs sous contrainte azotée. Determination et application d'un indice de nutrition. Dr These Académie de Nancy-Metz, Institut National Polytechnique de Lorraine.

Quincke, J.A., 2003. Soil microbial community responses during early season corn growth following supplemental N and P starters. M.S. thesis. University of Nebraska, Lincoln.

Rhoades J.D. and D.L. Corwin, 1990. Soil electrical conductivity: effects of soil properties and application to soil salinity appraisal. Comm. Soil Sci. Plant Anal. 21:873-860.

Ritchie, S.W., Hanaway, J.J., and Benson, G.O., 1997. How a corn plants develops. Spec. Publ. 48. Iowa State Univ. Coop. Ext. Serv., Ames.

Schutter, M.E. and Dick, R. P., 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Sci. Soc. Am. J. 64:1659-1668.

Singh, R. and Adholeya, A., 2001. Plant and fungal response to colonization. Arbuscular mycorrhizae. Interactions in plants, rhizosphere and soils. Ed: Sharma A.K. and Johri, B.N. Science publishers, Inc. Enfield, NH. Pp.7-28

Smith, S.E., Read, D.J., 1997. Mycorrhizal symbiosis. Academic Press, London pp.587.

Speeding, T.A., Hamel, G.R., Mehuys, G.R., Madramootoo, C.A., 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biol. Biochem. 36:499-512.

Stainer, R.Y., Duodorff, M., Adelberg, E.A., 1970. The microbial world. Prentice-Hall, Inc. (Eds.) Englewood Cliffs, NJ pp760.

Suresh, C.K and Bagyaraj, D.J., 2001. Mycorrhiza-microbe interactions: effect on rhizosphere. Arbuscular mycorrhizae. Interactions in plants, rhizosphere and soils. Ed: Sharma A.K. and Johri, B.N. Science publishers, Inc. Enfield, NH pp.7-28

Tinker, P.B., 1978. Effect of vesicular arbuscular mycorrhizas on plant nutrition and plant growth. Phisiologie Vegetable 16:743-749.

Chapter I

Soil Microbial Biomass Relationships with Organic Matter Fractions in a Nebraska Corn Field Mapped using Apparent Electrical Conductivity

María S. Grigera, Rhae A. Drijber, Kent M. Eskridge, and Brian J. Wienhold

Accepted for publication Soil Science Society of America Journal.

ABSTRACT

Soils exhibit spatial variability in their properties. One approach for delineating field-scale variability involves mapping the variation in apparent soil electrical conductivity (ECa). The objectives of this study were to evaluate the physical and chemical soil characteristics that define ECa classification and the association of specific soil microbiological communities with these soil properties. In May 2003 ECa was measured in a field in Buffalo County, Nebraska, and processed to create four ECa zones based on ranges of horizontal and vertical ECa measurements using unsupervised classification. Soil properties (0- to 90-cm) that showed higher correlations with vertical ECa included total C ($r = 0.87$), clay ($r = 0.83$), total dissolved solids ($r = 0.68$) and depth of topsoil ($r = 0.70$). These properties influence soil water availability in this field. Soil microbial groups were correlated with different soil C fractions in the 0- to 15-cm depth and were similar across ECa zones. Bacterial ($r = 0.85$) and actinomycetes ($r = 0.71$) biomarker concentrations were more highly correlated with fine particulate organic matter (POM) than coarse POM (bacteria $r = 0.69$ and actinomycetes $r = 0.48$). In contrast, fungal ($r = 0.77$) and mycorrhizal ($r = 0.48$) biomarker concentrations were only correlated with coarse POM. Given current knowledge of the spatial distribution of POM and aggregates we hypothesize that microorganisms are also spatially distributed and that fungal groups are closely associated with coarse POM early in the growing season.

Abbreviations: ECa: apparent soil electrical conductivity, FAMEs: fatty acid methyl esters, OM: organic matter, POM: particulate organic matter, TDS: total dissolved solids

Successful implementation of site-specific management requires an understanding of how variation in physical and chemical soil properties effects nutrient and water availability and ultimately crop production in a field. Intensive soil sampling may provide the necessary information on soil fertility, water retention capacity and other relevant soil properties that define potential productivity, but this approach is expensive and labor intensive (Kitchen et al., 1999). Apparent soil electrical conductivity (ECa) sensors have been used for delineating soil variability. Soil ECa is affected by salinity, texture, cation exchange capacity, and moisture content (Rhoades and Corwin, 1990). Soil classification using ECa provides an effective basis for delineating interrelated physical, chemical and biological soil attributes important to crop productivity and ecological sustainability (Kitchen et al., 1999; Johnson et al., 2001; Wienhold and Zhang, 2001; Eigenberg et al., 2002). Also, measurements of ECa are a good estimate of topsoil thickness and may be used to diagnose potential rooting and water-related problems affecting crop production (Kitchen et al., 1999).

Spatial variability in crop production within a field may impact soil biota through variation in crop residue production. Soil properties are also influenced by the diverse physical and chemical properties of organic matter (OM) in soil (Baldock and Nelson, 1999). Soil OM provides the chemical energy and nutrients for soil microorganisms. As crop residue decomposes, the resulting soil OM contributes to ion exchange, soil structural stability, water retention, and soil thermal properties important to soil resilience. Each pool of soil OM will contribute differently to these various functions (Baldock and Skjemstad, 1999). Particulate organic matter (POM) consists of partially decomposed plant residues and forms the first intermediate pool in the decomposition

process between crop residue and humified OM (Gregorich and Janzen, 1996). The extent to which OM progresses through the different stages of decomposition depends on the presence of protection mechanisms capable of enhancing biological stability (Baldock and Skjemstad, 2000). The decomposition of plant residues and the dynamics of POM are directly related to soil aggregate formation and C stabilization in relatively undisturbed systems (Gale et al., 2000). Long term studies showed that the amount of plant C retained in a soil was proportional to soil clay content (Ladd et al., 1985) and the mean residence time was also correlated with the specific surface area of the soil minerals (Saggar et al., 1996). The presence of clay particles in soil provides the surface area onto which OM may be adsorbed (Baldock and Skjemstad, 2000) and may also provide protection for microorganisms (Skene et al., 1996). In a recent study, the stabilizing effect of clay on soil microbial biomass was even greater than that for humus (Müller and Höper, 2004). In addition to clays, multivalent cations and other nutrients affects the biological stability of OM in soils (Oades, 1988; van Veen et al., 1989; Nelson et al., 1996; Baldock and Skjemstad, 2000).

Soil microbial communities respond to changes in the soil environment. Drijber et al. (2000) compared soil microbial communities in soils under mixed prairie sod to those in soil cropped to wheat or left fallow. They found that during the wheat cycle the composition of the soil microbial community was influenced mostly by inputs from wheat while during fallow it responded to physicochemical differences in the soil resulting from tillage. Zymogenous microorganisms (opportunists that grow immediately following addition of a readily decomposable organic substrate) feeding on easily decomposable OM may adapt very quickly (in term of days) to the availability of

substrate (Müller and Höper, 2004). Degens (1998) determined that microbial functional diversity in soils, measured as catabolic potential, was influenced by the addition of different types of organic C. Beare et al., (1992) concluded that when plant residue is incorporated into the soil, microenvironments are created that can strongly influence the composition of decomposer communities by changing the amount and the distribution of substrates and consumers. Thus, selected groups of soil microorganisms are likely to govern the transformations of the different labile OM pools, and the rate at which each OM pool responds to changes in management or other perturbations will vary considerably between soil types and according to the nature of the organic inputs (Bending et al., 2000).

Soil microbial communities influence nutrient cycling and aggregation processes, though it is important to determine to what extent environmental and edaphic factors govern microbial communities. Phospholipid fatty acid (PLFA) profiles showed differences in community composition that were consistent with differences in organic C input under organic and conventional systems (Bossio et al., 1998). They also found that soil type was the most important determinant of microbial communities due to the influence of clay content and aeration. Schutter and Dick (2000) also found that fatty acid methyl esters (FAMEs) profiles were able to separate soils differing in texture and total organic C content. The objectives of this study were to identify the soil physical and chemical properties influencing ECa in this field and to determine whether variation in these properties influenced the composition of soil microbial communities.

MATERIALS AND METHODS

Experimental Site

The field study was conducted during 2003 near Shelton NE (40º45'01" N, 98º46'01"W). Soils at the site are Hord silt loam (Fine-silty, mixed mesic Pachic Argiustoll, 0-1% slope) and a Blendon loam (Coarse-loamy, mixed mesic Pachic Haplustoll, 0-1% slope). The Hord series are soils developed on loess-capped alluvial stream terraces of the Platte and Wood River valleys, formed in silty alluvium or in loess. The Blendon series are soils formed on stream terraces along the Platte and South Loup Rivers. They are underlain by mixed sand and gravel along the Platte River and by medium and coarse sand along the South Loup River. Land leveling activities were performed on portions of the field around 1960 to facilitate furrow irrigation (J.S. Schepers, personal communication, 2005). Continuous corn (*Zea mays* L.) has been cropped since 1990 under conventional disk-tillage with furrow irrigation, and since 1996 under disk and row cultivation with sprinkler irrigation. Prior to planting 168 kg N ha^{-1} as urea ammonium nitrate solution was incorporated with a field cultivator. Corn (Pioneer 31B51) was planted on May 16th, 2003 with 13.1 kg N ha^{-1} and 19.5 kg P ha^{-1}as diammonium phosphate applied adjacent to the seed. Stand density was 71600 plants ha^{-1} and field average yield was 12.9 Mg ha^{-1}at 155 g kg^{-1} moisture content.

Field Mapping

In May 2003, ECa was measured with an EM 38 dual dipole conductance meter (Geonics Ltd., Mississauga, Ontario, Canada[1]) pulled behind an all terrain vehicle. Data was logged in vertical mode (0- to 90- cm approximately) and horizontal mode (0- to 30-cm) at 1-s intervals (every 2 m) and georeferenced using a differential global positioning system receiver (Trimble Navigation, Sunnyvale, CA) mounted near the EM38 sensor. The data collected in this 7.3 ha field was processed using ERDAS Imagine (ERDAS Inc., Atlanta, GA) to create four ECa classes based on ranges of EC measurements using unsupervised classification (ERDAS, 1997)(Johnson et al., 2001). Briefly, unsupervised classification identifies statistically similar clusters, and combines them into classes. (Figure 1, Table 1). Six locations in each ECa class were randomly selected for soil sampling.

Soil Sampling and Analysis

Soil samples were collected during the V6 stage of corn (Ritchie et al., 1997) on June 20th, 2003. Ten soil cores (2 cm diameter, 0- to 15-cm depth) were randomly taken from the furrow at each location in an area of 6 m^2. (Figure 1) and combined. The composite soil samples were transported in a cooler to the laboratory and passed through a 4 mm sieve to remove visible organic residues, thoroughly mixed, and divided in two subsamples. One subsample was stored at –20 ºC for microbial biomass and fatty acid analysis. The other subsample was passed through 2 mm sieve, air-dried, and analyzed

[1] Mention of commercial products and organizations in this paper is solely to provide specific information. It does not constitute endorsement by USDA-ARS over other products and organizations not mentioned.

for clay content (Kettler et al., 2001), total OM and POM (0.05-2 mm) by weight loss-on-ignition (Cambardella et al., 2001), total C and N analyzed with a Carlo Erba NA 100 (CE Elantech, Lakewood, NJ) (Schepers et al., 1989), pH and electrical conductivity ($EC_{(1:1)}$) in 1:1 soil:water slurry (Smith and Doran, 1996). NO_3-N was determined by a commercial laboratory (Brown, 1998). Soil bulk density was determined by dividing the oven dry weight of the soil by the volume of the sample before sieving. Bulk density was similar among ECa classes and the average value of 1.28 Mg m^{-3} was used to convert the concentration data to volumetric data.

After harvest a soil core (5 cm diameter, 0- to 90-cm) was collected from the center of each location using a truck-mounted hydraulic soil sampler. Topsoil depth was defined as the depth from the surface to the first change of soil color. Carbonate depth was determined by dropping a 10% hydrochloric acid solution on the soil core and measuring the distance from the soil surface to where effervescence occurred. No effervescence was observed in any of the cores. The sample was divided in four sections: 0- to 15-cm, 15- to 30-cm, 30- to 60-cm and 60- to 90-cm. Soil particle size, Bray P, $EC_{(1:1)}$, total C and N of each depth increment were determined on air-dried, 2 mm sieved soil samples using methods described above. Total dissolved solids (TDS) were estimated by multiplying the $EC_{(1:1)}$ at 25 °C (dS/m) value for each depth interval by 0.64 and summing these values to obtain a TDS value for the 0- to 90-cm depth (Smith and Doran 1996).

Quantification, Identification and Classification of FAMEs:

Microbial biomass was determined as extractable lipid P on duplicate 1-g samples using perchloric acid digestion and determining the released phosphate by the method of

Bartlett (Kates, 1986) and reported on dry weight soil basis. Microbial community structure was based on extraction of total FAMEs by mild alkaline hydrolysis. This process does not methylate free fatty acids, but only ester-linked fatty acids (Kates, 1986; Grogan and Cronan, 1997). Briefly, 10-g soil samples were hydrolyzed using freshly prepared 0.2 M potassium hydroxide in methanol and the resulting FAMEs were partitioned into hexane (White et al., 1979). Following saponification to release ester-linked FAMEs, methyl-nonadecanoate (0.05 μg μl^{-1}) was added to the extract as an internal standard. Released FAMEs were separated by gas chromatography, using helium as a carrier gas, and an Ultra 2 HP (50 m, 0.2 mm I.D., 0.33 μm film thickness) capillary column. The gas chromatograph was run in split mode (44:1) with a 0.75 min purge time. Injector and flame ionization detectors were maintained at 280º and 300º C, respectively, and oven temperature was ramped from 50º C to 160º C at 40º C min^{-1} and held for 2 min, then ramped at 3º C min^{-1} to 300º C and held for 30 min. The fatty acids were identified by retention-time and confirmed by mass spectrometry. Concentrations of FAMEs were calculated from peak areas and reported as nmol g^{-1} soil.

Fatty acids were designated as the total number of C atoms followed by a colon, the number of double bonds followed by the position of the double bond from the carboxyl end of the molecule and its *cis* or *trans* configuration (IUPAC-IUB, 1987). The prefixes *a* and *i* indicate antieso and iso branching, respectively; *cy* indicates cyclopropane fatty acids, *br* indicates an unknown branch position and 10Me indicates a methyl branch on the 10^{th} C atom from the carboxyl end of the molecule. A total of 41 FAMEs were detected and identified. Those selected to represent bacterial markers were *i*C15:0, *a*C15:0, C15:0, *i*C16:0, *i*C17:0, *a*C17:0, C17:0, *cy*C17:0, *cy*C19:0 and C16:1*cis*7.

Frostegård and Bååth (1996) recommend the use of several bacterial fatty acid markers since this is likely to produce less variable results than the use of only one marker. The markers selected to represent actinomycetes were *i*10MeC18:0 and 10MeC18:0 (Kroppenstedt 1992). Fungal biomarkers was represented by C18:2*cis*9,12 (Stahl and Klug, 1996) and arbuscular mycorrhizal biomarkers by C16:1*cis*11 (Olsson and Johansen, 2000).

Statistical Analysis

Relationships between crop and soil components and ECa class were assessed by ANOVA for a complete randomized design with vertical and horizontal ECa class (identified using unsupervised classification) as treatment factors (Johnson et al., 2001). Analysis of variance (using PROC MIXED procedure) was also performed, and to test for differences in physical and chemical soil properties among ECa class means we used Fisher's protected LSD test. Pearson correlation analysis was conducted to assess the linear association between ECa sample means for each location and soil physicochemical and microbiological properties. All statistical analyses were performed using SAS (SAS Inst., 1999) and differences were declared significant at the 0.05 level unless stated otherwise.

RESULTS AND DISCUSSION

Relationship of ECa Class to Soil Properties in the 0- to 90-cm depth

The range of vertical and horizontal ECa measurements and soil physicochemical properties are shown in Table 1. Of the physical and chemical soil properties measured we found that clay content in class I was lower than that observed in class III and IV (Figure 2a). Topsoil depth was similar for classes I, II and III and greater in class IV. The increase in ECa was correlated with the amount of clay and depth of topsoil; however the correlation coefficient between ECa and total clay was higher (Table 2). Total C content (and N, data not shown) followed the pattern of clay content in this profile (Figure 2c) being largest in class IV followed by class III and smallest in classes I and II. Total dissolved solids were lowest in class I and largest in class IV (Figure 2d).

Horizontal and vertical ECa measurements were highly correlated with clay, organic C content, TDS, and topsoil depth in the 0- to 90-cm samples (Table 2). Bray P was associated with vertical ECa but not with horizontal readings. Depth of topsoil was positively correlated with clay and C content. Bulk ECa readings integrated many soil properties and appear to be a useful tool for the delineation of overall soil condition (Johnson et al., 2001). The correlation among clay, total C, TDS, depth of topsoil and ECa may represent soil properties affecting water availability to the crop. Previous studies indicated that in the central Great Plains, vertical ECa was driven by soil water content, solids in the soil solution, and clay content at 0- to 90-cm depth and correlated with soil water-holding capacity (Johnson et al., 2003b). Similarly, Auerswald et al. (2001) used multivariate regression to determine the relationship among ECa and a

number of soil properties and found that clay content, $EC_{(1:1)}$, and water content explained 84% of the variability observed in ECa.

Relationship of ECa Class to Soil Properties in the 0- to 15-cm depth

While ECa appears to integrate soil properties over depth, management impacts are usually most evident in the surface soil layer. The physical, chemical and biological soil parameters evaluated to characterize the surface layer (0- to 15-cm) of this field are presented in Table 3. Soil pH was similar among the ECa classes with a mean value of 6.7 (p-value = 0.57). Simple correlation coefficients of spatially varying soil properties provide the first level of information needed to determine what factors covary with ECa. Horizontal and vertical ECa measurements were positively correlated with Bray P and TDS measured in the laboratory, and were negatively correlated with mycorrhizal fungal biomarker concentration. No significant correlations were observed between ECa and total C, fine and coarse POM, and clay content in this surface layer (Table 3). However, there was a significant relationship observed between total C, POM, lipid P and the individual microbial pools (fungi and bacteria biomarker concentrations) (Table 3).

Total C (Figure 3b) and the different fractions of POM (Figure 3d) in the 0- to 15-cm depth followed the same distribution as clay content (Figure 3a) with an increase in ECa class III. In contrast, Bray P in the 0- to 15-cm depth (Figure 3c) followed the same trend as the 0- to 90-cm variables. Thus, among the physical, chemical and biological soil properties in the 0- to 15-cm depth, clay content appeared to influence the size of the C pools of this stratum. The amount of clay in this surface layer increased as ECa increased

for the first three ECa classes but was less than expected in ECa IV likely due to land leveling activities altering texture in this part of the field (Figure 3a). This resulted in no correlation between ECa and clay content in the 0- to 15-cm depth (Table 3). Mueller et al. (2003) found stronger correlations for horizontal than for vertical ECa measurements with chemical soil properties, clay content, and volumetric water content. These authors found that deep EC readings were similarly affected by the variability in ECa at the surface (0- to 30- cm) and subsurface (30- to 90-cm) layers. Our results also showed a high correlation between horizontal and vertical ECa (Table 2); however, there was dissimilar pattern in clay and C distribution with ECa class when comparing the 0- to 90-cm and 0- to 15-cm depths (data not shown). The different response of ECa to soil characteristics reported by Mueller et al. (2003) may be due to differences in parent material and soil water content in that study.

Fine POM was negatively correlated with bulk density and positively correlated with TDS, total C, and Bray P, while coarse POM was somewhat correlated with NO_3-N ($r = 0.38$, $p = 0.07$) (Table 3). These results may indicate a linkage with crop biomass production, size of the POM fraction and fertility status of this soil. The relationship between POM and chemical soil properties important to OM cycling include deposition of plant residues and roots (coarse POM) as well as microbial residues produced during decomposition (Baldock and Skjemstad, 2000). Fine POM was also correlated with the amount of clay, and this relationship is likely determined by the amount of fine mineral particles in the surface layer and the physical and chemical protection of different C pools by clay particles (Baldock and Nelson, 1999). Wander and Bollero (1999) found that POM was the most sensitive indicator of soil management practices contributing to soil

quality in Illinois. They also found that biological and physical aspects of soils were the properties most altered by agronomic practices. Under dryland conditions, Johnson et al (2001) found that POM, total C and microbial biomass C were negatively correlated with ECa measurements.

Bray P increased as ECa readings increased (Figure 3c and Table 3). An aerial photo used in the soil survey (Buller et al.,1974) showed that the portion of the field that comprises ECa class IV was segregated by fencing under different management. Specific management practices for this part of the field are not known but manure applications or different fertilizer practices than the rest of the field may explain the differences. Heiniger et al. (2003) attributed part of the success of using ECa to measure P concentration in the field to salinity imparted by manure. Furthermore, ECa maps were able to differentiate rates of compost, manure and commercial fertilizer additions to a corn field (Eigenberg and Nienaber, 1998). Others have correlated ECa to tillage and cropping patterns (Clay et al., 2001; Johnson et al., 2003a).

Grain and stover yield were correlated variables. Vertical and horizontal ECa and Bray P (0- to 15-cm) were correlated with stover yield (Table 3). Stover production increased as vertical ECa increased (Stover = 3.58 + 0.11 ECa; $r^2 = 0.38$; $p < 0.001$). However, there were no differences among classes in grain production (mean 8.5 Mg ha^{-1}). The lack of a correlation between grain yield and ECa may be related to the high availability of water in this irrigated corn field. Under dry land conditions, Kravchenko et al. (2003) found that in years with optimum precipitation grain yield and EC were not correlated.

Relationship of Microbial Biomarkers to POM

Lipid P, indicative of soil microbial biomass, was higher in ECa classes III and IV, than in classes I and II (Figure 4a). At this early growth stage of the crop (V6 stage) total microbial biomass in the furrow environment seems to be driven by bacterial biomarker concentration (r=0.96, Figure 5). Bacterial and actinomycetes biomarker concentrations were highly correlated (r=0.88) and increased with increasing ECa (Figures 4b and 4c), although not significantly in all cases, mirroring that of clay and total C in this surface soil layer. Schutter et al. (2001) studied the soil microbial response to soil type, seasonal changes, and soil management using FAMEs. They found that FAMEs profiles were correlated with soil texture, cation exchange capacity and C content in early spring. Fungal and mycorrhizal biomarker concentrations were not different among ECa classes (Figure 4d) although mycorrhizal biomarker concentration was negatively correlated with both horizontal and vertical ECa readings (Table 3). Mycorrhizal biomarker concentration was negatively correlated with ECa values (Table 3) but mean values were similar across ECa classes (Figure 4d). Fine POM was highly correlated with bacterial and actinomycetes biomarkers, while coarse POM was highly correlated with fungal biomarker concentration, and to a lesser extent with actinomycetes, bacterial and mycorrhizal biomarkers (Figure 5). Similarly, several fungal biomarkers (C18:2*cis*9,12 and 18:3*cis*6,9,12) were enriched in soils after the incorporation of winter cover crops, suggesting that the addition of these residues were important for stimulating or maintaining fungal populations over time (Schutter et al.2001).

The positive correlation between coarse POM and the soil microbial biomarkers (Table 3, Figure 5) suggests that fungi, bacteria and actinomycetes all use coarse POM as a source of C. Coarse POM is mainly comprised of plant residues, but may contain seeds and microbial structural components such as fungal hyphae and spores (Dalal and Mayer, 1986a, b; Oades et al., 1987), indicating an early stage of decomposition and a microbial contribution, mostly fungal, to this coarse fraction (Six et al., 2001; Six et al., 2004). Baldock and Skjemstad (2000) used nuclear magnetic resonance to analyze the structure of POM and found that it is dominated by O-alkyl structures such as polysaccharides typically found in fresh plant and microbial tissues. They identified these organic compounds as being preferentially used by soil microorganisms: a decrease in particle size being associated with the accumulation of more chemically recalcitrant structures in the 2- to 20-μm size fraction. As plant residues decompose, lignin-degrading fungi are essential to continued decomposition; however, some bacteria can modify the nature of functional groups attached to lignin exposing more labile structures (Baldock and Skjemstad, 2000). Thus, the relationship among soil microbial biomass and the different fractions of POM reflects the decomposition dynamics and stability of C in the ecosystem. Our results indicate fungal groups are closely associated with coarse POM early in the growing season.

Fine POM was correlated with clay content, but the coarse POM fraction was not (Table 3). This supports an intimate association between fine POM and the mineral fraction in this soil that enhances stabilization of soil C (Ladd et al., 1985). This protection of soil C may also be afforded through the process of aggregation. Golchin et al. (1994) suggested that during the transformation of free C into intraaggregate light C

there is a selective decomposition of easily decomposable carbohydrates (i.e. O-alkyl C) and preservation of more recalcitrant long-chained C (i.e. alkyl C). During this process, POM is reduced in size and gradually becomes encrusted with clay particles and microbial products to form microaggregates (Golchin et al., 1994). Bacteria may be occluded inside these aggregates, protected from predation and linked to this pool of POM, which is less accessible to fungal biomass. Our results support the association of bacterial and actinomycetes biomarkers with the fine POM fraction (Figure 5, Table 3) indicating that pools of protected C with a slower decomposition rate might be preferentially colonized by bacteria instead of fungi.

The mycorrhizal marker was negatively correlated with Bray P in this surface layer (Table 3). Spore germination and hyphal growth of mycorrhizal fungi is known to be heavily dependent on the availability of soil P (De Miranda and Harris, 1994); thus, mycorrhizal biomass decreased as the availability of P increased in the surface soil. Since we collected samples in the furrow environment, our study would reflect remnants of the prior year's mycorrhizal network as well as any new hyphae formed at this early growth stage. Although mycorrhizal fungi are important for plant P uptake, the significance of this role under the high fertility status of this system is uncertain.

In conclusion, ECa classes were defined by the amount of clay, organic C, and TDS in the 0- to 90- cm depth as well as the depth of topsoil. EC was an effective tool to identify soil sampling sites that represent diverse soil physicochemical properties. Soil properties measured for the shallow depth were not highly correlated with ECa except for TDS and Bray P. Soil physicochemical characteristics evaluated in the 0- to 15-cm layer are influenced by management practices and prior history of this field. Soil microbial

biomass was related to total C and clay content in this surface layer. However, inherent soil chemical and physical properties in the 0- to 90-cm depth influenced potential production of corn dry matter, and hence, the amount of corn residue returned to the soil. Over time greater residue inputs in ECa IV should result in increases in OM and microbial biomass. Thus, soil biological properties in the surface layer are expected to correlate more strongly over time with ECa class. Soil microorganisms play important roles in residue decomposition, nutrient cycling, and maintenance of soil structure. Thus, knowledge of their spatial variability will lead to improved management of spatially variable fields.

ACKNOWLEDGMENTS

We thank Liz Jeske, Susan Siragusa, Susan Wagner, Paul Koerner for laboratory assistance and field data collection. We also thank Scott Stubblefield for allowing access to his field and willingness to provide management input data.

REFERENCES

Auerswald, K., S. Simon, and H. Stanjek. 2001. Influence of soil properties on electrical conductivity under humid water regimes. Soil Sci. 166:382-390.

Baldock, J.A. and J.O. Skjemstad. 1999. Soil organic carbon/soil organic matter. pp. 159-170. In: Peverill, K.I., Sparrow, L.A., Reuter, D.J. (Eds.), Soil Analysis: An Interpretation manual. CSIRO Publishing, Melbourne, Australia.

Baldock, J.A., and P.N. Nelson. 1999. Soil organic matter. p. D3-D18. In M.E. Summer et al.(ed.) Handbook of soil science. CRC Press, Boca Raton, FL.

Beare, M.H., R.W. Parmelee, P.F. Hendrix, and W. Cheng. 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecol. Monog. 62:569-591.

Bending, G.D., C. Putland, and F. Rayns. 2000. Changes in microbial community metabolism and labile organic matter fractions as early indicators of the impact of management on soil biological quality. Biol. Fert. Soils 31:78-84.

Bossio,D.A., K.M. Scow, and K.J. Graham. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipids fatty acid profiles. Microb. Ecol. 36:1-12.

Bray, R.H., and L.T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39-45.

Brown, J.R. 1998. (Ed.) Recommended chemical soil test procedures for the north central region. North Central Region Research Publication No. 221 (Revised), 72 pages. Missouri Agricultural Experiment Station SB 1001.

Buller, L.L., R.S. Pollock, R.A. Boccheciamp, C.L. Hammond, H. Hill, and J. A.Elder. 1974. Soil Survey of Buffalo County, Nebraska. USDA-SCS. U.S. Gov. Print. Office, Washington, D.C.

Cambardella, C.A., A.M. Gajda, J.W. Doran, B.J. Wienhold, and T.A. Kettler. 2001. Estimation of particulate and total organic matter by weight loss on ignition. p. 349-359. In R. Lal et al. (ed) Assessment methods for soil carbon. CRC Press, Boca Raton, FL.

Clay, D.E., J. Chang, D.D. Malo, C.G. Carlson, C. Reese, S.A. Clay, M. Ellsbury, and B. Berg. 2001. Factors influencing spatial variability of soil apparent electrical conductivity. Comm. Soil sci. plant anal. 32:2993-3008.

Dalal, R.C. and R.J. Mayer. 1986a. Long term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. III Distribution and kinetics of soil organic carbon in particle-size fractions. Aust. J. Soil Res. 24:293-300.

Dalal, R.C. and R.J. Mayer. 1986b. Long term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. IV Loss of organic carbon from different density functions. Aust. J. Soil Res. 24:301-309.

Degens, B.P. 1998. Microbial functional diversity can be influenced by the addition of simple organic substrates to soil. Soil Biol. Biochem. 30:1981-1988.

De Miranda, J.C.C and P.J. Harris. 1994. Effects of soil phosphorous on spore germination and hyphal growth of arbuscular mycorrhizal fungi. New Phytol. 128:103-108.

Drijber, R.A.; J.W. Doran, A.M. Parkhurst, and D.J. Lyon. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol. Biochem. 32:1419-1430.

Eigenberg, R.A., J.W. Doran, J.A. Nienaber, R.B. Ferguson, and B.L. Woodbury. 2002. Electrical conductivity monitoring of soil condition and available N with animal manure and a cover crop. Agr. Ecosyst. Envron. 88:183-193.

Eigenberg, R.A. and J.A. Nienaber. 1998. Electromagnetic survey of cornfield with repeated manure applications. J. Environ. Qual. 27:1511-1515.

ERDAS. 1997. ERDAS field guide. ERDAS, Inc., Atlanta, GA.

Frostegård, A., and E. Bååth. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22: 9-65.

Gale, W.J., C.A. Cambardella, and T.B. Bailey. 2000. Root-derived carbon and the formation and stabilization of aggregates. Soil Sci. Soc. Am. J. 64:201-207.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994. Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning electron microscopy. Aust. J.Soil Res. 32:285-309.

Grant, C.A., D.N. Flaten, D.J. Tomasiewicz, and S.C. Sheppard. 2001. The importance of easly season phosphorous nutrition. Can. J. Plant Sci. 81:211-224.

Gregorich, E.G., and H.H. Janzen. 1996. Storage of soil carbon in the light fraction and macroorganic matter. p. 167-190. *In* M.R. Carter, and B.A. Stewart (ed.) Structure and organic matter storage in soils. Lewis Publ., CRC Press, Boca Raton, FL.

Grogan, D.W., and J.E. Cronan, Jr. 1997. Cyclopropane ring formation in membrane lipids of bacteria. Microbiol. Mol. Biol. Rev. 61:429-441.

Heiniger, R.W., R.G. McBride, and D.E. Clay. 2003. Using soil electrical conductivity to improve nutrient management. Agron. J. 95:508-519.

IUPAC-UIB. 1987. The nomenclature of lipids. J. Lipid Res. 19:114-128.

Johnson, C.K., K.M. Eskridge, B.J. Wienhold, J.W. Doran, G.A. Peterson, and G.W. Buchleiter. 2003a. Using electrical conductivity classification and within-field variability to design field-scale research. Agron. J. 95:602-613.

Johnson, C.K., D.A. Mortensen, B.J. Wienhold, J.F. Shanahan, and J.W. Doran. 2003b. Site-specific management zones based on soil electrical conductivity in a semiarid cropping system. Agron. J. 95:303-315.

Johnson, C.K., J.W. Doran, H.R. Duke, B.J. Wienhold, K.M. Eskridge, and J.F. Shanahan. 2001. Field-scale electrical conductivity mapping for delineating soil condition. Soil Sci. Soc. Am. J. 65:1829-1837.

Kates, M., 1986. Techniques of lipidology: isolation, analysis and identification of lipids. *In*: Burdon, R.H., van Kippenberg, P.H. (eds.), Laboratory Techniques in Biochemistry and Molecular Biology, vol. 3, Part 2. Elsevier, NY.

Kettler, T.A., J.W. Doran, and T.L. Gilbert. 2001. A simplified method for soil particle size determination to accompany soil quality analyses. Soil Sci. Soc. Am. J. 65:849-852.

Kitchen, N.R., K.A. Sudduth, and S.T. Drummond. 1999. Soil electrical conductivity as a crop productivity measure for claypan soils. J. Prod. Agric. 12:607-617.

Kravchenko, A.N., K.D. Thelen, and N.R. Miller. 2003. Relationship among crop grain yield, topography, and soil electrical conductivity studied with cross-correlograms. Agron. J. 95:1132-1139.

Kroppenstedt, R.M., 1985. Fatty acid and menaquinon analysis of actinomycetes and related organisms. p. 173-199. *In*: Goodfellow, M., Minnikin, D.E. (eds.), Chemical Methods in Bacterial Systematics. Academic Press, London.

Ladd, J.N., M. Amato, and J.M. Oades. 1985. Decomposition of plant material in Australian soils. III. Residual organic and microbial biomass C and N from isotope-labeled legume material and soil organic matter, decomposing under field conditions. Aust. J. Soil Res. 23:603-611.

Mueller, T.G., N.J. Hartsock, T.S. Stombaugh, S.A. Shearer, P.L. Cornelius, and R.I. Barnhisel. 2003. Soil electrical conductivity map variability in limestone soils overlain by loess. Agron. J. 95:496-507.

Müller, T., and H. Höper. 2004. Soil organic matter turnover as a function of the soil clay content: consequences for model applications. Soil Boil. Biochem. 36:877-888.

Nelson, P.N., J.N. Ladd, and J.M. Oades. 1996. Decomposition of ^{14}C-labelled plant material in a salt affected soil. Soil Boil. Biochem. 28:433-441.

Oades, J.M., A.M. Vassallo, A.G. Waters, and M.A. Wilson. 1987. Characterization of organic matter in particle size and density fractions from Red-Brown Earth by solid-state 13C NMR. Aust. J. Soil Res. 25:71-82.

Oades, J.M. 1988. The retention of organic matter in soils. Biogeochemistry 5:35-70.

Olsson, P.A. and A. Johansen. 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycol. Res. 104:429-434.

Ritchie, S.W., J.J. Hanaway, and G.O. Benson. 1997. How a corn plants develops. Spec. Publ. 48. Iowa State Univ. Coop. Ext. Serv., Ames.

Rhoades J.D. and D.L. Corwin. 1990. Soil electrical conductivity: effects of soil properties and application to soil salinity appraisal. Comm. Soil Sci. Plant Anal. 21:873-860.

Saggar, S., A. Parshotam, G.P. Sparling, C.W. Feltham, and P.B.S. Hart. 1996. ^{14}C-labelled ryegrass turnover and residence times in soils varying in clay content and mineralogy. Soil Biol. Biochem. 28:1677-1686.

SAS Institute, 1999. SAS/STAT software: Changes and enhancements through release 8.0. SAS Inst., Cary, NC.

Schepers, J.S., D.D. Francis, and M.T. Thompson. 1989. Simultaneous determination of total C, total N, and 15N on soil and plant material. Commun. Soil Sci. Plant Anal. 20:949-959.

Schutter, M.E. and R. P. Dick. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Sci. Soc. Am. J. 64:1659-1668.

Schutter, M.E., J.M. Sandeno, and R.P. Dick. 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. Biology and Fertility of Soils 34:397-410.

Six, J., G. Guggenberg, K. Paustian, K., E.T. Elliot and W. Zech. 2001. Sources and composition of soil organic matter frecations between and within aggregates. Eur. J. Soil Sci. 52:607-618.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the linkage between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil Till. Res. 79:7-31.

Skene, T.M., J.O. Skjemstad, J.M. Oades, and P.J. Clarke. 1996. The influence of inorganic matrices on the decomposition of straw. Aust. J. Soil Res. 34:413-426.

Smith, J.L., and J.W. Doran. 1996. Measurement and use of pH and electrical conductivity for soil quality analysis. p. 169-186. *In* J.W Doran, and A.J. Jones (ed.) Methods for assessing soil quality. ASA, CSSA, and SSSA, Madison, WI.

Stahl, P.D. and M.J. Klug. 1996. Characterization and differentiation of filamentous fungi based on fatty acid composition. App. Envir. Microb. 62:4136-4146.

van Veen, J.A., R. Merckx, and S.C. van de Geijn. 1989. Plant- and soil related controls of the flow of carbon from the roots through the soil microbial biomass. Plant soil 115:179-188.

Wander, M.M. and G.A. Bollero. 1999. Soil quality assessment of tillage impacts in Illinois. Soil Sci. Soc. Am. J. 63:961-971.

White, D.C., W.M. Davis, J.S. Nickels, J.D., King, and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40: 51-62.

Wienhold, B.J. and Zhang, R. 2001. Use of electrical conductivity to estimate N-mineralization contributions to crop N availability. Proc. XII World Fertil. Congr. of China International Exhibition Center. 1:390-397. Beijing, China.

Table 1. Range of values of ECa vertical and horizontal readings, topsoil depth and variables studied in the depth 0- to 90-cm.

Class		ECav	ECah	Topsoil depth	Bray P	Clay content	TDS	TC	TN
		dS m^{-1}	dS m^{-1}	cm	kg ha^{-1}	----------Mg ha^{-1}----------			
I	Max	20	15	45	716	2031	2.0	131	15
	Min	19	14	35	78	1474	1.7	107	13
II	Max	22	17	42	139	2290	2.5	146	16
	Min	21	16	28	40	1482	1.8	88	11
III	Max	25	20	55	236	2561	2.5	165	17
	Min	23	19	30	28	1722	2.0	116	13
IV	Max	35	26	62	328	2922	3.1	204	19
	Min	28	22	37	33	2183	2.1	167	16

ECa(v)=ECa vertical readings, ECa(h)=ECa horizontal readings, TC=total C, and TN=total N, TDS=total dissolved solids, Bray P=extractable P.

Table 2. Correlation matrix for grain and stover yield, soil physical and chemical properties (0- to 90-cm)

	ECa(v)	ECa(h)	Grain	Stover	Bray P	Depth	TC	Clay	TDS
ECa(v)	1								
ECa(h)	0.98**	1							
Grain	0.30	0.27	1						
Stover	0.62**	0.61**	0.54**	1					
Bray P	-0.33	-0.43*	-0.13	-0.24	1				
Depth	0.70**	0.63**	0.30	0.65**	0.10	1			
TC	0.87**	0.88**	0.23	0.75**	-0.28	0.69**	1		
Clay	0.83**	0.81**	0.17	0.61**	-0.34	0.66**	0.84**	1	
TDS	0.68**	0.71**	0.13	0.38	-0.29	0.32	0.59**	0.55**	1

ECa(v)=ECa vertical readings, ECa(h)=ECa horizontal readings, Bray P=extractable P, Depth=topsoil depth, TC=total C, TDS= total dissolved solids. N = 24

*, ** Correlation between measured attributes is significant at the 0.05 and 0.01 levels, respectively.

Table 3. Correlation matrix for grain and stover yield, soil physical and chemical properties (0- to 15-cm)

	ECa(v)	ECa(h)	Grain	Stover	Clay	Bd	TDS	TC	N-NO_3	Bray P	CPOM	FPOM	Lipid P	Bact	Actin	Myco	Fungi
ECa(v)	1																
ECa(h)	0.98**	1															
Grain	0.30	0.27	1														
Stover	0.62**	0.61**	0.54**	1													
Clay	0.25	0.30	-0.16	0.29	1												
Bd	-0.33	-0.36	0.21	-0.16	-0.70**	1											
TDS	0.43*	0.45*	-0.11	0.15	0.56**	-0.61**	1										
TC	0.26	0.30	0.0	0.21	0.85**	-0.63**	0.58**	1									
N-NO_3	0.12	0.14	-0.01	-0.09	0.12	-0.23	0.61**	0.24	1								
Bray P	0.79**	0.81**	0.17	0.65**	0.37	-0.42*	0.56**	0.27	0.17	1							
CPOM	-0.6	-0.2	-0.24	-0.18	0.21	-0.21	0.30	0.50*	0.38	0.0	1						
FPOM	0.28	0.33	-0.13	0.07	0.51*	-0.62**	0.54**	0.53**	0.17	0.43*	0.47*	1					
Lipid P	0.27	0.34	-0.22	0.08	0.66**	-0.66**	0.65**	0.72**	0.30	0.46*	0.67**	0.87**	1				
Bact	0.22	0.29	-0.13	0.10	0.68**	-0.59**	0.61**	0.73**	0.28	0.40†	0.69**	0.85**	0.96**	1			
Actin	0.11	0.18	-0.25	-0.4	0.74**	-0.61**	0.71**	0.72**	0.40†	0.34	0.47*	0.71**	0.82**	0.88**	1		
Myco	-0.45*	-0.43*	-0.29	-0.57**	0.06	0.06	-0.07	0.18	0.17	-0.53**	0.48*	0.25	0.29	0.37	0.34	1	
Fungi	-0.37	-0.37	-0.31	-0.50*	0.0	-0.04	0.21	0.24	0.46*	-0.35	0.77**	0.25	0.43*	0.44*	0.35	0.74**	1

ECav=ECa vertical readings, ECah=ECa horizontal readings, TC=total C, Bray P=extractable P, Bd=bulk density, FPOM= fine particulate organic matter (0.053-0.5 mm); CPOM=coarse particulate organic matter (0.5-2mm), TDS= total dissolved solids, Lipid P=total microbial biomass; Bact=bacterial markers (*i*C15:0, *a*C15:0, C15:0, *i*C16:0, *i*C17:0, *a*C17:0, C17:0, *cy*C17:0, *cy*C19:0,C16:1*cis*7); Actin=actinomycetes markers (*i*10MeC18:0, 10MeC18:0), Myco=mycorrhizal marker (C16:1*cis*11); Fungi=fungal marker (C18:2*cis*9,12). N = 24.

*, ** Correlation between measured attributes is significant at the 0.05 and 0.01 levels, respectively.

† Correlation between measured attributes is significant at the 0.06 level.

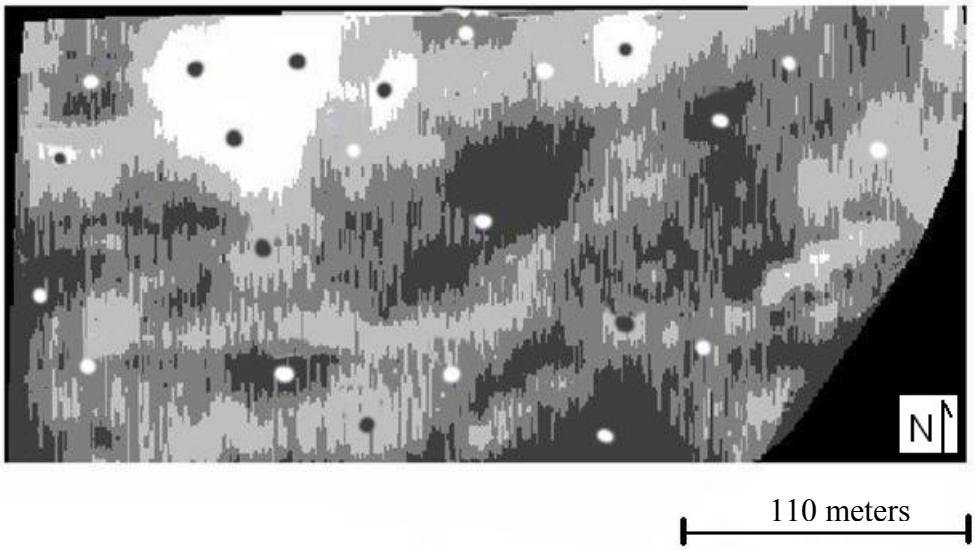


Figure 1. Four ECa classes and location of sampling sites (circles) in each class at the Buffalo Co., NE study site. ECa class I (19 to 20 dS m^{-1}), ECa class II (21 to 22 dS m^{-1}), ECa class III (23 to 25 dS m^{-1}), and ECa class IV (28 to 35 dS m^{-1}).

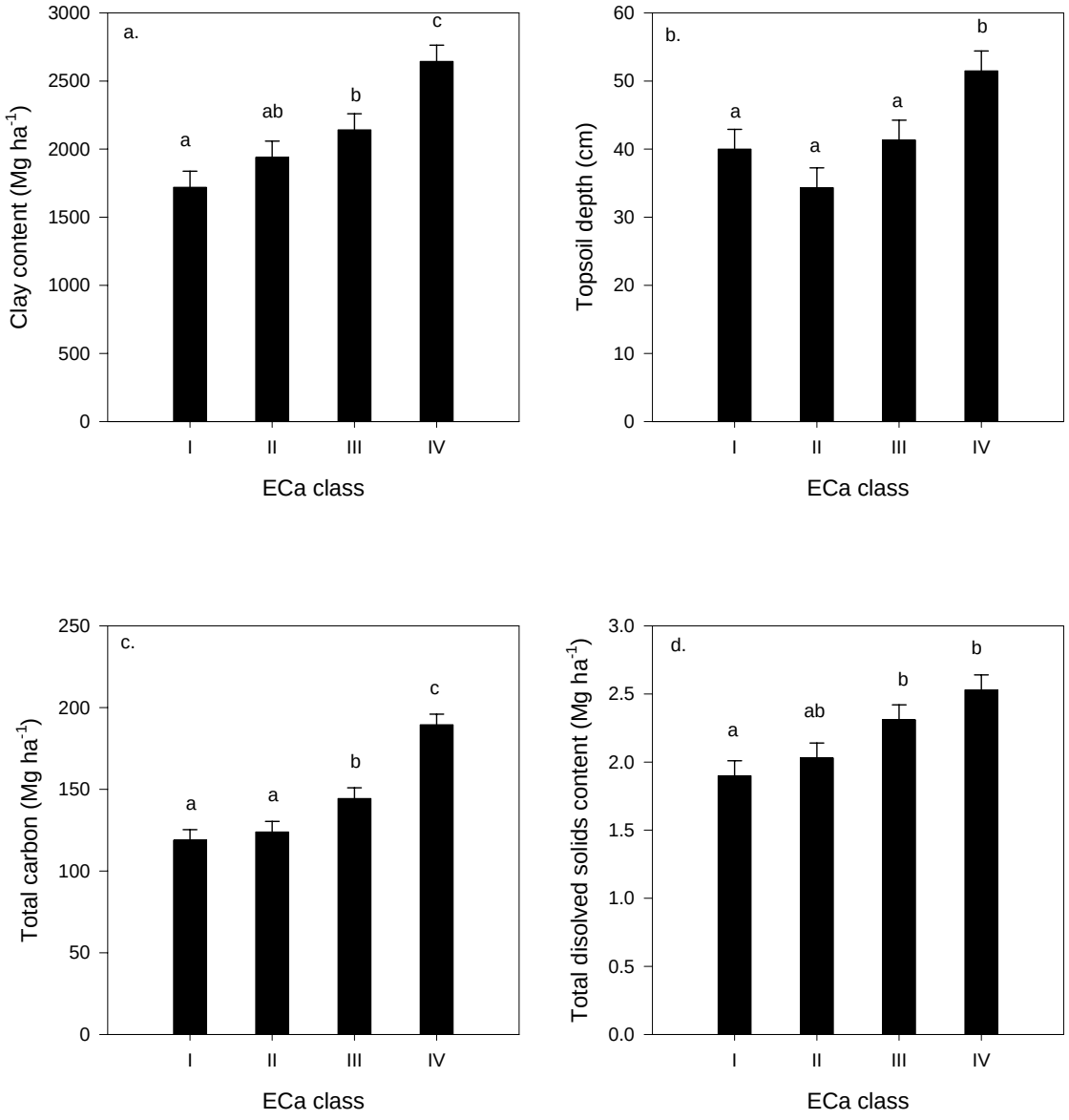


Figure 2. Apparent electrical conductivity (ECa) classes and mean values for a) clay content (0- to 90-cm)(SE = 118), b) topsoil depth (SE = 2.9), c) total C (0- to 90-cm)(SE = 6.5), and d) total dissolved solids content (0- to 90-cm)(SE = 0.1). Bars followed by the same letter within ECa classes are not different ($p<0.05$). N = 24. Error bars represent ± one standard error of the mean.

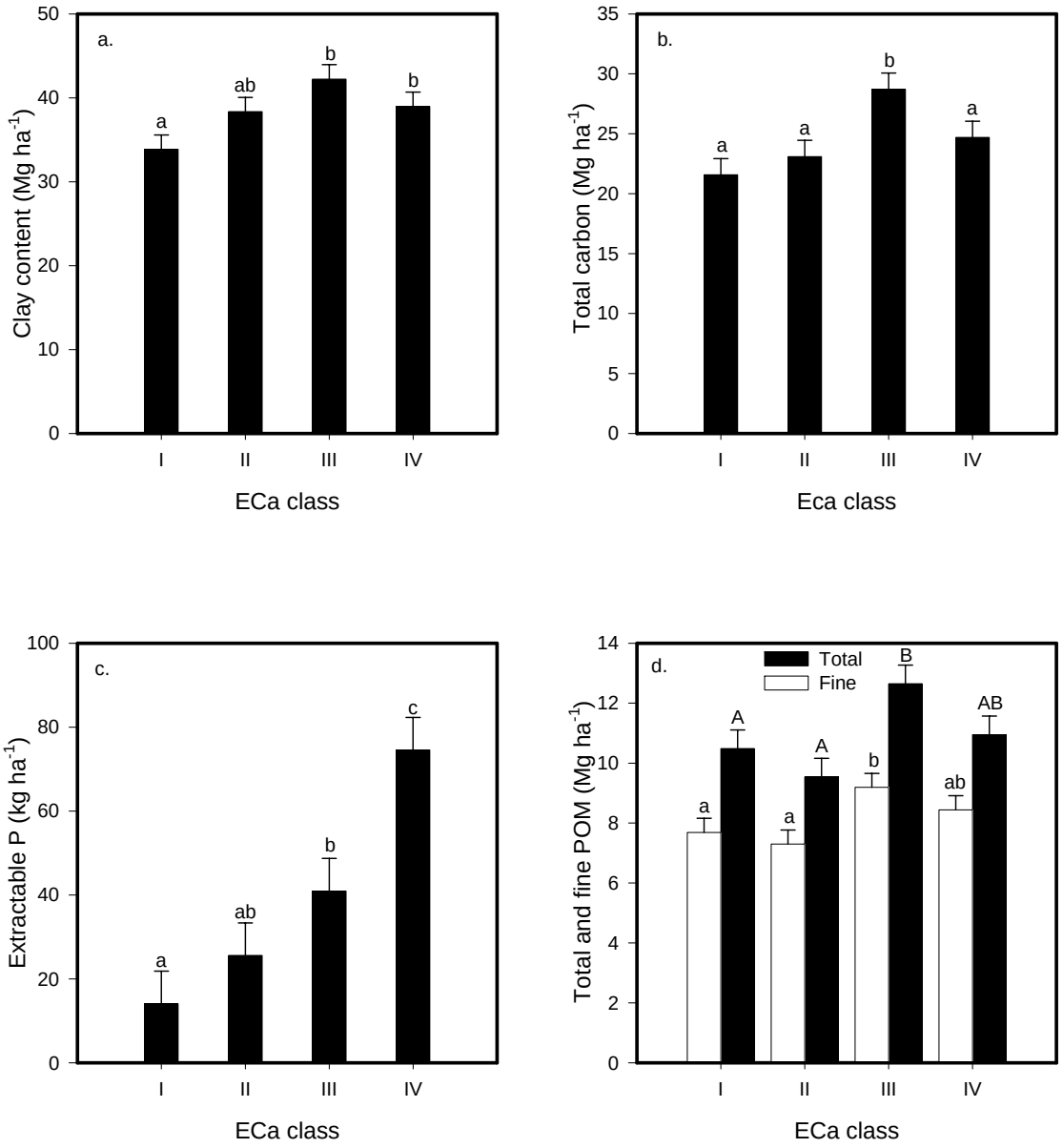


Figure 3. Apparent electrical conductivity (ECa) classes and mean values for a) clay content (0- to 15-cm)(SE = 1.7), b) total C (0- to 15-cm)(SE = 1.35), c) extractable P (0- to 15-cm)(SE = 7.76) and d) total and fine POM (0- to 15-cm)(SE = 0.6 and 0.5 respectively). Bars followed by the same letter within ECa classes are not different ($p<0.05$). N = 24.Error bars represent ± one standard error of the mean.

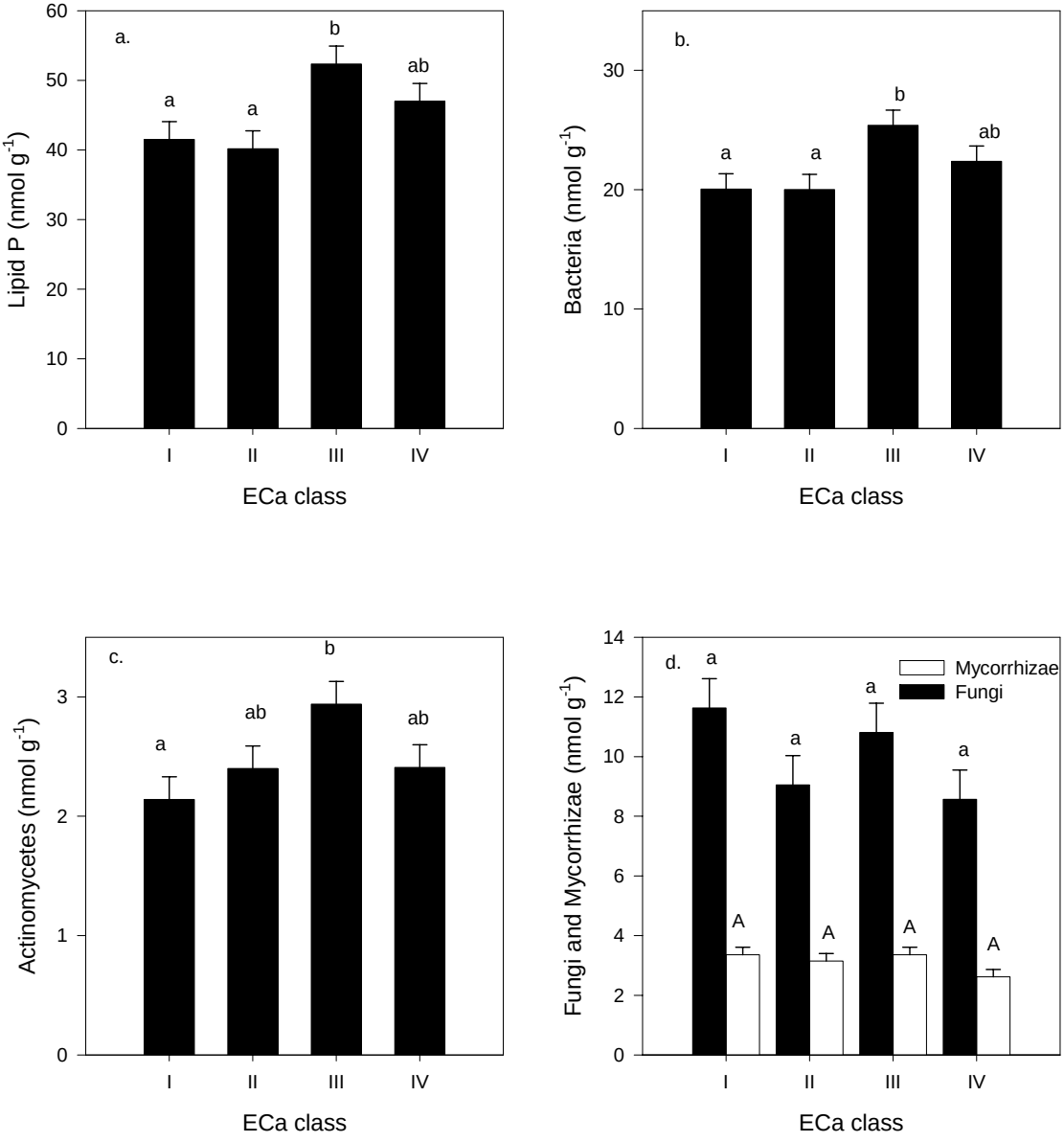


Figure 4. Apparent electrical conductivity (ECa) classes and mean values for biological soil properties 0- to 15-cm depth: a) total microbial biomass measured as lipid P (SE = 2.6); b) Bacteria=bacterial fatty acid markers (*i*C15:0, *a*C15:0, C15:0, *i*C16:0, *i*C17:0, *a*C17:0, C17:0, *cy*C17:0, *cy*C19:0,C16:1*cis*7)(SE = 1.5); c) Actinomycetes= actinomycetes fatty acid markers (*i*10MeC18:0, 10MeC18:0)(SE = 0.2); and d) Fungi=fungal fatty acid marker (C18:2*cis*9,12) (SE = 1.0) and Mycorrhizae=mycorrhizal fatty acid marker (C16:1*cis*11)(SE = 3.4). Bars followed by the same letter within ECa classes are not different ($p<0.05$). N = 24. Error bars represent ± one standard error of the mean.

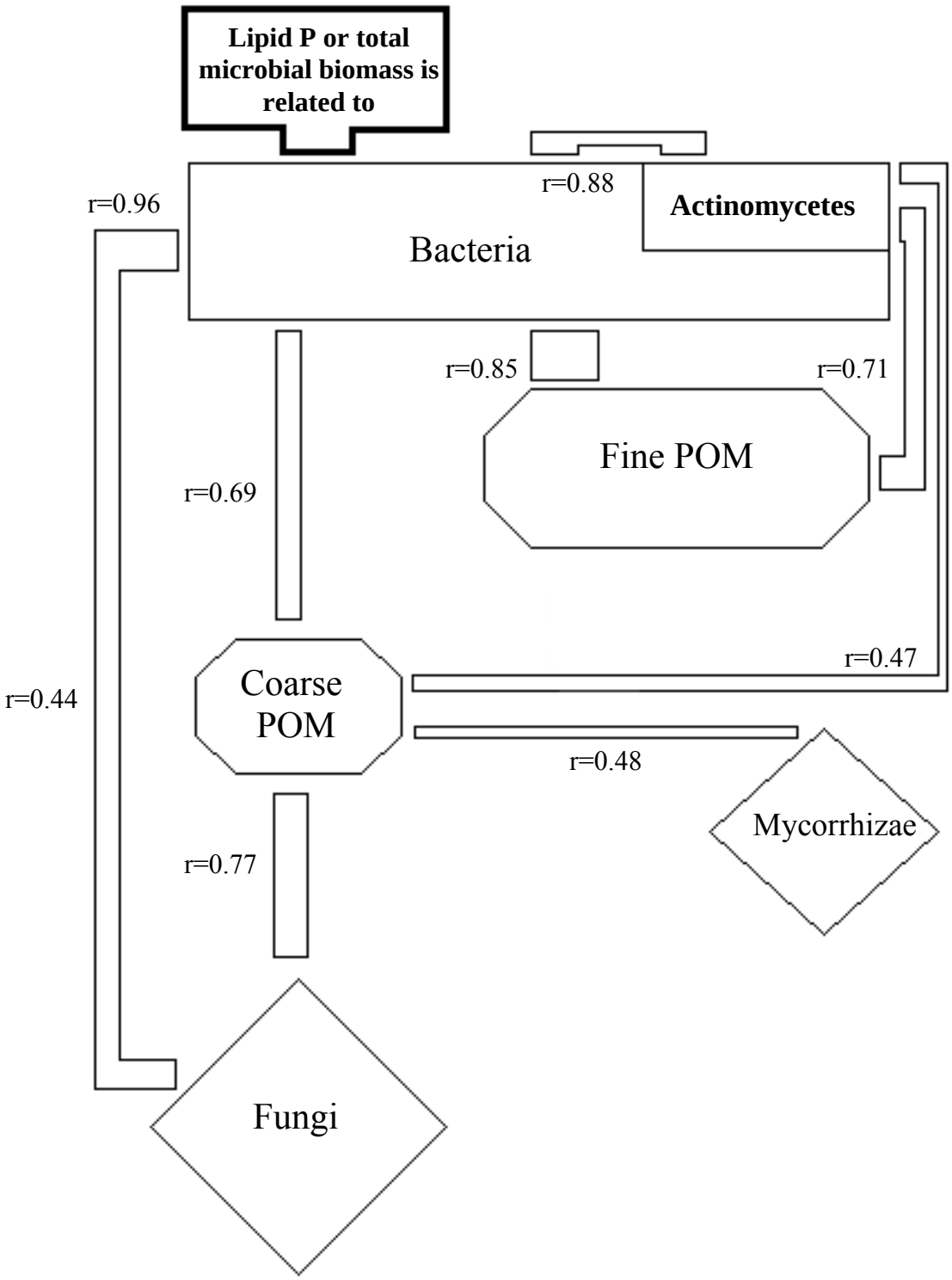


Figure 5. Diagram of correlations among coarse and fine POM fractions and different microbial groups in the early stages of the crop. Bacteria=bacterial fatty acid markers (*i*C15:0, *a*C15:0, C15:0, *i*C16:0, *i*C17:0, *a*C17:0, C17:0, *cy*C17:0, *cy*C19:0,C16:1*cis*7); Actinomycetes=actinomycetes fatty acid markers (*i*10MeC18:0, 10MeC18:0); Fungi=fungal fatty acid marker (C18:2*cis*9,12) and Mycorrhizae=mycorrhizal fatty acid marker (C16:1*cis*11), as nmol g^{-1} of soil. N = 24.

Chapter II

Redistribution of crop residues during row cultivation creates a biologically enhanced environment for soil microorganisms

M. Susana Grigera, Rhae A. Drijber, Brian J. Wienhold

Submitted to Soil and Tillage Research

Abstract

Formation of ridges during row cultivation creates microsites to existing spatial heterogeneity for soil properties such as organic C that may influence soil microbial communities. A study was conducted during 2003 near Shelton, NE, on a corn field mapped using apparent electrical conductivity (ECa). New ridges are built each year with a row cultivator when corn reaches the V3-V4 growth stage. Cultivation increased labile carbon pools and soil microbial biomass in the row position for all ECa classes. Canonical discrimination analysis showed no clear differences in relative abundance of specific microbial groups among ECa classes or between row and furrow position, except for enhanced mycorrhizal biomass in the row. Microbial biomass responded strongly to changes in C redistribution, this was not accompanied by a significant change in the abundance of specific microbial groups. The influence of labile C from corn roots and the redistribution of crop residues in diverse stages of decomposition are associated with diverse microbial groups. Thus, row cultivation for weed control creates a biologically enhanced root zone that may improve the early season performance of corn.

Key words: row cultivator, FAMEs, C availability, C redistribution.

Introduction

Conventional tillage with mechanical cultivation has historically been used to control weeds between the rows in maize, sorghum, and soybean production for a long time (Burton et al., 2006). Ridges and furrows formed by row cultivation create spatial heterogeneity for biological and physical soil properties (Wander and Bollero, 1999).

Ridge formation and the associated residue movement modify soil water and solute movement (Bargar et al., 1999), and may influence soil microbial communities.

Soil microbial biomass represents only a small proportion (<5%) of SOM, but due to its dynamic nature acts as a major sink and source of labile nutrients (Jenkinson and Ladd, 1981). Spatial and temporal dynamics of soil microbial biomass are good indicators of changes in soil biological processes (Pankhurst et al., 2002) related to organic matter transformations and nutrient cycling (Gupta et al., 1994). Microbial biomass has been shown to respond to tillage practices, phase of a crop-fallow cycle, and distribution of crop residue (Drijber et al., 2000; Pankhurst et al., 2002). Understanding how redistribution of C pools during row cultivation affects soil microbial groups is important due to the effect microorganisms have on soil chemical and physical properties. Here we test the hypothesis that row cultivation redistributes crop residues creating row and furrow environments that differ in C availability and associated soil microbiological communities.

Materials and methods

A field study was conducted during 2003 near Shelton, NE (40º45’01” N, 98º46’01” W). Soils at the site are Hord silt loam (Fine-silty, mixed mesic Pachic Argiustoll, 0-1% slope) and Blendon loam (Coarse-loamy, mixed mesic Pachic Haplustoll, 0-1% slope). Continuous corn (*Zea mays* L.) was grown from 1990 to 1996 under conventional disk-tillage with furrow irrigation and since 1996 under reduced tillage with sprinkler irrigation. Current tillage practices include disking the field in the fall following harvest to level past year ridges and incorporate crop residue, prepare the

seedbed in the spring using a field cultivator, and plant corn in mid to late May. Corn is grown using agronomic practices common to the region, including row cultivation for weed control when the corn reaches the V3-V4 growth stage (Ritchie et al., 1997).

In May 2003, spatial variability in soil properties was mapped using apparent electrical conductivity (ECa) measured with an EM 38 conductance meter (Geonics Ltd., Mississauga, Ontario, Canada). The ECa data was processed to delineate four classes within the field (Grigera et al., 2006). Within each ECa class six sites were randomly selected for sampling. Soil samples were collected during the V6 stage of corn (Ritchie et al., 1997) on 20 June, 2003. At each of the 24 locations, ten soil cores (0- to 15-cm depth) were collected and composited from the row and ten from the furrow positions. The composite soil samples were placed in a cooler for transport to the laboratory. Soil was passed through a 4 mm sieve to remove visible organic residues, thoroughly mixed, and divided into two subsamples. One subsample was stored at –18 ºC for fatty acid analysis and the other was air-dried, passed through 2 mm sieve, and analyzed for clay content (Kettler et al., 2001), total soil organic matter (SOM), fine (0.053-0.5 mm) particulate organic matter (POM), and coarse (0.5-2 mm) POM by loss-on-ignition (Cambardella et al., 2001). Electrical conductivity ($EC_{1:1}$) was measured in a 1:1 soil:water slurry (Smith and Doran 1996). Total dissolved solids (TDS) were estimated by multiplying the $EC_{1:1}$ at 25 °C (dS/m) by 0.64 (Smith and Doran 1996). Soil bulk density was determined by dividing the oven dry weight of the soil by the volume of the sample. Organic matter, TDS, fine POM, and coarse POM were expressed on an equivalent mass basis (Ellert and Bethany 1995).

Microbial biomass was determined as extractable lipid P on duplicate 1 g samples using perchloric acid digestion and then quantifying the released phosphate (Kates, 1986). Microbial community structure was based on extraction of total fatty acid methyl esters (FAMEs) by mild alkaline hydrolysis (Kates, 1986; Grogan and Cronan, 1997). The fatty acids were identified by retention-time and confirmed by mass spectrometry. Five microbial groups were defined based on known FAME biomarkers. Bacteria were represented by FAME markers *i*C15:0, *a*C15:0, C15:0, *i*C16:0, *i*C17:0, *a*C17:0, C17:0, and C16:1*cis*7, with the cyclopropane fatty acids cy17:0 and cy19:0 included as biomarkers for Gram-negative bacteria (Grogan and Cronan 1997). Actinomycetes were represented by FAME markers *i*10MeC18:0 and 10MeC18:0 (Kroppenstedt, 1992). Fungal communities were represented by C18:2*cis*9,12 (Stahl and Klug, 1996) and vesicular arbuscular mycorrhizal fungi were represented by C16:1*cis*11 (Olsson and Johansen, 2000).

Relationships between physical, chemical, and biological soil properties and ECa class were assessed by ANOVA (using PROC MIXED procedure) for a complete randomized design with ECa class and position (row vs. furrow) as factors. Pearson correlation analysis was conducted to assess the linear association between ECa sample means for each site and soil physicochemical and microbiological properties. Discriminant analysis was conducted on relative abundances of the defined microbial groups (bacteria, Gram negative bacteria, actinomycetes, fungi, vesicular arbuscular mycorrhizal fungi) to determine linearly uncorrelated axes and to prioritize the separation of two or more groups of variables. All statistical analyses were performed using SAS

(SAS Inst., 1999), and differences were declared significant at the 0.05 level, unless stated otherwise.

Results and discussion

The relationship between physical and chemical soil properties and ECa classes or row-furrow position in the 0- to 15-cm depth varied (Table 1). Clay content was greater in ECa class III than in class I and TDS were higher in classes III and IV than in classes I and II (Table 1). In contrast, bulk density and SOM were similar across ECa classes. Components of SOM also differed in their relationship to ECa class with fine POM being greater in ECa class III than in ECa classes I and II but coarse POM being similar among ECa classes. The relationship of physical and chemical soil properties to row-furrow position was consistent. Coarse POM, fine POM, SOM, and TDS were greater in the row and bulk density was greater in the furrow. The increase in POM and SOM and lower bulk density in the row position (Table 1) is likely due to redistribution of soil and crop residue C with row cultivation. Clay content was similar in the row and furrow positions (Table 1). The interaction between ECa class and row-furrow position was not significant for any of the soil physical and chemical properties measured.

Microbial biomass and FAME biomarkers for bacteria, gram negative bacteria, and actinomycetes were higher in ECa class III than classes I, II and IV (Table 1). The FAME biomarker for mycorrhizae was greater in ECa II than in ECa IV. The FAME biomarker for fungi was similar across ECa classes. The interaction between ECa class and row-furrow position was marginally significant for the mycorrhizal FAME biomarker

(p-value = 0.048)(Table 2). When FAME biomarkers for all microbial groups were summed we found that the total correlated with microbial biomass (r=0.77) and was greater in the row position (24.46 mmol m^{-2}) than in the furrow position (19.18 mmol m^{-2}). The correlation between microbial biomass and total FAME biomarkers was stronger in the furrow (r=0.85) than in the row (r=0.60). Microbial biomass and FAME biomarkers for bacteria, gram negative bacteria, actinomycetes, fungi, and mycorrhizae were all greater in the row than in the furrow.

Although microbial biomass responded strongly to changes in C redistribution, this was not accompanied by a significant change in the abundance of specific microbial groups. The canonical discriminant analysis identified two discriminant functions ($p<0.05$). We used the first and second canonical functions (eigenvalues 1.32 and 0.95) that together explained 83% of the total variance. As observed in Figure 1a there is no clear discrimination among the row and furrow position or by classes. However, the samples from the row position were displaced towards the left within each ECa class analyzed,. The most important fatty acid involved in this shift was the relative amount of mycorrhizal FAME biomarker, C16:1*cis*11 (Figure 1b). Increased relative abundance of mycorrhizal fungi in the row position was likely due to the influence of living roots in this environment.

In conventional tillage systems, preplant tillage operations create more homogeneous soil conditions and redistribute applied fertilizers throughout the tilled layer (Kaspar et al., 1991). John et al. (2004) observed homogeneity of the older soil organic C in the Ap horizon across row and furrow positions due to 40 years of plowing. A similar condition would be present in our field prior to row cultivation. Thus,

redistribution of labile C fractions and associated microbial biomass would be largely due to row cultivation. There would be less influence by the crop on the composition of the soil community (with the exception of mycorrhizae) at this early stage of growth.

The redistribution of crop residues to form a biologically enhanced root zone may have significant implications for early season crop growth. John et al. (2004) observed that the total emission of corn-derived CO_2 (due to the mineralization of C_4-soil organic C and corn residues) was three times greater for the row than for the furrow, while no differences were observed between row and furrow positions in the mineralization of native soil C. They also found that uneven distribution of corn residues resulted in increased heterotrophic activity in the row compared to the furrow. Likewise, we showed an increase in both POM fractions and microbial biomass in the row position. Consequently, row cultivation may enhance mineralization of corn residues during the growing season and thereby increase nutrient availability. Kaspar et al. (1991) observed that fertilizer placement altered root distribution, and that root length and weight in non tracked furrows were greater than tracked furrows. They stated that fertilizer injected into the row caused a proliferation of small-diameter roots. Thus, row cultivation may also increase corn root development through lowered soil bulk density, higher nutrient availability and improved mycrorrhizal relations.

Uneven redistribution of corn residues due to ridge formation resulted in increased fine POM, coarse POM, and soil microbial biomass and decreased bulk density in the row environment when compared to the furrow. Although microbial biomass responded strongly to changes in C redistribution, this was not accompanied by a significant change in the abundance of specific microbial groups, indicating a shift in

quantity rather than diversity of microorganisms. Increased microbial abundance provides greater opportunity for nutrient capture and turnover. Thus, row cultivation for weed control creates a biologically enhanced root zone that may improve the early season performance of corn.

Acknowledgments

We thank Liz Jeske, Susan Siragusa, Susan Wagner, Paul Koerner for laboratory assistance and field data collection. We also thank Scott Stubblefield for allowing access to his field and willingness to provide management input data.

References

Bargar, B., Swan, J. B., Jaynes, D., 1999. Soil water recharge under uncropped ridges and furrows. Soil Sci. Soc. Am. J. 63:1290–1299.

Burton, M.G., Mortensen , D.A., Lindquist, J.L., 2006. Effect of cultivation and within-field differences in soil conditions on feral *Helianthus annuus* growth in ridge-tillage maize. Soil Till. Res. in press.

Cambardella, C.A., Gajda, A.M., Doran, J.W., Wienhold, B.J., Kettler T.A., 2001. Estimation of particulate and total organic matter by weight loss on ignition. In: R. Lal et al. (Eds.) Assessment methods for soil carbon. CRC Press, Boca Raton, FL., pp. 349-359.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol. Biochem. 32:1419-1430.

Ellert, B.H., Bettany, J.R., 1995. Calculations of organic matter and nutrients stored in soil under contrasting management. Can. J. Soil Sci. 75:529-538.

Grigera, M.S., Drijber, R.A., Eskridge, K.M., Wienhold, B.J., 2006. Soil Microbial Biomass Relationships with Organic Matter Fractions in a Nebraska Corn Field Mapped using Apparent Electrical Conductivity. Soil Sci. Soc. Am.J. in press.

Grogan, D.W., Cronan, Jr. J.E., 1997. Cyclopropane ring formation in membrane lipids of bacteria. Microbiol. Mol. Biol. Rev. 61:429-441.

Gupta, V. V. S. R., Roper, M. M., Kirkegaard, J. A., Angus, J. F., 1994. Changes in microbial biomass and organic matter levels during the first year of modified tillage and stubble management practices on a red earth. Aust. J. Soil Res. 32**:**1339-54

Jenkinson, D. S., Ladd, J. N., 1981. Microbial biomass in soil: measurement and turnover. In: E. A. Paul and J. N. Ladd (Eds.), Soil Biochemistry. Marcel Dekker, NY, pp. 415-71.

John, B., Ludwig, B., Potthoff, M., Flessa, H., 2004. Carbon and nitrogen mineralization after maize harvest between and within maize rows: a microcosm study using ^{13}C natural abundance. J. Plant Nutr. Soil Sci. 167:270-276.

Kaspar, T.C., Brown, H.J., Kassmeyer, E.M., 1991. Corn root distribution as affected by tillage, wheel traffic, and fertilized placement. Soil Sci. Soc. Am. J. 55:1390-1394.

Kates, M. 1986. Techniques of lipidology: isolation, analysis and identification of lipids. In: Burdon, R.H., van Kippenberg, P.H. (Eds.), Laboratory Techniques in Biochemistry and Molecular Biology, vol. 3, Part 2. Elsevier, NY, 464 pp.

Kettler, T.A., Doran, J.W., Gilbert, T.L., 2001. A simplified method for soil particle size determination to accompany soil quality analyses. Soil Sci. Soc. Am. J. 65: 849-852.

Kroppenstedt, R.M., 1985. Fatty acid and menaquinon analysis of actinomycetes and related organisms. In: Goodfellow, M., Minnikin, D.E. (Eds.), Chemical Methods in Bacterial Systematics. Academic Press, London, pp. 173-199.

Olsson, P.A., Johansen, A., 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycol. Res. 104:429-434.

Pankhurst, C.E., Kirkby, C.A., Hawke, B.G., Harch, B.D., 2002. Impact of a change in tillage and crop residue management practice on soil chemical and microbiological properties in a cereal-producing red duplex soil in NSW, Aust. Biol. Fertil. Soils 35:189–196.

Ritchie, S.W., Hanaway, J.J., Benson, G.O., 1997. How a corn plants develops. Spec. Publ. 48. Iowa State Univ. Coop. Ext. Serv., Ames, IA.

SAS Institute, 1999. SAS/STAT software: Changes and enhancements through release 8.0. SAS Inst., Cary, NC.

Smith, J.L., Doran, J.W., 1996. Measurement and use of pH and electrical conductivity for soil quality analysis. In: J.W Doran, and A.J. Jones (Eds.) Methods for assessing soil quality. ASA, CSSA, and SSSA, Madison, WI., pp. 169-186.

Stahl, P.D., Klug, M.J., 1996. Characterization and differentiation of filamentous fungi based on fatty acid composition. App. Envir. Microb. 62:4136-4146.

Troeh, Z.I., Loynachan, T.E., 2003. Endomycorrhizal fungal survival in continuous corn, soybean, and fallow. Agron. J. 95:224-230.

Wander, M. M., Bollero, G. A., 1999. Soil Quality Assessment of Tillage Impacts in Illinois. Soil Sci. Soc. Am. J. 63:961–971.

Table 1 Mean values for soil physicochemical and biological properties in the furrow and in the row.

Main effect	Clay	CPOM	FPOM	SOM	TDS	BD	MB	Bact	Gram-	Actino	Fungi
	kg m^{-2}	g m^{-2}				g cm^{-3}	mmol m^{-2}	nmol m^{-2}			
Class											
ECa I	32.2b	297.7a	770.5b	5010.5a	55.2b	1.28a	44.1b	3220.3b	963.9b	428.2b	2234.1a
ECa II	36.0ab	268.1a	776.9b	5228.1a	54.4b	1.25a	45.5b	3439.7b	1011.6b	495.3ab	2038.6a
ECa III	39.9a	354.2a	929.2a	5959.0a	63.7a	1.21a	57.0a	4118.1a	1183.0a	573.2a	2160.7a
ECa IV	36.5ab	269.9a	871.9ab	5381.4a	60.7a	1.23a	49.9ab	3507.4b	949.1ab	469.5b	1729.0a
SE	1.7	25.7	35.2	247.7	1.8	0.02	2.4	174.0	43.4	32.3	137.3
Position											
Furrow	36.3A	260.2B	770.9B	5308.6B	54.6B	1.28A	45.3B	3198.1B	949.1B	448.6B	1818.3B
Row	36.0A	334.7A	903.3A	5480.9A	62.3A	1.21B	53.0A	3944.7A	1158.6A	534.4A	2262.9A
SE	0.9	14.3	20.3	126.2	1.2	0.01	1.3	94.8	23.4	16.9	89.3

FPOM= fine particulate organic matter (0.053-0.5 mm); CPOM=coarse particulate organic matter (0.5-2 mm), SOM=soil organic matter, TDS= total dissolved salts, BD= bulk density, MB=total microbial biomass measured as Lipid P, Bact=bacterial markers (*i*C15:0, *a*C15:0, C15:0, *i*C16:0, *i*C17:0, *a*C17:0, C17:0, C16:1*cis*7); Gram-= cyclopropane bacterial markers (*cy*C17:0, *cy*C19:0), Actino=actinomycetes markers (*i*10MeC18:0, 10MeC18:0),); Fungi=fungal marker (C18:2*cis*9,12). Means followed by the same letter within ECa or Position are not different ($p<0.05$). N = 48.

Table 2 Mean values for the relative concentration of arbuscular mycorrhizal marker interaction Site*Position .

Site*Position interaction	Myco
	_____nmol m^{-2}_____
ECa I - Furrow	609.3cd
ECa I - Row	849.1ab
ECa II - Furrow	572.2cd
ECa II - Row	1043.4a
ECa III - Furrow	609.9cd
ECa III - Row	871.1a
ECa IV - Furrow	475.2d
ECa IV - Row	649.8bc

Myco=mycorrhizal marker (C16:1*cis*11). Means followed by the same letter are not different ($p<0.05$). N = 48. SE of the mean = 74.7.

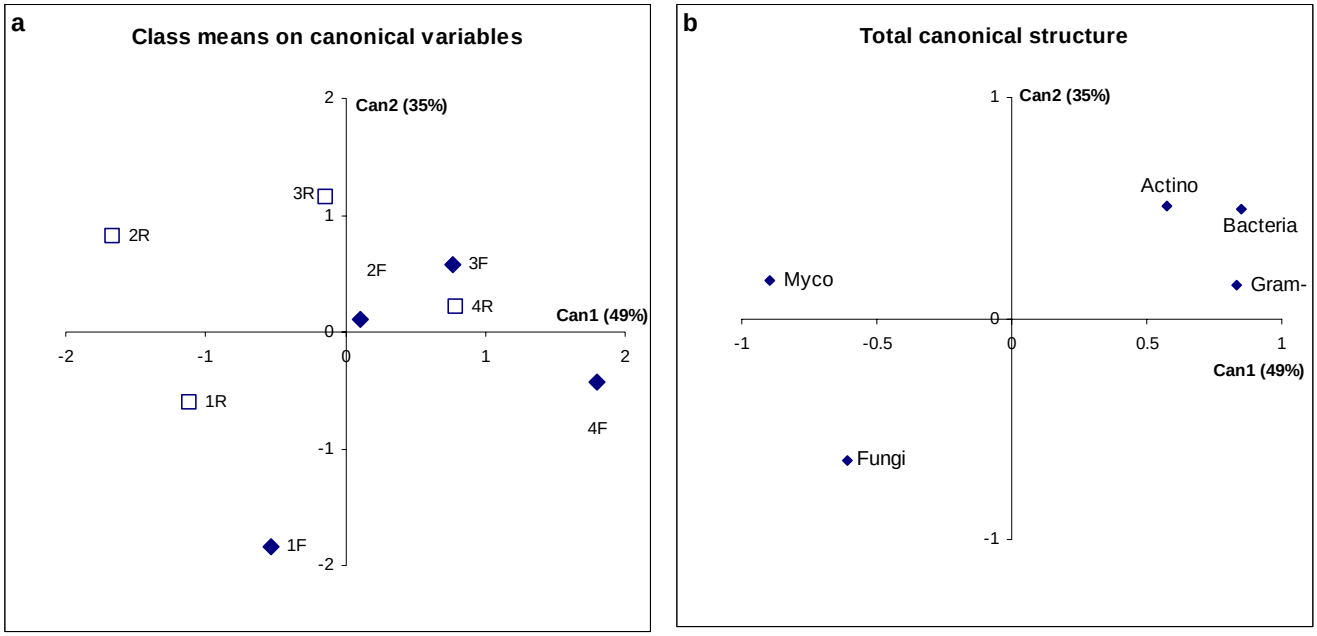


Figure 1. Discriminant scores of treatments for (a) class means on canonical variables and (b), showing the two significant discriminant functions, Can1 and Can2. 1F= apparent electrical conductivity (ECa) class I furrow, 1R=ECa class I row, 2F=ECa class II furrow, 2R=ECa class II row, 3F=ECa class III furrow, 3R=ECa class III row, 4F=ECa class IV furrow, and 4R=ECa class IV row). Bacteria=bacterial markers (*i*C15:0, *a*C15:0, C15:0, *i*C16:0, *i*C17:0, *a*C17:0, C17:0,C16:1*cis*7); Gram- = cyclopropane bacterial markers (*cy*C17:0, *cy*C19:0); Actino=actinomycetes markers (*i*10MeC18:0, 10MeC18:0), Myco=mycorrhizal marker (C16:1*cis*11); Fungi=fungal marker (C18:2*cis*9,12). N = 48.

Chapter III

Increased abundance of arbuscular mycorrhizal fungi in soil coincides with the reproductive stages of corn

M. Susana Grigera, Rhae A. Drijber, Brian J. Wienhold

Prepared for Soil Biology and Biochemistry

Abstract

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with most land plants and are recognized for their positive effects on plant growth, playing an important role in plant P nutrition. We used C16:1*cis*11 and C18:1*cis*11 fatty acid methyl ester (FAME) biomarkers to monitor the dynamics of AM fungi during the reproductive stages of maize (*Zea mays* L.) grown at high yield in Nebraska, USA. Two fields with four different levels of P availability were sampled throughout the reproductive stages. Chambers, made of PVC enclosed mesh fabric to allow passage of roots and hyphae (+R) or hyphae alone (-R) and amended with either KH_2PO_4 (+P) or distilled water (-P), were installed in the field at tasselling and removed after three, six and nine weeks. Our objectives were (i) to study the influence of available P and roots on extramatrical mycorrhizal fungal growth, (ii) to provide evidence for C allocation to AM fungi during the reproductive stages of high productivity maize, and (iii) to link AM fungal growth dynamics with changes in soil P availability. We observed that initial AM FAME concentration was lower at sites with a high availability of P. During the reproductive growth of maize, AM FAME biomarkers increased inside the chambers and was consistent with the biomarker increase observed in adjacent field soil. This confirms that there is C allocation from the plant to the symbiont during the reproductive stages of maize. We also observed a reduction in available P in +R and –R chambers. This observation implies that hyphae were as efficient as roots and hyphae in reducing the P concentration in chambers. These results demonstrate that AM fungi are active during the reproductive growth stages of maize and may benefit high productivity maize crops by facilitating P uptake. **Key words: AM fungi, FAMEs, Bray P, maize (*Zea mays* L.)**

1. Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with up to 80% of land plants and are also recognized for their positive effects on plant growth and soil quality (Smith and Read, 1997). The extramatrical growth of the mycelium is a key factor in nutrient acquisition by the mycorrhizal symbiont (Olsson et al., 1997). The plant-AM fungal relationship has been studied under controlled conditions in the greenhouse (Evans and Miller, 1990; Liu et al., 2000; Smith et al., 2003; Smith et al., 2004), in early stages of crop development (Miller, 2000; 1990), and under different levels of disturbance (Evans and Miller, 1988; 1990). The effectiveness of inoculation versus managing the indigenous AM populations has also been assessed (Liu et al., 2000; Smith et al., 2003; Smith et al., 2004). Field studies are lacking that focus on the temporal dynamics of the interaction between plant and indigenous AM fungi in cropping systems

Phosphorous is an essential plant nutrient and, following N, is the second most common fertilizer nutrient applied in crop production. Although P supply during early development has an effect on crop yield potential (Grant et al., 2001), there may also be a requirement for additional P later in crop growth. In maize (*Zea mays* L.), P accumulates steadily until maturity, with a high proportion (approximately 60%) being absorbed during the reproductive period (Karlen et al., 1988). Various forms of P present in soil have very low solubility and the concentration of P in soluble forms is particularly low (Singh and Adholeya, 2001). Phosphate transport across the root is usually faster than diffusive transport in soil. This lowers the concentration of phosphate in the soil solution surrounding the root forming a P depletion zone (Barber, 1977). In addition, it was observed that the amount of dry matter in maize roots remains almost constant shortly

after reproductive growth begins at R1 (Ritchie et al., 1997). The below ground dry matter/above ground dry matter ratio is maximum at V10 (61%) and decreases with time, being 25 to 34% 7 days before R1, 17 to 20% at R1, and 10 to 13% at R5 (Plenet, 1995).

Low input agricultural systems were shown to be more dependent on AM fungi than conventionally managed systems, affecting the AM fungus population, mycorrhiza formation, and nutrient use in maize (Galvez et al., 2001). Jansa et al. (2003) observed that under field conditions colonization of maize roots by the genus *Scutellospora* was depressed by intensive tillage, while the opposite was observed for some members of the genus *Glomus*. These changes may result from disturbance of hyphal integrity by tillage, changes in nutrient content of the soil, changes in microbial activity, or changes in weed populations. It was also shown that increasing soil P concentrations resulted in decreased fatty acid signatures for AM fungi in both soil and roots (Olsson et al., 1997). Under field conditions the variation in physical, chemical and biological status, including multiple plant species and soil microorganisms, make studying mycorrhizal associations complex. Understanding the composition of ecosystems and identifying the controls that operate over spatial and temporal scales, may be useful tools to identify the patterns of plant-fungal interactions (Allen et al., 2003).

The uniqueness and utility of fatty acid methyl ester (FAME) biomarkers for AM fungi has been demonstrated by several authors. Larsen et al. (1998) used fatty acid signatures to study mycelial interactions between AM fungi and saprotrophic fungi in root-free soil. The dominant fatty acid for saprotrophic fungi was C18:2*cis*9,12 while it was negligible in mycelium of *Glomus intraradices*. The fatty acids C16:1*cis*11, C20:4, and C20:5 were found in this AM fungi, but not in the saprotrophic one. FAME analysis

performed on the spores of four AM fungi (*Glomus coronatum, Glomus mosseae, Gigaspora margarita* and *Scutellospora calospora*) showed C16:1*cis*11 to be the dominant fatty acid present (Madan et al., 2002). Furthermore, Olsson and Johansen (2000) found that the amount of phospholipid fatty acid (PLFAs) C16:1*cis*11 per unit biomass of two AM species (*Glomus intraradices* and *G. clarideum*) remained rather constant as the mycelium aged, and its distribution between the mycelium and hyphae was highly consistent making it a suitable biomass indicator. Van Aarle and Olsson (2003) observed that NLFA and PLFA were similarly correlated with AM % root colonization of *Plantago lanceolata* L in monoxenic cultures. In soils, NLFA C16:1*cis*11 may be a more sensitive indicator of AM fungal biomass due to high background concentrations of PLFA C16:1*cis*11 (Olsson, 1999).

The relative amount of this fatty acid in roots may provide an index of the amount of C allocated for fungal growth and lipid storage in the root during the colonization process (Graham et al., 1995). We used this approach to understand the dynamics of mycorrhizal fungi during the reproductive stages of high productivity maize. Management systems that enhance natural mechanisms for P acquisition will help optimize use of P fertilizer resources. Although AM fungi play an important role in plant P nutrition, factors influencing that role and associated temporal dynamics are poorly understood. Plant growth dynamics and nutrient accumulation patterns leave uncertainty about the mechanism for nutrient uptake. We hypothesize that AM fungi are essential to meet the demand for P during the reproductive stages of maize managed for high yield. Our objectives were (i) to study the influence of available P and roots on extramatrical mycorrhizal fungal growth, (ii) to provide evidence for C allocation to AM fungi during

the reproductive stages of high productivity maize, and (iii) to link AM fungal growth dynamics with changes in soil P availability.

2. Materials and methods

2.1 Study area

The field study was conducted during 2004 near Shelton NE (40º45'01" N, 98º46'01"W) and Lincoln, NE (40º49'12" N, 95º39'W). Soils at Shelton are Hord silt loam (Fine-silty, mixed mesic Pachic Argiustoll, 0-1% slope) and Blendon loam (Coarse-loamy, mixed mesic Pachic Haplustoll, 0-1% slope). Continuous maize has been cropped since 1990 under conventional disk-tillage with furrow irrigation, and since 1996 under reduced tillage (disk and field cultivator) with sprinkler irrigation. Prior to planting 168 kg N ha^{-1} as urea ammonium nitrate solution was incorporated with a field cultivator. Maize (Pioneer 33B51) was planted on 2 May, 2004 with 19.5 kg P ha^{-1}, and 13.1 kg N ha^{-1} as diammonium phosphate applied adjacent to the seed. Stand density was 71600 plants ha^{-1} and field average yield was 12.2 Mg ha^{-1}at 155 g kg^{-1} moisture content.

In May 2003, apparent electrical conductivity (ECa) was measured with an EM 38 dual dipole conductance meter (Geonics Ltd., Mississauga, Ontario, Canada) pulled behind an all terrain vehicle. Data was logged at 1-s intervals (every 2 m) and georeferenced using a differential global positioning system receiver (Trimble Navigation, Sunnyvale, CA) mounted near the EM38 sensor. The data collected was processed using ERDAS Imagine (ERDAS Inc., Atlanta, GA) to create four ECa classes based on ranges of ECa measurements using unsupervised classification (ERDAS,

1997)(Johnson et al., 2001). Six sites in ECa class II and class IV were randomly selected for soil sampling and to study mycorrhizal dynamics.

Soil at Lincoln is a deep Kennebec silty clay loam (Fine-silty, mixed, superactive, mesic Cumulic Hapludolls 0-1% slope). Continuous maize has been cropped since 1999 under conventional tillage with sprinkler irrigation. The field was plowed in the fall using a Salford conservation tillage plow (Salford Farm Machinery Limited, Salford, ON, Canada) (about 25-30 cm deep), and field-cultivated before planting. The experiment was arranged in a randomized complete block design with four replicates. Two levels of fertilizer-nutrient management were applied: recommended fertilizer rates (M1) and intensive nutrient management (M2). For the M1 treatment 200 kg N ha^{-1} as ammonium nitrate were applied, 100 kg N ha^{-1} incorporated with a field cultivator prior to planting, and 100 kg N ha^{-1} at V6. For the M2 treatment 280 kg ha^{-1} of N were applied as follows: 50 kg N ha^{-1} in October 2003 as UAN on crop residue (before plowing), 80 kg ha^{-1} as ammonium nitrate prior to planting, 60, 50 and 40 kg N ha^{-1} as ammonium nitrate at V6, V10 and V14 respectively. In addition, 45 kg ha^{-1} P as single super phosphate and 85 kg ha^{-1} K as KCl were broadcasted before planting and incorporated. Maize (Pioneer 31N28) was planted on 12 May, 2004. Stand density was 74000 plants ha^{-1}, and field average yield for M1 was 15.5 Mg ha^{-1} and for M2 was 15.6 Mg ha^{-1}at 155 g kg^{-1} moisture content.

2.2 Preparation and installation of soil chambers

Two bulk soil samples were collected from the 0- to 15-cm depth on 22 June in ECa II and ECa IV at Shelton and from M1 and M2 at Lincoln. Field moist soil was passed through a 6 mm mesh sieve to remove plant residues and stored at 4ºC until

chambers were prepared. These soil samples were analyzed for Bray P (Bray and Kurtz, 1945), total, and organic P (Olsen and Sommers, 1982). The chambers were made of PVC with a wall thickness of 0.6 cm, a diameter of 10 cm, and a height of 4.5 cm. The sides of the rings were enclosed with 1 mm mesh fabric to allow (+R) or 0.04 mm mesh fabric to exclude (-R) maize roots (Sefar America Inc., Depew, NY). Both mesh sizes allowed hyphae penetration. Rings were filled with approximately 425 g of bulk soil from either ECa II, ECa IV, M1 and M2, amended with either 20 ml of 0.014 M KH_2PO_4 (+P) or 20 ml of distilled water (-P), thoroughly mixed, and packed to a bulk density of 1.2 g cm^{-3}. The chambers were placed in plastic bags and stored at 4ºC until they were placed in the field.

In each plot, twelve chambers constructed using soil from that plot, were installed (three of each treatment: +R+P, +R-P, -R+P, and -R-P) at the VT stage of maize, on 15 July at Shelton and 13 July at Lincoln. The chambers were buried so that the upper edge was 5 cm below the soil surface.

2.3 Removal of chambers and field sampling

One chamber of each treatment was removed three, six, and nine weeks after installation (4 and 23 August, and 13 September at Shelton and 3 and 26 August and 16 September at Lincoln). Ten soil cores (0- to 15-cm depth) were taken adjacent to the chambers and combined on each date that chambers were removed. Additional soil samples were taken at Shelton at V6 and R1-2. The chambers and the composite soil samples were transported in a cooler to the laboratory, where chambers where opened. The soil was passed through a 4 mm sieve to remove visible organic residues, thoroughly

mixed, and divided in two subsamples. One subsample was air dried for Bray P analysis, and the other was stored at –18 ºC until they were analyzed for AM fatty acid biomarkers.

2.4 AM biomarker quantification and identification

Mycorrhizae were quantified using fatty acid biomarkers extracted by mild alkaline hydrolysis of the soil (Bradley et al., 2006). This process does not methylate free fatty acids, but only ester-linked fatty acids (Kates, 1986; Grogan and Cronan, 1997). Briefly, samples were hydrolyzed using freshly prepared 0.2 M potassium hydroxide in methanol and the resulting FAMEs were partitioned into hexane (White et al., 1979). Following saponification to release ester-linked FAMEs, methyl-nonadecanoate (0.05 µg μl^{-1}) was added to the extract as an internal standard.

Released FAMEs were separated by gas chromatography, using helium as a carrier gas, and an Ultra 2 HP (50 m, 0.2 mm I.D., 0.33 µm film thickness) capillary column. The gas chromatograph was run in split mode (44:1) with a 45 sec purge time. Injector and flame ionization detectors were maintained at 280º and 300º C, respectively, and oven temperature was ramped from 50º C to 160º C at 40º C min^{-1} and held for 2 min, then ramped at 3º C min^{-1} to 300º C and held for 30 min. The fatty acids were identified by retention-time and confirmed by mass spectrometry. Concentrations of FAMEs were calculated from peak areas and reported as nmol g^{-1} soil.

Fatty acids were designated as the total number of C atoms followed by a colon, the number of double bonds followed by the position of the double bond from the carboxyl end of the molecule and its *cis* or *trans* configuration (IUPAC-IUB, 1987). The prefixes *a* and *i* indicate antieso and iso branching, respectively; *cy* indicates cyclopropane fatty acids, *br* indicates an unknown branch position and 10Me indicates a

methyl branch on the 10th C atom from the carboxyl end of the molecule. The FAMEs selected as mycorrhizal biomarkers were C16:1*cis*11 (Olsson and Johansen, 2000) and C18:1*cis*11 (Olsson, 1999). Although, C18:1*cis*11 is common to several gram negative bacteria, C16:1*cis*11 is found in only a few bacterial genera (Zelles, 1999).

2.5 AM biomarker concentration in selected lipid classes from Shelton chambers

A modified one-phase extraction procedure of Bligh and Dyer (White et al., 1979) was used to extract lipids from soil at Shelton. Two randomly selected chambers +R,-P from three dates, 4 August, 23 August and 13 September, were extracted with chloroform-methanol (1:1 vol/vol) and separated into neutral lipid fatty acids (NLFA), glycolipid fatty acids (GLFA) and phospholipid fatty acid (PLFA) classes by silica gel chromatography (Drijber et al., 2000). Lipids were saponified and quantified as described above for FAMEs.

2.6 Statistical analysis

Statistical analyses were performed using SAS (SAS Inst., 1999) and differences were declared significant at the 0.05 level unless stated otherwise. At Shelton, analysis of variance was performed to test for differences in fatty acid concentration and Bray P in soil samples for a complete randomized design with ECa class as treatment factors (Johnson et al., 2001). The chamber experiment was analyzed as a split plot in time and space, with EC as the whole plot in a complete randomized design. At Lincoln, field soil was tested with a complete randomized block design. The chamber experiment was analyzed as a split plot in time and space, with fertility treatment as the whole plot in a complete randomized block design. Seasonal and treatment differences between the absolute amounts of individual fatty acids and Bray P were evaluated using repeated-

measures mixed model ANOVA (using PROC MIXED procedure). As the correlation between observations did not follow a clear pattern over time, compound symmetry covariance structure was used in the repeated-measures mixed model ANOVA. The ANOVA tables are described at the beginning of each section.

3. Results

3.1 Properties of soil used to construct chambers

Bulk soil collected from field sites at Shelton and Lincoln differed in available P and AM biomarker concentration. At Shelton, Bray and organic P were lower in ECa II than ECa IV (Table 1). At Lincoln M1 had lower Bray and organic P than M2 (Table 1). FAME biomarker concentration was inversely related to available P.

3.2 Dynamics of AM fungal biomarkers in field soil

The concentration of C16:1*cis*11 and C18:1*cis*11 biomarkers over time from Shelton field soil are reported in Table 2. For both biomarkers the main factor 'ECa' and the interaction 'ECa*time' were not significant. The biomarkers concentrations from Lincoln are shown in Table 3. The main factor 'fertility' (M1 vs. M2) was significant ($P = 0.04$) for C16:1*cis*11, and the interaction 'fertility*time' was not. The mean concentration of C16:1*cis*11 decreased from 3.42 ± 0.15 nmol g^{-1} in M1 to 2.88 ± 0.15 nmol g^{-1} soil in M2. For C18:1*cis*11, only the main factor 'time' was significant (Table 3).

The FAME biomarker C16:1*cis*11 increased from August to September at Shelton (Table 2) and from July to September at Lincoln (Table 3). This increase

demonstrates transport of C from the plant to the fungal symbiont during the reproductive stages of maize. At both sites, C18:1*cis*11 increased from August to September. Field samples from Shelton showed a higher concentration of AM FAME biomarkers than samples from Lincoln.

3.3 Dynamics of AM fungal biomarker in chamber soils

Chamber mesh size effectively excluded or allowed root passage into the soil chambers. Roots were found in the +R chambers three weeks after VT at Shelton and Lincoln. We observed roots on the outer walls of –R chambers, but none penetrated the mesh.

At Shelton the C16:1*cis*11 and C18:1*cis*11 biomarkers concentration in chambers over time are reported in Table 4. The main factors 'time' (P = 0.01), 'ECa' (P = 0.01) and 'mesh size' (+R, -R) (P = 0.02) were significant for C16:1*cis*11; the addition of P (+P, -P) and the interactions among these factors were not significant. For C18:1*cis*11, the main factors 'mesh size' and 'time', and the interaction 'mesh*time' (P = 0.01) were significant. At Lincoln the main factor 'time' was significant for C16:1*cis*11 (Table 5), while P addition, mesh size, ECa or the interactions among them were not significant. The main factor 'time' was marginally significant for C18:1*cis*11 (P = 0.07) and significant for mesh size (P = 0.04).

At Shelton, C16:1*cis*11 increased inside the chambers from VT onwards (Table 4). The addition of P to the chambers had no effect on the concentration of C16:1*cis*11 or C18:1*cis*11 over time. At Shelton the symbiotic plant-fungi relationship responded to the presence and absence of roots inside the chambers; the +R chambers had an 11% higher C16:1*cis*11 marker concentration than in the –R chambers. We also observed that

C16:1*cis*11 was 36% greater in ECa class II than class IV. The concentration of C18:1*cis*11 showed a 'mesh*time' interaction (Table 4). This interaction resulted from similar concentration of the biomarker in chambers at the first and second sample times but greater biomarker concentration in +R than –R on 13 September. The increase in C18:1*cis*11 in +R chambers in September, accompanied the increase in the C16:1*cis*11 over time. However C18:1*cis*11 was less responsive than C16:1*cis*11, possibly related to the influence of other soil microorganisms on the concentration of C18:1*cis*11.

At Lincoln, C16:1*cis*11 was greater on 16 September than on earlier sampling dates (Table 5). In this field, there were no differences in C16:1*cis*11 concentration due to mesh size or soil P availability. The C18:1*cis*11 biomarker also increased over time ($P = 0.07$) (Table 5), and had a higher concentration at M1 sites (5.22 ± 0.13 nmol g^{-1} soil) than M2 sites (5.01 ± 0.13 nmol g^{-1} soil).

When comparing the concentration of the C16:1*cis*11 biomarker over time in the chambers to that in the field soil, we observed a lower initial concentration of this marker in the chambers and a slower rate of increase over time. The soil used to assemble the chambers was sieved, stored in the refrigerator (before and after chamber assembly), packaged into chambers, and in some cases amended with P. This manipulation possibly caused a loss and/or alteration of AM structures during soil processing. Field samples had a 22% (Shelton) or 32% (Lincoln) higher concentration of C16:1*cis*11 (mean of the three sampling dates) compared to the chambers.

3.4 Dynamics of P availability in soil chambers

The addition of P to the chambers increased the amount of available P for both Shelton (Table 6) and Lincoln (Table 7). For Shelton chambers without additional P (-P),

available P was significant for the main factors 'ECa' and 'time', and was not significant for mesh size, or the interactions among these factors. The concentration of Bray P was 14.5 mg kg^{-1} soil $\pm$ 2.5 mg kg^{-1} soil for ECa II and 38.0 $\pm$ 2.5 for ECa IV. Similarly, in chambers with additional P (+P) the significant factors were 'ECa' and 'time'. The concentration of Bray P was 22.9 mg kg^{-1} soil $\pm$ 3.6 for ECa II and 49.3 mg kg^{-1} soil $\pm$ 3.6 for ECa IV. At Lincoln, available P in chambers without additional P (-P) was significant for the main factors 'mesh size' and 'time' and the interactions 'fertility*mesh' ($P = 0.04$) and 'mesh*time' ($P = 0.04$) (Table 7). For the chambers with additional P (+P) the ANOVA showed no significant differences.

We did not observe an increase in available P when comparing the soil used to make the chambers and the soil from the first sampling date in –P chambers at Shelton or Lincoln, demonstrating that soil manipulation (sieving, storage in refrigerator, packaging) did not enhance P mineralization (Tables 1, 6 and 7). At Shelton, available P inside the chambers decreased from August to September (Table 6). Phosphorous availability was not influenced by mesh size, thus AM fungi (-R chambers) appeared as effective as roots *plus* mycorrhizae (+R chambers) in reducing chamber P concentration.

In –P chambers at Lincoln, there was a 'mesh*time' interaction (Table 7). There was an increase in available P on 26 August in the +R chambers that was not observed in the –R chambers. A 'site*mesh' interaction was also observed due to an increase in P availability in the –R, M2 site. In this field, the availability of P in +P chambers was similar over time (Table 7).

3.5 AM fungal biomarker distribution in +R-P chambers at Shelton

There was an increase in the total concentration of AM fungal biomarker C16:1*cis*11 (measured as the addition of C16:1*cis*11 in the NLFA, GLFA and PLFA lipid classes) over time at Shelton. The concentration of PLFA C16:1*cis*11 was similar for the three sampling dates. Although mean concentrations of C16:1*cis*11 increased over time for both the NLFA and GLFA fractions, they were not significant. However, when summed, the mean concentrations increased significantly over time. The combined concentration of the NLFA and GLFA fractions was 204% higher in September than the mean of the August sampling dates. In addition, the relative contribution of these two fractions to total C16:1*cis*11 was 52% in August, and 76% in September.

4. Discussion

4.1 Concentration of AM fungal biomarkers in field soil and chambers

Mycorrhizal colonization of soil and plant roots is a complex multi-step process, in which the mycorrhizal association is influenced not only by the host plant and the AM fungi, but also by soil and other environmental conditions (Barea, 1991). The higher concentration of C16:1*cis*11 found in field samples from Shelton, when compared to Lincoln, may stem from differences in soil type and the lower P availability (Bray P and organic P) at Shelton. Although P availability may be responsible for decreased concentrations of AM biomarkers at Shelton ECa IV and Lincoln M2 sites, the higher availability of N in Lincoln M2 may also contribute to the reduction in C16:1*cis*11. In spring 2003, the 0- to 30-cm surface layer contained 8.9 and 31.2 kg of NO_3-N ha^{-1}, for M1 and M2 respectively (A. Dobermann, personal communication, 2006). Liu et al. (2000) studied the influence of soil N and P levels in three maize hybrids. After two

months, P application decreased the percentage of AM colonization, the fungal colonization had a negative interaction with N application and was affected by the hybrid. Bradley et al. (2006) studied soil microbial response to N fertilization throughout time in a grassland ecosystem. They found that mycorrhizal FAME biomarkers decreased in response to anthropogenic N long before responses were evident in plant communities.

A second factor could be the presence of AM species that differ in their responses to cropping history and edaphic factors. Johnson et al. (1991) attributed the large difference in evenness of AM communities between two sites to differences in the soil cultural practices. The frequency and intensity of the tillage practices at Shelton and Lincoln are different. Soil disturbance by fall-plowing, as is practiced at Lincoln, was shown to reduce over-winter survival of hyphae and mycorrhizal potential, probably as a consequence of physical disruption of roots and hyphal network (Kabir et al., 1997).

A third factor may be plant genotype. Liu et al. (2003) showed that P uptake by AM fungi in maize was strongly hybrid-dependant. Furthermore, carbohydrate allocation was shown to affect colonization rate in citrus and this process apparently was controlled by plant genotypes (Graham et al., 1991; Graham and Eissenstat, 1994). Thus, the different maize hybrids used at Shelton and Lincoln may have different affinity for AM infection.

The concentration of AM biomarkers in field samples was greater than in chambers for all dates and sites. This was probably due to disturbance of the soil before assembling the chambers, and the cost attributed to the formation of a new hyphal network inside these chambers. Disturbance reduces total hyphal density, metabolically active hyphal density, and the proportion of hyphae that is viable (Kabir et al., 1997).

Mycelia network appears to be an important component of the inoculum potential in undisturbed soil, and its disturbance effects mycorrhizal colonization and P absorption (Evans and Miller, 1990; Miller, 2000). Alternatively, in the field soil where the hyphal network was disturbed prior to V6 by field operations, the AM fungi would exist in several growth stages and the development of specialized structures, or accumulation of storage lipids could account for the higher biomarker concentrations than in chambers..

4.2 Evidence for C allocation to AM fungi during reproductive growth of maize.

In field soils at both sites, C16:1*cis*11 increased during the reproductive growth stages of the crop. A parallel increase in C18:1*cis*11 occurred only during the later reproductive growth stages. Both of these FAME biomarkers are indicative of AM fungi, although C18:1*cis*11, and to a lesser extent C16:1*cis*11, are found in bacteria. Although net increase in AM biomarkers could arise from reallocation of C within the fungal network, a more likely immediate source would be the plant. This is supported by AM fungal colonization of the soil chambers installed just prior to the reproductive stages of growth.

Preparation of the chambers with bulk field soil would have thoroughly disrupted the mycelial network of existing AM fungi in the field leaving only mycelial fragments and spores as inoculum for the current maize crop. This inoculum would be subject to degradation over time leading to declining amounts of the AM fungal biomarker C16:1*cis*11 in the absence of a host plant. Thus any net increase in the AM fungal marker is evidence for hyphal penetration of the chamber (-R or +R) from adjacent field soil or successful spore germination (in terms of establishment of the plant-fungi symbiosis), and the formation of arbuscules and vesicles within the root structure in +R chambers.

Olsson (1999) reviewed the signature fatty acid composition of lipids in mycorrhizal fungi and stated that in most fungi, a large proportion of the total fatty acids are found as C16:1*cis*11 and C18:1*cis*11. In our study, the smaller response of C18:1*cis*11 compared to C16:1*cis*11, was possibly related to the influence of Gram-negative bacteria (Zelles, 1999) on the concentration of this marker in the chambers. The correlation observed between these two AM biomarkers at Shelton in bulk soil samples was r = 0.78 (P = 0.01), in chamber soil samples was r = 0.66 (P = 0.01), and at Lincoln in field soil was r = 0.38 (P = 0.02) and in chamber soil was r = 0.35 (P = 0.01). The correlation observed in our study between C16:1*cis*11 and C18:1*cis*11 confirmed the existence of complementary markers, that in conjunction with C16:1*cis*11 are good indicators of AM fungi dynamic in soils.

4.3 Distribution of AM fungal biomarkers among lipid classes

Two important types of lipids found in fungi are phospholipids (membrane constituents) and neutral lipids (energy storage). Both contain ester-linked fatty acids to a glycerol backbone (Olsson, 1999). Extraction of FAMEs by mild alkaline hydolysis includes both these lipid pools, in addition to glycolipid-linked fatty acids. The distribution of the AM biomarker C16:1*cis*11 among lipid classes (Figure 1) clearly shows an increase in the NLFA and GLFA fractions over time and is likely the source of increased C16:1*cis*11 extracted by mild alkaline hydrolysis.

Gavito and Olsson (2003) observed that allocation of C to hyphal proliferation or storage lipids in AM fungi was related to plant variations in both nutrient supply and plant C availability. Fungal lipids present in the external mycelium are derived from C taken up as hexose by the fungal tissues within the host root (Pfeffer et al., 1999). The

AM fungi convert these sugars into lipids that are then translocated to the extraradical mycelium in lipid bodies. Up to 50% of the hyphal volume may be lipid bodies (Bago et al., 2000). Using ^{13}C as a tracer, Pfeffer et al. (1999) demonstrated ^{13}C enrichment of NLFA C16:1*cis*11 in mycelium. This indicates that lipids are the main C compounds translocated in AM fungal mycelia. Olsson and Johansen (2000) observed that the proportion of neutral lipids in old AM cultures was found to be higher in external mycelium than in internal mycelium. They conclude that neutral lipids accumulate in old mycelium as energy storage products, possibly preceding the sporulation processes. The neutral lipids within the extramatrical hyphae would largely coincide with cytoplasmic regions of the hyphae bound by phospholipid membranes.

Our study confirmed that, even if the biomarker comes from hyphae, and branched structures in the –R chambers, or arbuscules and vesicles in the +R ones, the plant has allocated C to the fungus after tasseling. Clearly there is an important functional difference if this marker comes from storage structures or from external functional structures (i.e. absortive hyphae), and these issues should be elucidated in future studies.

4.4 AM fungal colonization of chambers and available P

A large proportion of P uptake by maize occurs during the reproductive period (Karlen et al., 1988), while approximately three weeks after tasseling root biomass reaches a maximum (Plenet, 1995). The reduction in available P inside the +R and –R chambers from August to September at Shelton was likely due to hyphal-mediated P uptake.

Phosphorous uptake by root cells is influenced by the presence of AM fungi in cucumber plants, and this effect varies between fungal species (Pearson and Jakobsen,

1993). While P uptake by cucumber roots seemed to be reduced when they were colonized by *Glomus caledonium*, root P uptake was stimulated when colonized by *Scutellospora calospora*. Additionally, Bago et al. (1998) observed the presence of acidic zones in the cultures coinciding with a higher proportion of spores (low branched absorbing structures/spore ratios), possibly due to a greater P (and other nutrients) uptake by spore associated branched absorbing structures. That may indicate that P uptake by AM fungi is not inhibited by the occurrence of events prior to the sporulation phase of the fungi.

Application of P has resulted in an increase in the nutrient concentration in both the shoots and the roots of cucumber plants (Olsson et al. 1997). High P availability in soils reduced the C allocation to AM in plants (Olsson et al., 1997). Similarly, several studies have shown that higher availability of P in soils reduced extraradical hyphae growth and spore germination (De Miranda and Harris, 1994; Liu et al., 2000). As plant P content is influenced by soil P availability (Liu et al., 2000), possibly the P status of the plants from our study defined the allocation of C in vesicular arbuscular mycorrhizas.

An alternative explanation is that the formation and distribution of different specialized structures may respond to fine morphogenetic regulation processes (Bago et al., 1998). Graham et al. (1997) found that heavily colonized mycorrhizal-dependent citrus species at low P supply had lower total non-structural carbohydrates in root tissues (implying that they expended more C to acquire P) than those plants of similar biomass and P status grown at high P supply. In addition, the fact that spore-forming hyphae often grew adjacent to others that produced almost no spores, suggests the existence of a signal carried cytoplasmatically, inducing a differential morphogenesis in certain hyphae (Bago

et al., 1998). Thus, accumulation of NLFAs prior to sporulation may or may not be related to P availability but to internal physiological signals from the maize plant.

5. Conclusions

We conclude that the influence of soil P availability on AM fungal development may be dependent on maize P status and its linkage to crop physiological signals. The increase in the AM biomarker concentration in field soil and in chambers during the reproductive stages of maize confirmed the allocation of C from the plant to the mycorrhizal symbiont. Since root biomass does not change from two to three weeks after tasselling to maturity, AM fungi may play an essential role in P uptake of maize later in the growing season. Chambers that allowed only passage of AM hyphae were as efficient as roots and AM hyphae in extracting P from soil within the chambers. Further work is needed to identify mechanisms controlling the temporal allocation of C from plant to AM fungus, and the mycorrhizal contribution to P uptake during the reproductive stages of maize. The dynamics of lipid accumulation and formation of specialized structures in AM fungi appears to be essential to this process.

Acknowledgements

We want to express our gratitude to Liz Jeske, Susan Siragusa, Josh Cool, Sarah Berg, Susan Wagner for their essential help in the laboratory and in the field.

References

Allen, M.F., Swenson, W., Querejeta, J.I., Egerton-Warbuton, L.M., Treseder, K.K., 2003. Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. Annual Review of Phytopatology 41, 271-303.

Bago, B., Azcón-Aguilar, C., Piché, Y., 1998. Architecture and developmental dynamics of the external mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown under monoxenic conditions. Mycologia 90, 52-62.

Bago, B., Pfeffer, P.E., Shachar-Hill, Y., 2000. Update on symbiosis. Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiology 124, 949-957.

Barber, S. A., 1977. Application of phosphate fertilizers: methods, rates and time of application in relation to the phosphorus status of soils. Phosphorus in Agriculture. 70, 109-115.

Barea, J.M., 1991. Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. p 8-18. In: B.A. Stewart (Eds.), Advances in Soil Sicence, vol.16. Springer-Verlag, NY.

Bradley, K., Drijber, R.A., Knopps, J., 2006. Long and short-term increases in nitrogen availability alter the composition of soil microbial communities and decrease the abundance of arbuscular mycorrhizae in a grassland ecosystem. Soil Biology & Biochemistry in press.

Bray R.H., Kurtz, L.T., 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Science 59, 39-45.

De Miranda, J.C.C., Harris, P.J., 1994. Effects of soil phosphorous on spore germination and hyphal growth of arbuscular mycorrhizal fungi. New Phytologist 128, 103-108.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology & Biochemistry 32, 1419-1430.

Evans, D.G., Miller, M.H., 1988. Vesicular arbuscular mycorrhizas and the soil-disturbance induced reduction of nutrient absorption in maize. III. Influence of P amendments to soil. New Phytologist 110, 67-74.

Evans, D.G., Miller, M.H., 1990. The role of the external micelia network in the effect of soil disturbance upon vesicular-arbuscular mycorrhizal colonization of maize. New Phytologist 114, 65-71.

Galvez, L., Douds, Jr. D.D., Drinkwater, L.E., Wagoner, P., 2001. Effect of tillage and faming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. Plant and Soil 228, 299-308.

Gavito, M.E., Olsson, P.A., 2003. Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. FEMS Microbiology Ecology 45, 181-187.

Graham, J.H., Duncan, L.W., Eissenstat, D.M., 1997. Carbohydrate allocation patterns in citrus genotypes as affected by phosphorous nutrition, mycorrhizal colonization and mycorrhizal dependency. New Phytologist 135, 335-343.

Graham, J.H., Eissenstat, D.M., 1998. Field evidence for the carbon cost of citrus mycorrhizas. New Phytologist 140, 103-110.

Graham, J.H., Eissenstat, D.M., Drouillard, D.L., 1991. On the relationship between a plant's mycorrhizal dependency and rate of vesicular-arbuscular mycorrhizal colonization. Functional Ecology 5, 773-779.

Graham, J.H., Hodge, N.C., Morton, J.B., 1995. Fatty acid methyl ester profiles for characterization of Glomalean fungi and their endomycorrhizae. Applied and Environmental Microbiology 61, 58-64.

Grant, C.A., Flaten, D.N., Tomasiewicz, D.J., Sheppard, S.C., 2001. The importance of early season phosphorus nutrition. Canadian Journal of Plant Science 81, 211–224

Grogan, D.W., Cronan, Jr. J.E., 1997. Cyclopromane ring formation in membrane lipids of bacteria. Microbiology and Molecular Biology Reviews 61, 429-441.

IUPAC-UIB. 1987. The nomenclature of lipids. Journal of Lipid Research 19, 114-128.

Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2003. Soil tillage affects the community structure o mycorrhizal fungi in maize roots. Ecological Applications 13, 1164-1176.

Johnson, C.K., J.W. Doran, H.R. Duke, B.J. Wienhold, K.M. Eskridge, and J.F. Shanahan. 2001. Field-scale electrical conductivity mapping for delineating soil condition. Soil Science Society America Journal 65, 1829-1837.

Johnson, N.C., Pfleger, F.L., Crookston, R.K., Simmons, S.R., Copeland, P.J., 1991. Vesicular-arbuscular mycorrhizas respond to corn and soybean cropping history. New Phytologist 117, 657-663.

Kabir, Z., O'Halloran, I.P., Hamel, C., 1997. Overwinter survival of arbuscular mycorrhizal hyphae is favored by attachment to roots but diminished by disturbance. Mycorrhiza 7, 197-200.

Karlen, D.L., Flannery, R.L., Sadler, E.J., 1988. Aerial accumulation and partition of nutrients by corn. Agronomy Journal 80, 232-242.

Kates, M., 1986. Techniques of lipidology: isolation, analysis and identification of lipids. p. 123-127. In: Burdon, R.H., van Kippenberg, P.H. (Eds.), Laboratory Techniques in Biochemistry and Molecular Biology, vol. 3, Part 2. Elsevier, NY.

Larsen, J., Olsson, P.A., Jakobsen, I., 1998. The use of fatty acids signatures to study mycelial interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the saprotrophic fungus *Fusarium culmorum* in root-free soil. Mycological Research 102, 1491-1496.

Liu, A., Hamel, C., Begna, S.H., Ma, B.L., Smith, D.L., 2003. Soil phosphorus depletion capacity of arbuscular mycorrhizae formed by maize hybrids. Canadian Journal of Soil Science 83, 337-342.

Liu, A., Hamel, C., Hamilton, R.I., Smith, D.L., 2000. Mycorrhizae formation and nutrient uptake of new corn (*Zea mays L.*) hybrids with extreme canopy and leaf architecture as influenced by soil N and P levels. Plant and Soil 221, 157-166.

Miller, M. H., 2000. Arbuscular mycorrhizae and the phosphorus nutrition of maize: A review of Guelph studies. Canadian Journal of Plant Science 80, 47–52.

Madan, R., Parkhurst, C., Hawke, B., Smith, S., 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM Spores in soil. Soil Biology & Biochemistry 34, 125-128.

Olsen, S.R., Sommers, L.E., 1982. Phosphorus. p. 403-430. In: A.L. Page. R.H. Miller, and D.R. Keeney (Eds.), Methods of Soil Analysis. 2nd ed. Agronomy Series No 9, Part 2. Soil Science Society of America, Inc., Madison, WI.

Olsson, P.A., 1999. Minireview. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29, 303-310.

Olsson, P.A., Bååth, E., Jakobsen, I., 1997. Phosphorous effects on mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by fatty acids signatures. Applied and Environmental Microbiology 63, 3531-3538.

Olsson, P.A., Johansen, A., 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different gowth stages. Mycological Research 104, 429-434.

Pearson, J.N. Jakobsen, I., 1993. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labeling with ^{32}P and ^{33}P. New Phytologist 124, 489-494.

Pfeffer, P.E., Douds, D.D., Bécard, G., Shachar-Hill, Y., 1999. Carbon uptake and the metabolism and transport of lipids in and arbuscular mycorrhiza. Plant Physiology 120, 587–598

Plenet, D., 1995. Fonctionnement des cultures de maïs sous contrainte azotée. Determination et application d'un indice de nutrition. Dr These Académie de Nancy-Metz, Institut National Polytechnique de Lorraine.

Ritchie, S.W., Hanaway, J.J., Benson, G.O., 1997. How a corn plants develops. Spec. Publ. 48. Iowa State Univ. Coop. Ext. Serv., Ames.

SAS Institute Inc, 1999. SAS/STATs User's Guide, Version 8. SAS Institute Inc., Cary, NC.

Singh, R., Adholeya, A., 2001. Plant and fungal response to colonization. p. 7-28. In: Sharma A.K. and Johri, B.N (Eds.), Arbuscular mycorrhizae. Interactions in plants, rhizosphere and soils. Science publishers, Inc. Enfield, NH.

Smith S.E, Smith, F. A., Jakobsen I., 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake paghway is not correlated with mycorrhizal responses in growth or total P uptake. New Phytologist 162, 511-524.

Smith S.E, Smith, F.A., Jakobsen, I., 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology 133, 16-20.

Smith, S.E., Read, D.J., 1997. Mycorrhizal symbiosis. Academic Press, London, 587 pp.

van Aarle, I.M., and Olsson, P.A., 2003. Fungal lipid accumulation and development of mycelial structures by two arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 69, 6762-6767.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51-62.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111–129.

Table 1. Concentration of C16:1*cis*11 FAME biomarker and availability of P in soil samples from Shelton and Lincoln used to make the chambers.

Field		C16:1*cis*11	Bray P	Organic P
		---nmol g^{-1} soil---	----------mg kg^{-1} soil----------	
Shelton	ECa class II	5.25	13.26	115.30
	ECa class IV	3.63	41.93	126.82
Lincoln	M1	2.19	57.59	191.18
	M2	2.07	84.06	235.59

ECa class II= apparent electrical conductivity class II; ECa class IV= apparent electrical conductivity class IV; M1=recommended fertilizer rates and M2= intensive nutrient management.

Table 2. Concentration of C16:1*cis*11 and C18:1*cis*11 FAME biomarkers in soil samples from Shelton over time.

Field soil					
	19 June	15 July	4 August	23 August	13 September
	----------nmol g^{-1} soil----------				
C16:1*cis*11 (SE = 0.32)	4.69C	4.53C	4.99C	5.54B	6.47A
C18:1*cis*11 (SE = 0.57)	9.58b	10.34b	10.7b	10.62b	11.96a

For ach fatty acid, means followed by the same letter among dates are not different ($P < 0.05$). N = 60.

Table 3. Concentration of C16:1*cis*11 and C18:1*cis*11 FAME biomarkers in soil samples from Lincoln over time.

Field soil				
	15 July	3 August	26 August	16 September
	----------nmol g^{-1} soil----------			
C16:1*cis*11 (SE = 0.16)	2.21D	2.67C	3.21B	4.51A
C18:1*cis*11 (SE = 0.47)	5.22b	5.64b	5.61b	6.76a

For each fatty acid, means followed by the same letter among dates are not different ($P < 0.05$). N = 32.

Table 4. Concentration of C16:1*cis*11 and C18:1*cis*11 FAMEs biomarker in chambers from Shelton over time.

Chambers	4 August	23 August	13 September
	----------nmol g^{-1} soil----------		
C16:1*cis*11 (SE = 0.29)	3.81B	4.18B	5.9A
C18:1*cis*11 + R (SE = 0.53) ┣	7.7b	8.34b	11.02a
C18:1*cis*11 - R	7.61b	7.79b	8.84b

C16:1cis11 biomarker means followed by the same letter among dates are not different ($P < 0.05$).

┣ Significant interaction mesh*time ($P = 0.02$) C18:1*cis*11 FAME biomarker. Rings enclosed with a 1 mm mesh fabric to allow (+R) or 0.04 mm mesh fabric to exclude (-R) maize roots. Fatty acid C18:1*cis*11 means followed by the same letter are not different ($P < 0.05$). N = 144.

Table 5. Concentration of C16:1*cis*11 and C18:1*cis*11 FAMEs biomarker in chambers from Lincoln over time.

Chambers	3 August	26 August	16 September
	----------nmol g^{-1} soil----------		
C16:1*cis*11 (SE = 0.20)	1.84B	2.14B	3.08A
C18:1*cis*11 (SE = 0.16) ‡	4.95b	5.04ab	5.36a

Biomarker concentration means followed by the same letter among dates are not different. For C16:1cis11 ($P < 0.05$), N = 96.

‡ For C18:1cis11 ($P = 0.07$).

Table 6. Availability of P in soil from chambers at Shelton over time.

	4 August	23 August	13 September
	----------mg kg^{-1} soil----------		
+ P chambers (SE = 2.83)	38.73A	37.23A	32.3B
- P chambers (SE = 1.94)	27.35a	27.53a	24.0b

Within P treatments, mean followed by the same letter among dates are not different ($P < 0.05$). N = 72

Table 7. Availability of P in soil from chambers at Lincoln over time.

	3 August	26 August	16 September
	----------mg kg^{-1} soil----------		
+ P chambers (SE = 4.93)	84.51A	85.98A	83.22A
- P,+R chambers (SE = 4.63) ┣	68.47b	74.57a	70.14bc
- P,-R chambers	70.98ac	74.36a	71.26ab

Within P treatments, mean followed by the same letter among dates are not different ($P < 0.05$).

┣ Significant interaction mesh*time ($P = 0.04$) –P chambers. Rings enclosed with a 1 mm mesh fabric to allow (+R) or 0.04 mm mesh fabric to exclude (-R) maize roots. N = 48.

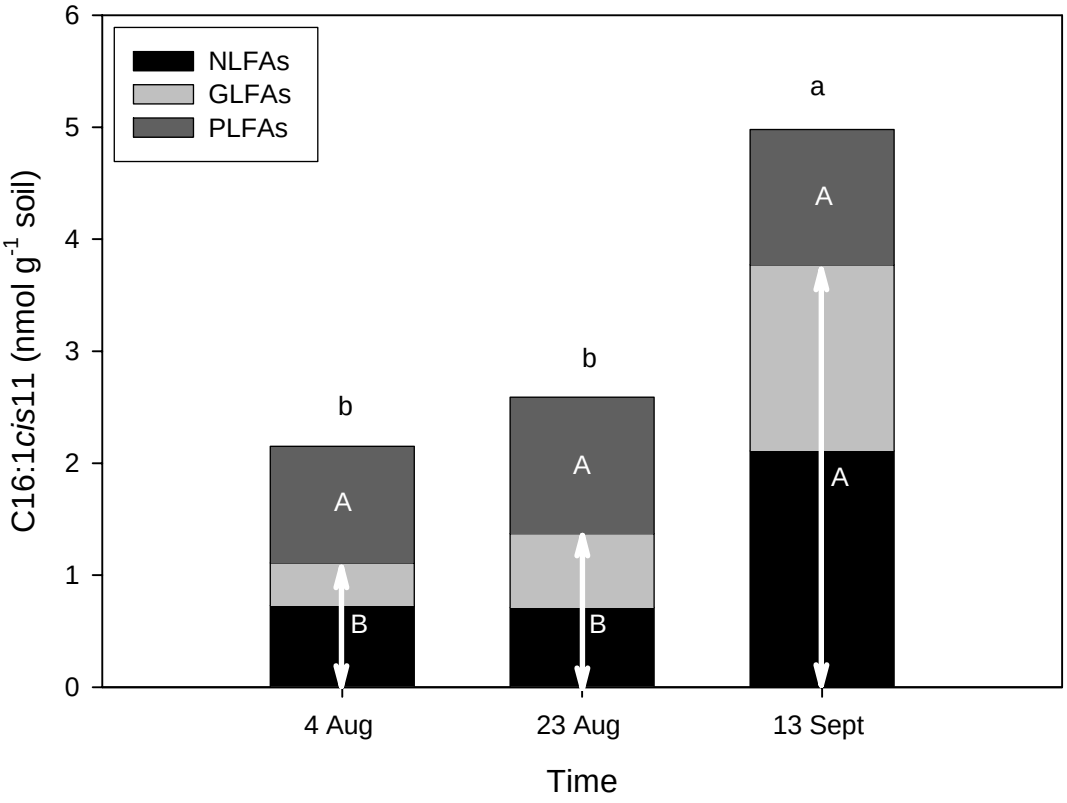


Figure 1. Concentration of chloroform methanol extracted C16:1*cis*11 over time in Shelton (+R,-P) chambers. Bars followed by the same letter are not different among dates ($P < 0.05$). Capital letters are used to compare PLFA over time (SE = 0.06), and the combined concentration of NLFA and GLFA over time (SE = 0.34). Lower case letters represent differences among the addition of the three fractions (SE = 0.37). N = 6.

Chapter IV

The distribution of arbuscular mycorrhizal biomarkers among lipid classes extracted from soil during the reproductive growth of maize

M. Susana Grigera, Rhae A. Drijber, Rebecca H. Shores-Morrow,

Brian J. Wienhold

Prepared for Soil Biology and Biochemistry

Abstract

Arbuscular mycorrhiza (AM) fungi form symbiotic relationships with the majority of land plants and are known for their positive effects on plant P acquisition and soil quality. The extramatrical growth of the mycelium is a key factor in nutrient acquisition by the symbiont. Identifying patterns in plant-fungi growth is necessary to understand how these symbionts operate in agroecosystems over time. The objective of this work was to study C partitioning in AM fungi in soil samples during the reproductive growth stage of corn (*Zea mays* L.). Chambers made of PVC enclosed with a 1 mm mesh fabric to allowed roots and hyphae passage amended with distilled water were installed in two sites at tasselling and removed after three, six and nine weeks in Nebraska. Soil samples were analyzed using ester linked fatty acid (EL-FAME) and chloroform-methanol extraction (CM-FAME) analysis. We also separated and analyzed the neutral (NLFA), glyco (GLFA) and phospho (PLFA) lipid fractions. We observed that AM fungi have resistant structures that are hydrolyzed only after the soil sample is roller-milled (RM). These particular structures were enriched of NLFA and GLFA C16:1*cis*11, but not PLFA, indicating that storage lipids and possibly membrane lipids are released by RM. EL- and CM-FAME C16:1*cis*11 increased later in the corn season, effectively showing the C allocation from the plant to the fungi under field conditions. We successfully utilized EL-FAME and CM-FAME C16:1*cis*11 to study the dynamics of AM fungi in soils under field conditions, however the C partition was not clearly shown by the different fractions. PLFA C16:1*cis*11 did not change over time or due to RM. NLFA C16:1*cis*11 increased with RM treatment; and did not change over time. GLFA C16:1*cis*11 increased over time

and due to RM treatment. Further studies are needed to elucidate the role of GLFA C16:1*cis*11 in AM fungi.

Key words: C16:1cis11, FAME, EL-FAME,CM-FAME, NLFA, GLFA, PLFA, corn.

1. Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with most land plants and are recognized for their positive effects on plant growth and soil quality (Smith and Read, 1997). This relationship benefits both the plant and the fungus. The plant acquires nutrients more efficiently than can be accomplished by the root system alone and the fungi are provided a C source. Extramatrical growth of the mycelium is a key factor in nutrient acquisition by the mycorrhizal symbiont (Olsson et al., 1997). In AM fungi C is stored and transported as lipid bodies. Lipid synthesis occurs in intraradical fungal structures using plant C. The fungi convert plant sugars into lipids that are translocated to the extraradical mycelium (Pfeffer et al., 1999) to be used for mycelial growth and sporulation.

Lipid fractions are useful tools in the study of AM fungal growth and morphogenesis. The proportion of neutral lipids in old AM hyphae was found to be higher in external mycelium than in internal mycelium (Olsson and Johansen, 2000). Triacylglycerol degradation during spore germination was demonstrated by Gaspar et al. (1994). After ten days of germination, the triacylglycerols were reduced to nearly one-fourth of their initial concentration. Simultaneously, phospholipids tripled their concentration during the first five days of germination, and continued to increase for two weeks. During germination triacylglycerols provide energy and C for the synthesis of phospholipids, which are structural molecules of membranes needed to form the germ tube (Gaspar et al., 1997). Thus, neutral and phospholipids dynamics are of interest when studying the temporal allocation of C from plant to fungus.

The fatty acid methyl ester (FAME) C16:1*cis*11 is often used as a biomarker for AM fungi in roots (Graham et al., 1995; Olsson and Johansen, 2000) and soils (Olsson, 1999). Graham et al. (1995) examined the FAME composition of spores of the order *Glomales* and found that C16:1*cis*11 accounted for more than 50% of the total fatty acids in all isolates of *Acaulospora* and *Eutrophospora* and most isolates of *Glomus* and *Scutellospora*. The amount of C16:1*cis*11 per unit biomass in the mycelium varied greatly for neutral lipids, but not for phospholipids, making PLFA C16:1*cis*11 a better indicator of mycelial biomass (Olsson, 1999). However, NLFA 16:1*cis*11 may be more sensitive than PLFA 16:1*cis*11 as an indicator of AM mycelium in soil systems since the ratio between NLFA and PLFA C16:1*cis*11 is high in AM fungi (between 1 and 200), while it is low in bacteria (<1) (Olsson, 1999).

In soil systems, levels of PLFA are often too low and insensitive to detect changes in mycorrhizal fungal biomass (Olsson, 1999). Olsson's extensive research into the utility of PLFA and NLFA C16:1*cis*11 has shown both to have reasonable correlations with hyphae and spores in soil-sand mixes, and to assess hyphae, arbuscules and vesicles in roots (Olson et al., 1997; Olson and Johansen, 2000; van Aarle and Olson, 2003). However, only 0.2% of the spore and 0.4% of the hyphae biomass consisted of phospholipids. Given these low concentrations, a low extraction efficiency may also affect the final estimation of fungal biomass by PLFAs (Olsson and Johansen, 2000). It is also important to note that these experiments were done under controlled conditions. In agricultural soils under field conditions the extraction efficiency of phospholipids may be even smaller, making interpretation more uncertain.

The FAME biomarker C16:1*cis*11 provides an index of C partitioning during the colonization process based on relative amounts of this fatty acid in different structures for fungal growth and lipid storage (Graham et al., 1995). We used this approach to study C allocation to AM fungi in soil samples collected during the reproductive growth stage of corn (*Zea mays* L.). The AM fungal signature NLFA C16:1*cis*11 in *Plantago lanceolata* roots was weakly correlated to vesicle and arbuscules numbers with time, even though the NLFA/PLFA ratio indicated the accumulation of storage lipids in vesicles and intraradical spores (van Aarle and Olsson, 2003). Others have concluded that neutral lipids are produced not only to be stored, but also to support translocation of C within the mycelium (Bago et al., 2002; van Aarle and Olsson, 2003). Comparing lipid accumulation in different genera of AM fungi must be done with care since taxonomically related differences in fatty acid composition may affect the results. At the community level, as under field conditions, the complexity of the system increases and several AM fungal groups may interact with the crop. In these complex systems, lipid analysis may be essential to understand AM fungal dynamics over time.

We hypothesize that direct extraction of FAMEs from soil using mild alkaline hydrolysis (EL-FAMES) is minimally effective at extracting spores (a main reservoir of NLFA's), thus reflecting largely extramatical hyphae in the soil. The neutral lipids within the extramatrical hyphae would largely coincide with cytoplasmic regions of the hyphae bound by phospholipid membranes. Furthermore, an increase in C16:1*cis*11 FAMEs would reflect an increase in neutral lipids typically found in presporulation stages and not to the presence of spores *per se*. Thus, FAMEs extracted from this cytoplasmic region would represent the 'physiologically active' portion of the fungus important to nutrient

acquisition by the plant. Neither PLFAs nor EL-FAMEs truly represent the 'biomass' of mycorrhizal hyphae as most hyphae are devoid of cytoplasm, acting mainly for transport of nutrients to and from the host plant.

2. Materials and methods

2.1 Experimental site

A field study was conducted during 2004 near Shelton, NE (40º45'01" N, 98º46'01" W). Soils at the site are Hord silt loam (Fine-silty, mixed mesic Pachic Argiustoll, 0-1% slope) and Blendon loam (Coarse-loamy, mixed mesic Pachic Haplustoll, 0-1% slope). Continuous corn was grown from 1990 to 1996 under conventional disk-tillage with furrow irrigation and since 1996 under reduced tillage with sprinkler irrigation. Prior to planting, 168 kg N ha^{-1} as urea ammonium nitrate solution was incorporated with a field cultivator. Corn (Pioneer 33B51) was planted on 2 May, 2004 with 19.5 kg P ha^{-1}, and 13.1 kg N ha^{-1} as diammonium phosphate applied adjacent to the seed. Stand density was 71600 plants ha^{-1} and field average yield was 12.2 Mg ha^{-1} at 155 g kg^{-1} moisture content.

2.2 Preparation, installation and removal of chambers

A bulk soil sample was collected from the 0- to 15-cm depth on 22 June and passed through a 6 mm mesh sieve to remove plant residues then stored moist at 4ºC until the chambers were prepared. The chambers were made of PVC with a wall thickness of 0.6 cm, a diameter of 10 cm, and a height of 4.5 cm. The sides of the rings were enclosed with a 1 mm mesh fabric to allow corn roots and hyphae penetration (Sefar America Inc., Depew, NY). The ring was filled with approximately 425 g of soil and packed to a bulk

density of 1.2 g cm^{-3}. Soil in each ring was amended with 20 ml of distilled water and thoroughly mixed. The chambers were placed in a plastic bag and stored at 4ºC until they were placed in the field. Three chambers were installed randomly on 15 July in two field locations at the VT stage of corn (Ritchie et al., 1997).

One chamber from each site was removed three, six, and nine weeks after installation (4 August, 23 August, and 13 September). The chambers were transported in a cooler to the laboratory, and opened. The soil was passed through a 4 mm sieve to remove visible organic residues, thoroughly mixed, air dried and divided in two: one subsample was roller-milled (RM) and the other was not. Briefly, 10 stainless steel rods inside square bottles (7.0-cm tall, 4.0-cm sides) containing 2.5 g of soil per bottle were rolled for 16 h. Roller-milling results in a sample with smaller particles (99.2 % <100 mesh) than with a ball-milling device (83% <100 mesh) and thus provides a more uniform sample (Arnold and Schepers, 2004).

2.3 Quantification, identification, and classification of FAMEs

2.3.1 Ester linked (EL) FAMEs analysis

Briefly, samples were hydrolyzed using freshly prepared 0.2 M potassium hydroxide in methanol and the resulting FAMEs were partitioned into hexane (Kates, 1986; White et al., 1979). Following saponification to release ester-linked FAMEs, methyl-nonadecanoate (0.05 μg μl^{-1}) was added to the extract as an internal standard.

Released FAMEs were separated by gas chromatography, using helium as a carrier gas and an Ultra 2 HP (50 m, 0.2 mm I.D., 0.33 μm film thickness) capillary column. The gas chromatograph was run in split mode (44:1) with a 45 s purge time. Injector and flame ionization detectors were maintained at 280º and 300º C, respectively,

and oven temperature was ramped from 50º C to 160º C at 40º C min^{-1} and held for 2 min, then ramped at 3º C min^{-1} to 300º C and held for 30 min. The fatty acids were identified by retention-time and confirmed by mass spectrometry. Concentrations of FAMEs were calculated from peak areas and reported as nmol g^{-1} soil.

*2.3.2 Chloroform-methanol extraction (CM-FAME) and separation into lipid classes*A modified one-phase extraction procedure of Bligh and Dyer (White et al., 1979) was used to extract lipids from the soil. An aliquot (150 µl) of total lipid extract (CM-FAMEs) was saponified and quantified as described previously for FAMEs. The remaining lipid extract (300 µl) was separated into neutral lipid fatty acids (NLFA), glycolipid fatty acids (GLFA) and phospholipid fatty acid (PLFA) classes by silica gel chromatography (Drijber et al., 2000). Chloroform used to elute NLFA was freshly distilled. Lipids were saponified and quantified as described above for FAMEs.

Fatty acids were designated as the total number of C atoms followed by a colon, the number of double bonds followed by the position of the double bond from the carboxyl end of the molecule and its *cis* or *trans* configuration (IUPAC-IUB, 1987). The prefixes *a* and *i* indicate antieso and iso branching, respectively; *cy* indicates cyclopropane fatty acids, *br* indicates an unknown branch position and 10Me indicates a methyl branch on the 10^{th} C atom from the carboxyl end of the molecule. The C16:1*cis*11 FAME biomarker was selected to represent AM fungi (Olsson and Johansen, 2000).

2.4 Statistical analysis

Statistical analysis was performed with SAS PROC MIXED (SAS Institute, 1999) assuming time of the year and treatment (RM, not RM) as fixed effects. Pearson

correlations were also performed using SAS. Significance level for all comparisons was set a priori at $P < 0.05$, and exceptions are stated.

3. Results

The concentration of the biomarker C16:1*cis*11 was greater for all dates in EL-FAMEs compared to CM-FAMEs, and increased over time in the EL-FAMEs, CM-FAMEs, and marginally ($P < 0.07$) in the GLFA fraction (Table 1). The concentration of C16:1*cis*11 in PLFAs was similar over time, and while NLFA C16:1*cis*11 was higher in September this difference was not significant (Table 1). In addition, the ratio of NLFAs to PLFAs was similar over time. The relative concentration of NLFA over the combined concentration of NLFA, GLFA and PLFA C16:1*cis*11 was the same for all three sampling dates (Table 1). The relative concentration of GLFA C16:1*cis*11 was 45% of the combined concentration of the NLFA, GLFA and PLFA C16:1*cis*11 lipid fractions, and did not change over time. Because PLFA C16:1*cis*11 did not increase over time, the relative concentration of PLFA C16:1*cis*11 decreased by 44% in September compared to the two sampling dates in August. The percent recovery of the NLFAs, GLFAs, and PLFAs fractions was high ($y_0 = -0.06$, $a = 1$, $R^2 = 0.99$, $P < 0.001$) (Figure 1).

Roller-milling increased the concentration of C16:1*cis*11 in the EL-FAMEs by 129%, and CM-FAMEs by 98% (Table 1). This increase was due to the combined NLFA and GLFA C16:1*cis*11, which increased by 185% on RM. When considering only the NLFA C16:1*cis*11 biomarker, the increase was only 103%.

3.1 Efficiency of extraction of FAMEs in RM soil

Roller-milling increased efficiency of extraction of specific EL- and CM-FAMEs (Figure 2). The fatty acids *i*C15:0, *a*C15:0, C15:0, *i*C16:0, C16:1*cis*7, C16:1*cis*9, 10MeC17:0, *i*C17:0, *a*C17:0, C17:1*cis*9, cyC17:0(9,10), i10MeC18:0, 10MeC18:0, C18:0, *cy*C19:0(11,12) and C20:4(5,8,11,14) followed a 1 to 1 line indicating similar efficiency of extraction before and after RM. The linear regression was significant for both EL-FAMEs ($y_0 = 0.51$, a = 1, $R^2 = 0.96$) and CM-FAMEs ($y_0 = 0.28$, a = 1, $R^2 = 0.94$). The following fatty acids did not follow this distribution: C16:0, C16:1*cis*11, C18:1*cis*9, C18:1*cis*11, and, C18:2*cis*9,12. The linear regression was not significant for these fatty acids: EL-FAMEs ($y_0 = 9.8$, a = 1, $R^2 = 0.62$) and the CM-FAMEs ($y_0 = 4.9$, a = 1, $R^2 = 0.30$)

3.2 Distribution of C16:1cis11 among lipid fractions

The C16:1*cis*11 fatty acid present in EL-FAMEs covaried with the concentration in the CM-FAMEs, and showed that both methods of lipid analysis extracted fatty acids from similar pools (Table 2), even if the concentrations differed (Table 1). The correlation was also high between GLFA and NLFA C16:1*cis*11, separately or combined, and the EL- and CM-FAMEs. The concentration of C16:1*cis*11 in the most polar lipid fraction (PLFA) covaried with CM-FAMEs and EL-FAMEs, however this correlation was marginally ($P < 0.1$) significant. The correlation among the NLFAs, GLFAs and PLFAs was not significant.

3.3 Dynamics of C16:1cis11 in RM vs. non RM soil

The mean concentration of CM-FAME C16:1*cis*11 in non RM samples increased 157% from August to September and the combined concentration of NLFA and GLFA C16:1*cis*11 increased 204% (Figure 3). The increase in EL-FAME C16:1*cis*11 from

August to September was 55%. In RM samples, the increase from August to September was 42% for CM-FAME, 39% for EL-FAME and 45% for the combined concentration of NLFA and GLFA C16:1*cis*11 (Figure 3). The concentration of PLFA C16:1*cis*11 showed no increase over time or due to RM.

4. Discussion

4.1 RM enhances extraction of fungal biomarkers

Roller-milling the soil increased the recovery of EL- and CM-FAMEs common to saprophytic fungi (C16:0, C18:1*cis*9, C18:2*cis*9,12) and AM fungi (C16:0, C16:1*cis*11, C18:1*cis*11) but not those specific to bacteria or the faunal marker C20:4. This increase was attributed to increased extraction efficiency of resistant fungal structures, such as rhizomorphs and spores, disrupted by the milling action. In contrast, Madam et al. (2002) found that crushing the spores prior to lipid extraction caused a small (but non significant) decrease in the yield of fatty acids detected in several AM fungi. Disruption of soil aggregates by RM did not enhance recovery of bacterial FAMEs indicating adequate penetration of solvent by both extraction methods into dry soil aggregates of this medium textured soil.

Roller-milling significantly increased the amount of C16:1*cis*11 in the NLFA and GLFA fractions, but not the PLFA fraction. Olsson and Johansen (2000) extracted lipids from 3-mo-old AM mycelium and found that ball-milling enhanced the yield of NLFA 16:1*cis*11 by 733%, and the NLFA/PLFA ratio was increased by 249%. The estimated biomass of spores versus mycelium was high and would account for these values. In our study, the yield of NLFA increased by 102% and the NLFA/PLFA ratio was 91% higher

in RM than in non RM samples. This would suggest a greater mycelial (or non-spore) biomass in the soil chambers. This is supported by the NLFA C16:1*cis*11 accounting for the same proportion of the total CM-FAMEs in RM and non-RM soils. This ratio would be expected to increase significantly with a high spore population. Thus, increased concentration of C16:1*cis*11 in RM soil reflects increased overall extraction efficiency from all AM fungal structures within the chambers, not enhanced extraction from selected resistant structures such as spores. Furthermore, Olsson and Johansen (2000) also observed that most PLFAs are easily removed from mature spores using the Bligh and Dyer procedure, while ball-milling increased the yield of PLFA C16:1*cis*11 extracted from mycelium.

Roller milling increased the proportion of C16:1*cis*11 recovered in GLFA (292%) compared to NLFA (102%). This suggests more than one source for these complex lipids, or that glycolipids account for a greater proportion of the fungal biomass disrupted by RM than neutral lipids. Jabaji-Hare et al. (1984) studied the total lipid content of *Glomus* vesicles, and found that glycolipids represented 44.5% of the total lipid. Greater disruption of plant roots by RM may have led to increased recovery of AM lipids from intraradical fungal structures. Further studies are required to identify the sources of these complex lipids in fungal structures.

4.2 Suitability of lipid fractions for measuring AM biomass in field soil

Olsson and Johansen (2000) showed that the amount of PLFA 16:1*cis*11 per unit of biomass remained constant as the mycelium aged and was a good indicator of AM biomass. Although PLFA C16:1*cis*11 has been used successfully to quantify the dynamics of AM biomass in sand-culture systems, its utility has been questioned for soils

(Olsson, 1999). Our study supports this premise. Olsson and Wilhelmsson (2000) buried mesh bags and tubes containing initially mycelium-free sand in sand dunes near the roots of *Ammophila arenaria* L., and removed them after 35 and 68 days. They observed mean concentrations of C16:1*cis*11 PLFA of 46 pmol g^{-1} sand after 35 days and 41 pmol g^{-1} sand after 68 days. If we assume similar net accumulations of PLFA in the chambers over time, background concentrations of C16:1*cis*11 PLFA were several orders of magnitude higher making detection of new AM growth using PLFA impossible. This limitation led to the use of NLFA C16:1*cis*11 as a measure of AM biomass in field soils (Olsson, 1999).

Lipid synthesis occurs in intraradical fungal structures using plant C that is translocated to the extraradical mycelium (Pfeffer et al., 1999). Neutral lipids accumulate as mycelium ages and are evidence of successful root colonization, and a prerequisite for subsequent sporulation (Olsson and Johansen, 2000). Olsson and Wilhelmsson (2000) observed that NLFA C16:1*cis*11 was a sensitive tool for the detection of AM fungi mycelium in their field study. Although mean concentrations of NLFA C16:1*cis*11 increased during the 9 weeks of our experiment, it was not significant. Our results contradict previous reports under field conditions, possibly due a high background concentration of NLFA. We did observe a significant doubling in GLFA C16:1*cis*11 later in the corn season; however, there is minimal mention of this lipid fraction in the literature. We suggest that NLFA and GLFA C16:1*cis*11 have a complementary dynamic under field conditions and during the reproductive stages of corn, but future studies are required to understand its significance in terms of fungal growth and development.

4.3 Dynamics of AM biomarkers in chamber soil during the reproductive stages of corn

4.3.1 GLFA C16:1cis11

The increase in GLFA C16:1*cis*11 observed in our research may be related to the synthesis of specific membranes and structures as part of the pre-sporulation process. Nemec (1981) studied the histochemical characteristics of *Glomus etunicatus*. He suggested that the arbuscule wall is composed primarily by glycolipids, and its osmophilic and acidic properties would facilitate bi-directional flow of nutrients.

The relative concentration of GLFA C16:1*cis*11 was 45% and was higher than the proportion of NLFA and PLFA. Jabaji-Hare et al. (1984) studied the total lipid content of *Glomus* vesicles, and found that glycolipids represented 44.5% of the total lipid. They also found that the most abundant fatty acid was 16:1 in the neutral and glycolipid fractions. In our study, gycolipids composed 28% of the total fatty acids extracted from our non-RM samples and 50% from the RM samples. The percentage we report for soil extracts is similar to previous studies for pure culture extracts.

Drijber et al. (2000) observed that PLFA and EL-FAMEs clearly separated native sod plots from wheat fallow plots, and changes in the EL-FAMEs, particularly C16:1*cis*11 explained most of this separation. They inferred that the EL-FAME C16:1*cis*11 biomarker was probably ester linked to a glycolipid or another complex lipid, eluted from silica gel with acetone. Similarly, in our field study, we observed that GLFA C16:1*cis*11 followed the pattern of EL- and CM-FAME C16:1*cis*11. There is a need to better understand the role of glycolipids in AM fungi.

The increase in GLFA C16:1*cis*11 later in the corn season was likely due to a change in lipid synthesis pathways, with a clear dominance of gycolipids (observed in the

RM samples) over the other fractions. Mock and Gradinger (2000) studied the photosynthetic C allocation of Arctic-ice algae, and found that glycolipids constituted up to 80% of the total lipid production under nutrient and light stress. One hypothesis may be that perhaps a reduction in C supply to AM fungi during the R5-6 stage of corn may trigger the production of glycolipids in spore-related structures formed by symbiotic fungi. A second hypothesis is that stress affected the AM fungi during senescence of the corn crop. In plants, steryl glucosides and their acylated derivatives are phytoesterols (glycolipids) located in microsomal and mitochondria membranes (Harwood, 1977). Additionally, glycolipids (sulpholipids) may be vital in protecting certain plants from environmental stresses, e.g. drought tolerance. Further studies should be made to characterize and understand the function of glycolipids in AM fungi.

A third hypothesis is that the increase in GLFAs over time in AM fungi may be related to the development of mitochondria and related organelles, at the expense of storage lipids (NLFAs). Bago et al. (1998) observed in *Glomus intraradices* that arbuscules develop along intraradical, intercellular hyphae or intracellular coils, while branched absorbing structures (BAS) areformed on extraradical mycelia. Arbuscules and BAS share several cytological characteristics: contain numerous nuclei, mitochondria, glycogen deposits, lipid globules, abundant polyvesicular bodies, and electrodense granules inside small vacuoles (Bonfante-Fasolo, 1984; Bago et al. 1998). BAS structures are possibly associated with further spore formation. Gavito et al (2001) concluded that the shortage of C belowground when Pea (*Pisum sativum* cv. Solara) was flowering was associated with an increase in root senescence and changes in AM fungal phenology. The fungi switched from an active colonization phase to a reproductive phase, where storage

structures such as vesicles and spores (and possibly BAS) are typically formed. In our study, this switch may explain the increase in GLFA and triggered by the shortage of C at R5-6.

4.3.2 The combined concentration of NLFA and GLFA C16:1cis11

Glycolipids are easily extracted from fungal cells with organic solvents, but they exhibit many of the solubility properties of neutral lipids and are weakly absorbed by silicic acid. For these reasons they were included in the neutral lipid fraction in much of the earlier work and did not received detailed attention (Brennan et al., 1974). Today, it is difficult to find references to fungal glycolipids. Variations in methodology (i.e. column selection, solvent preparation, solvent elution volumes, etc.) for the separation of the neutral and glycolipid fractions makes it difficult to make comparisons among studies. A standardized protocol is needed before comparison of lipid fractions among studies is possible.

Given the methodological controversy stated above, the increase in the concentration of NLFA C16:1*cis*11 over time, the lack of knowledge of GLFA C16:1*cis*11, and the significance of the correlation among NLFA and GLFA with EL- and CM-FAME C16:1*cis*11, we combined the concentration of the NLFA and GLFA C16:1*cis*11 fractions to explain the dynamics of C partition during the reproductive stages of corn. There was an increase over time of the storage/structural lipids (represented by the increase in the combined concentration of NLFA and GLFA C16:1*cis*11) over time, that confirms an allocation of C from the plant to the symbiont. The increase over time of the combined concentration of NLFA and GLFA biomarker

was magnified in the non RM samples, but was also observed on a smaller scale in the RM samples.

4.4 EL-FAMEs as a simple measure of AM colonization of soil

We propose the simple EL-FAME extraction procedure for routine measurement of AM biomass in soils based on the following. (1) The concentration of the AM fungal marker, C16:1*cis*11, was greater in EL-FAMEs, but highly correlated to CM-FAMEs ($r=0.92$, $p=0.01$) in both RM and non RM soils suggesting extraction from similar structures within AM biomass. This higher concentration of C16:1*cis*11 in EL-FAMEs probably reflects easier solubilization of fatty acid FAMEs compared to intact complex lipids from fungal cells. (2) The amount of C16:1*cis*11 extracted by both methods is largely driven by GLFA, and to a lesser degree by NLFA. (3) In soils without prior grinding, both methods would be relatively inefficient at extracting spores and thus reflect largely the extramatical AM hyphal network important for nutrient acquisition by the plant.

5. Conclusion

Under field conditions, fungi may have lipid-containing resistant structures that become more extractable after RM the soil. These more resistant structures are possibly spores or rhizomorphs, or intramatrical AM structures within roots. The PLFA C16:1*cis*11 was not responsive to AM colonization of the soil. EL-FAME and CM-FAME C16:1*cis*11 biomarkers reflected the increase in NLFA and GLFA, and were related to storage lipids and resistant structures synthesized *de novo* during the

reproductive stages of corn. Further studies are needed to elucidate the role of GLFA C16:1*cis*11 in AM fungal dynamics.

To study the dynamics of AM fungi in soils during the reproductive stages of corn, we successfully used EL-FAMEs, CM-FAMEs and lipid fractions. NLFA C16:1*cis*11 alone was not adequate to measure the dynamics of indigenous AM fungi in soils. We suggest using NLFA and GLFA C16:1*cis*11 to understand C dynamics in AM fungi in agroecosystems. The simple EL-FAME extraction procedure is recommended for routine measurement of AM biomass in soils

References

Arnold, S.L. and Schepers, J.S. 2004. A simple roller-miller grinding procedure for plant and soil samples. Communication in Soil Science and Plant Analysis 35, 537-545.

Bago, B., Azcón-Aguilar, C., Goulet, A. and Piché, Y., 1998. Branched absorbing structures (BAS): a feature of the extraradical mycelium of symbiotic arbuscular mycorrhizal fungi. New Phytologist 139, 375-388.

Bago, B., Zipfel, W., Williams, R. M., Jun, J., Arreola, R., Lammers, P. J., Pfeffer, P. E., Shachar-Hill, Y., 2002. Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. Plant Physiology 128, 108-124.

Bonfante-Fasolo, P. 1984. Anatomy and morphology of VA mycorrhizae. p. 5-33. In: Powell, C.L., Bagayaraj, D.J., (Eds.) VA Mycorrhiza. Boca Raton, FL, USA. CRC Press.

Brennan, P.J., Friffin, P.F.S., Losel, D.M. Tyrrell, D., 1974. The lipids of fungi. Pergamon Press Ltd., Oxford, 89 pp.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology & Biochemistry 32, 1419-1430.

Gaspar, M.L., Pollero, R.J., Cabello, M.N., 1994. Triacylglycerol consumption during spore germination of vesicular-arbuscular mycorrhizal fungi. Journal of the American Oil Chemists' Society 71, 449-452.

Gaspar, M.L., Pollero, R.J., Cabello, M.N., 1997. Partial purification and characterization of a lipolytic enzyme from spores of the arbuscular mycorrhizal fungus Glomus versiforme. Mycologia 89, 610-614.

Gavito, M.E., Curtis, P.S., Jakobsen, I., 2001. Neither mycorrhizal inoculation nor atmospheric CO2 concentration has strong effects on pea root production and root loss. New Phytologist 149, 283-290.

Graham, J.H., Hodge, N.C., Morton, J.B., 1995. Fatty acid methyl ester profiles for characterization of Glomalean fungi and their endomycorrhizae. Applied and Environmental Microbiology 61, 58-64.

Harwood, J.L. 1977. Glycolipids of fungi and Plants. Biochemical Society Transactions 5, 1677-1682.

IUPAC-UIB. 1987. The nomenclature of lipids. Journal of Lipid Research 19, 114-128.

Jabaji-Hare S, Perumalla CJ, Kendrick WB. 1984. Auto-fluorescence of vesicles, arbuscules, and intercellular hyphae of a vesicular-arbuscular fungus in leek (*Allium porrum*) roots. Canadian Journal of Botany 62, 2665-2669.

Kates, M., 1986. Techniques of lipidology: isolation, analysis and identification of lipids. p. 123-127. In: Burdon, R.H., van Kippenberg, P.H. (Eds.), Laboratory Techniques in Biochemistry and Molecular Biology, vol. 3, Part 2. Elsevier, NY.

Madan, R., Parkhurst, C., Hawke, B., Smith, S., 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM Spores in soil. Soil Biology & Biochemistry 34, 125-128.

Mock, T. and Gradinger, R. 2000. Changes in photosynthetic carbon allocation in algal assemblages of Arctic sea ice with decreasing nutrient concentrations and irradiance. Marine Ecology Progress Series 202, 1-11.

Nemec, S. 1981. Histochemical characteristics of *Glomus etunicatus* infection of *Citrus limon* fibrous roots. Canadian Journal of Botany 59, 609-617.

Olsson, P. A., Bååth, E., Jakobsen, I., 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. Applied and Environmental Microbiology 63, 3531–3538.

Olsson, P.A., 1999. Mini review. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29, 303-310.

Olsson, P.A. and Johansen, A., 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycological Research 104, 429-434.

Olsson, P.A. and Wilhelmsson, P., 2000. The growth of external AM fungal mycelium in sand dunes and in experimental systems. Plant and Soil 226, 161-169.

Pfeffer, P.E., Douds, D.D., Bécard, G., Shachar-Hill, Y., 1999. Carbon uptake and the metabolism and transport of lipids in and arbuscular mycorrhiza. Plant Physiology 120, 587–598

Ritchie, S.W., Hanaway, J.J., Benson, G.O., 1997. How a corn plants develops. Spec. Publ. 48. Iowa State Univ. Coop. Ext. Serv., Ames.

SAS Institute Inc, 1999. SAS/STATs User's Guide, Version 8. SAS Institute Inc., Cary, NC.

Smith, S.E., Read, D.J., 1997. Mycorrhizal symbiosis. Academic Press, London, 587 pp.

van Aarle, I.M., and Olsson, P.A., 2003. Fungal lipid accumulation and development of mycelial structures by two arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 69, 6762-6767.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51-62.

Table 1. Concentration (nmol g^{-1} soil) of C16:1*cis*11 biomarker over time and by treatment.

Factor		EL-FAME	CM-FAME	NLFA	GLFA	PLFA	NLFA/ PLFA	NLFA/ N+G+PLFA
Time	4 Aug	7.19b	4.35b	1.62	1.46b‡	1.11	1.41	0.38
	23 Aug	7.51b	4.02b	1.63	1.66b	1.26	1.26	0.33
	13 Sep	10.57a	7.27a	2.10	3.52a	1.23	1.74	0.34
	SE	0.37	0.47	0.41	0.54	0.04	0.35	0.08
Roller-mill	No	5.12B	3.50B	1.18B	0.90B	1.15	1.01B‡	0.35
	Yes	11.72A	6.93A	2.39A	3.53A	1.25	1.93A	0.35
	SE	0.30	0.39	0.34	0.44	0.03	0.29	0.07

EL-FAME = ester liked fatty acid by mild alkaline hydrolysis; CM-FAME = ester linked fatty acid by chloroform-methanol extraction; NLFA = neutral lipid fraction by chloroform-methanol extraction; GLFA = glycolipid fraction by chloroform-methanol extraction, PLFA = phospholipid fraction by chloroform-methanol extraction; N+G+PLFA = is the combined concentration of the NLFA, GLFA and PLFA fractions. Different lower case letters stand for differences over time (P = 0.05 unless stated otherwise), and different capital letters represent differences between roller-milled treatments.

‡ Significant at P = 0.07 for GLFA over time, and for the ratio NLFA/PLFA by RM treatment. N = 12.

Table 2. Correlation matrix for different fractions of C16:1cis11 FAME biomarker.

	NLFA	GLFA	PLFA	GLFA + NLFA	CM-FAME	EL-FAME
NLFA	1					
GLFA	0.24	1				
PLFA	0.36	0.46	1			
GLFA + NLFA	0.62*	0.91**	0.52Φ	1		
CM-FAME	0.59*	0.88**	0.50Φ	0.96**	1	
EL-FAME	0.61*	0.89**	0.49Φ	0.98**	0.92**	1

EL-FAME = ester liked fatty acid by mild alkaline hydrolysis; CM-FAME = ester linked fatty acid by chloroform-methanol extraction; NLFA = neutral lipid fraction by chloroform-methanol extraction; GLFA = glycolipid fraction by chloroform-methanol extraction, PLFA = phospholipid fraction by chloroform-methanol extraction; NLFA +GLFA = is the combined concentration of these two fractions. N = 12

Φ, *, **, Correlation between measured attributes is significant at the 0.1, 0.05, and 0.01 levels, respectively

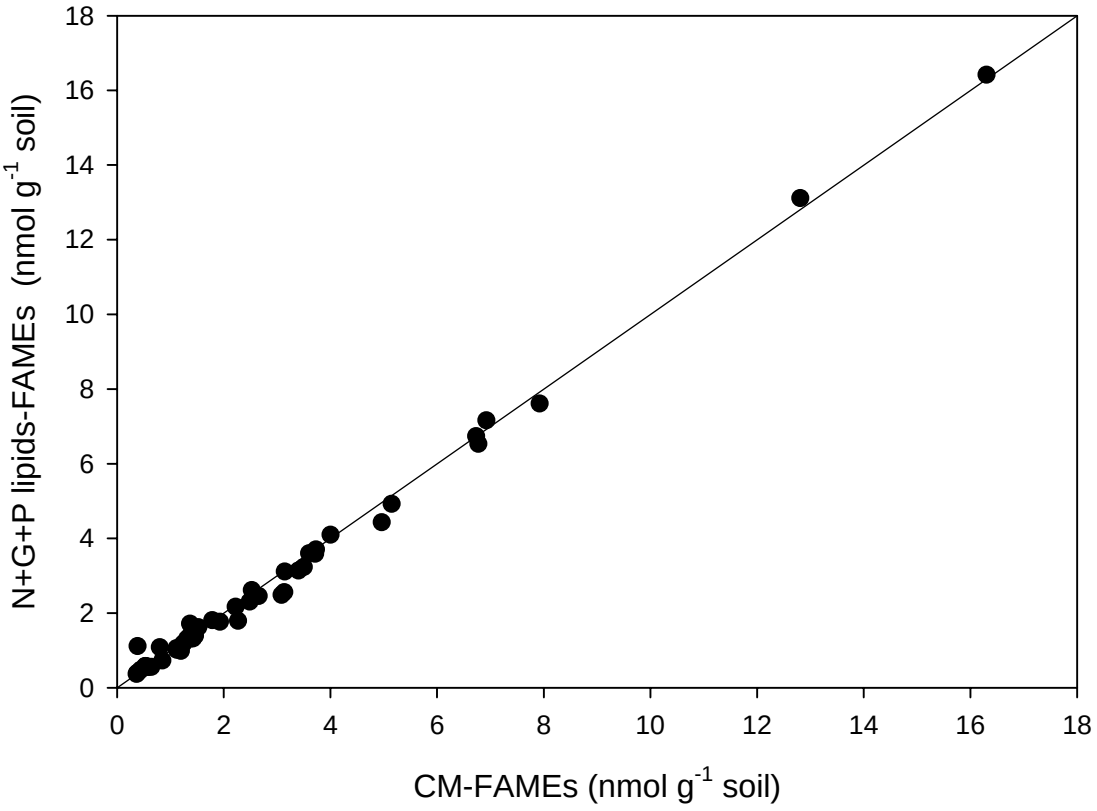


Figure 1. Recovery of fatty acids from silica gel chromatography. Correlation between the sum of neutral (N), glyco- (G) and phospholipids (P) fatty acid methyl esters (FAMEs) and chloroform methanol (CM) FAMEs. N = 12.

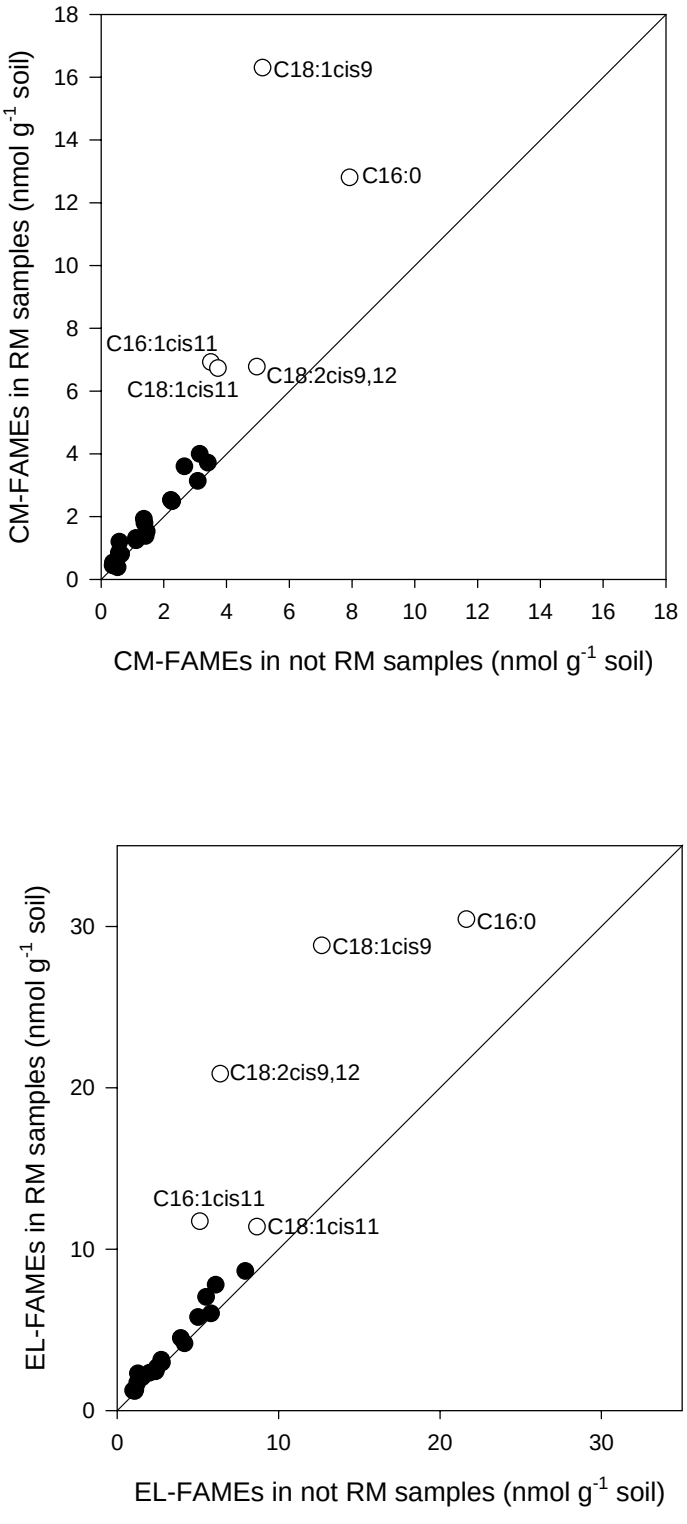
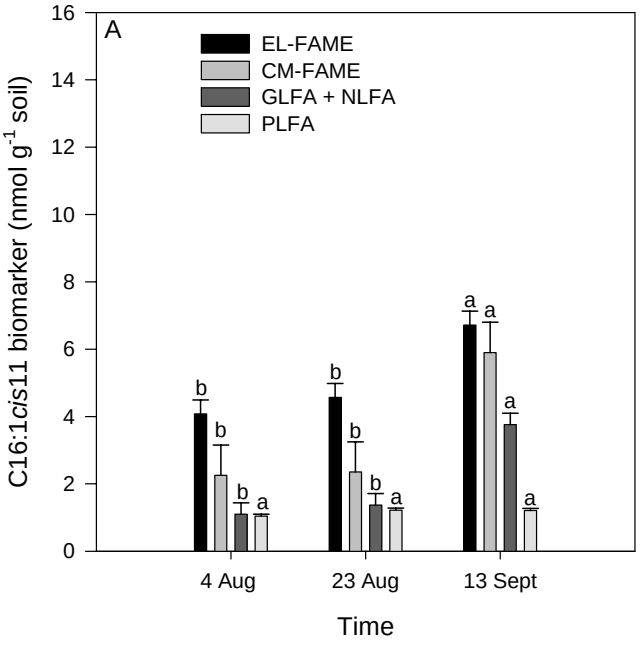


Figure 2. Correlation between non roller-milled (RM) and RM soil samples for chloroform methanol extracted fatty acid methyl esters (CM-FAMEs) or ester linked FAMEs (EL-FAMEs). N = 6.



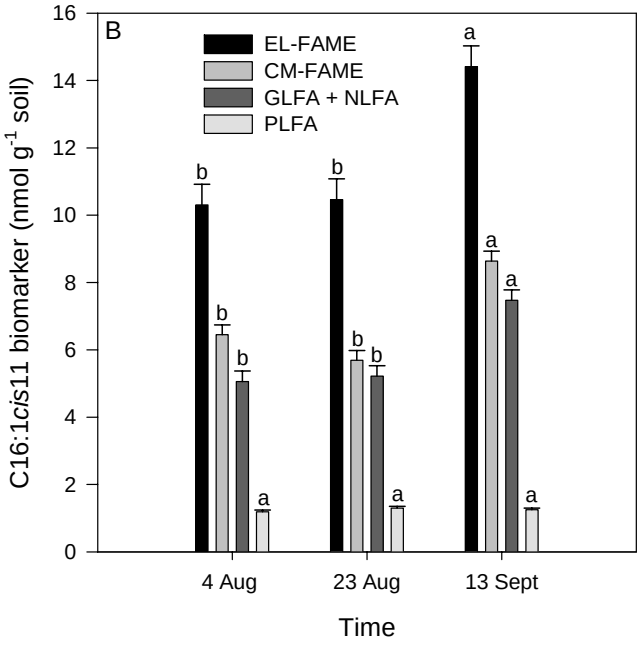


Table of Standard errors of the mean

	Non RM	RM
EL-FAME	0.41	0.62
CM-FAME	0.9	0.29
GLFA + NLFA	0.34	0.31
PLFA	0.06	0.05

Figure 3. Dynamics of the C16:1*cis*11 biomarker over time in A) non roller-milled (RM) soil samples, and B) in RM soil samples. EL-FAMEs = ester liked fatty acids by mild alkaline hydrolysis; CM-FAMEs = ester linked fatty acids by chloroform-methanol extraction; NLFA = neutral lipid fraction by chloroform-methanol extraction; GLFA = glycolipid fraction by chloroform-methanol extraction, PLFA = phospholipid fraction by chloroform-methanol extraction. Letters stand for significant differences (P = 0.05) among sampling dates.N = 6. .

Synthesis

Soils exhibit spatial variability in physical and chemical properties, and this variability affects soil microorganisms that play a role in nutrient cycling. The objectives of this work were (i) to identify the soil properties that influenced ECa, (ii) to determine the relationship between these soil properties and specific soil microbiological communities, and (iii) to evaluate AM fungi dynamics during the reproductive stages of high productivity corn in soils with different P availability.

The variability observed in a field at Shelton was related to soil properties that influenced soil water availability in the 0- to 90- cm depth: total C and clay content, total dissolved solids, and depth of topsoil. Soil microbial activity is important in organic matter turnover, nutrient cycling, and may be a good indicator of the functional capacity of a soil. Soil microbial communities were associated with diverse C pools. The concentration of bacterial and actinomycetes biomarkers covaried primarily with fine POM, while fungal and mycorrhizal biomarkers covaried with coarse POM distribution.

I also evaluated whether the changes in C redistribution caused by row cultivation influenced the different microbial groups, and if these changes were consistent across ECa delineated zones. Cultivation increased labile C pools and soil microbial biomass in the row position, independently of the ECa classification. Four weeks after row cultivation I observed a shift in the relative concentration of AM fungal biomarkers in the row position, but the discrimination analysis showed no clear differences in abundance of a specific group in the row or the furrow.

In addition to the spatial variability in soil properties, soil microbial groups may exhibit temporal dynamics. Two sites with different P availability were selected to study

AM fungi dynamics during the reproductive stages of corn. These two highly productive fields were under irrigation. I installed chambers with different mesh size that allowed or excluded roots (not hyphae) and with and without additional P. These compartments were designed to facilitate the study of the development of new hyphae in soils, and to evaluate the effect of roots and roots and hyphae on the P concentration from these chambers. The purpose of the chamber study was: (i) to study the influence of available P on extramatrical mycorrhizal fungi, (ii) to analyze the influence of the presence of roots in AM fungi dynamics, and (iii) to evaluate the utility of FAME C16:1cis11 biomarker to study the dynamics of AM fungi under field conditions.

I observed that AM fungi FAMEs concentration decreased as availability of P in field soil increased. The increase in the AM fungi FAMEs concentration in field soil and in chambers during the reproductive stages of corn confirmed the allocation of C from the plant to the mycorrhizal symbiont. It is possible that the partitioning of C from the plant to the AM fungi is related to the accumulation of P in plant tissue and is associated with physiological signals released from the crop. I also observed that chambers that allowed the passage of AM hyphae were as efficient as the chambers that allowed passage of both roots and hyphae in extracting P from the chambers. These results demonstrate that AM fungi are active during the reproductive growth stages of corn and may benefit high productivity corn crops by facilitating P uptake.

Finally I showed that FAME C16:1cis11 successfully described the dynamics of AM fungi in soils under field conditions during the reproductive stages of corn. FAME C16:1cis11 (mild alkaline hydrolysis or the chloroform extractable lipids method) reflected the increase in NLFAs and GLFAs, though related to storage lipids and some

resistant structures that are synthesized *de novo* during the reproductive stages of corn. I also observed that resistant structures of AM fungi are hydrolyzed only after roller milling the soil samples. Thus traditional FAME analysis does not release lipids from resistant structures unless soil samples are roller milled. These more resistant structures are rich in neutral and glycolipids, and are likely spores or rhizomorphs.

Preserving and optimizing soil fertility while minimizing negative environmental effects are goals of sustainable agroecosystem management. The development of sustainable agricultural practices depends on promoting the long-term fertility and productivity of soils at economically viable levels. It is important to determining the sustainability of these practices across a broad range of crops, soil types, and climatic regimes (Gliessman, 1998).

Soils physical and chemical properties help determine yield potential, because they influence processes like mineralization, immobilization, nitrification, leaching, denitrification, and plant uptake. Apparent electrical conductivity sensor technologies allow mapping of natural and management-induced patterns of soil properties across a field. These maps can be classified into management zones encompassing zones with similar yield potential. However, yield potential is not always expressed because it is affected not only by nutrient availability and soil–water content, but also by edaphic factors and weather (temperature and solar radiation).

The use of soil management zones has been proposed as a means to direct variable rate nutrient application to better match nutrients supply with spatial variation in crop requirements. Management zones may also be used as a basis for planting different

varieties, and varying seeding rates. However, as weather also influences crop growth, it must be considered when assessing the effect of spatial variation in fields. Further studies are needed to evaluate the influence of physicochemical properties on soil microbial communities under diverse land conditions, and different soil types. Given the results presented in the first and second chapters, the relationship among different soil C pools and soil microbial biomass appears to be strong. Similarly, fatty acids profiles showed differences in community composition that were consistent with differences in organic C input under organic and conventional systems (Bossio et al., 1998). I would expect the same strong correlation among soil biological properties and C availability in diverse agroecosystems.

Soil quality "is the capacity of soil to function" (Karlen et al. 1997). For a long time, soil scientists have identified diverse soil physical, chemical and biological properties, and methods to quantify soil quality. Soil microbial populations are fundamental for maintaining soil quality by mediating the processes of organic matter turnover and nutrient cycling, and may be a good indicator of the functional capacity of a soil. Furthermore, soil microorganisms are involved in aggregation processes and aggregation is tightly linked to soil C pools.

To understand the agronomic implications of my research, I compared my results to the aggregation and C dynamics model proposed by Gale et al. (2000). This model proposes that many macroaggregates form around root-derived POM, in other words coarse POM, while fine POM is more related to residue decomposition within macroaggregates leading to microaggregate formation. From my work, I substantiate previous research that stated the influence of fungal groups on macroaggregation

processes, and fungal-derived exudates as stabilizing agents. The POM entrapped inside macroaggregates continues to decompose and become encrusted with clay particles forming microaggregates. Bacterial groups are the main microorganisms related to this stage of residue decomposition. Future research is needed to evaluate the participation of soil microorganisms in the dynamics of aggregation processes in agroecosystems. I suggest separating the aggregates by size and stability in duplicate soil samples. One set would be analyzed by FAMEs to evaluate the relative concentration of fungal, bacterial, or actinomycetes groups in each fraction, and the other would be used to determine C and N content.

Soil microbial biomass is a good indicator of changes in soil biological processes related to organic matter transformations. Agronomic practices such as row cultivation have traditionally been used to control weeds in row crops. Row cultivation created two environments, row and furrow, which I sampled to evaluate the biological activity in the root zone. I found that cultivation created a biologically enhanced root zone in the row that may improve early season nutrient availability for corn, and influence aggregation processes. Row cultivation is known to be effective in reducing weed density, but also may have a positive effect on crops nutrient availability. Future research should be directed at following changes in microbial communities over time in these two environments and determine the effect of this discrimination on residue decomposition and nutrient dynamics. Soil sampling over time could be used (i) to follow the soil microbial biomass dynamics, (ii) to track changes in soil physicochemical properties, and (iii) to distinguish the specific point where the microbial groups discriminate row and furrow environments.

AM fungi form symbiotic relationships with most land plants and play an important role in plant P nutrition. In corn, P accumulates steadily until maturity, and more than 50% is absorbed during the reproductive period, thus AM fungi may have an important role in P acquisition. Phosphorus nutrition in high productivity corn fields is important because it plays a critical role in energy reactions in the plant. Deficits can influence not only energy requiring processes in plant metabolism but may negatively influence the use efficiency of other nutrients.

Since root biomass does not change from two to three weeks after tasselling to maturity, AM fungi may play an essential role in P uptake of corn later in the growing season. Effective manipulation of corn P uptake depends on identifying the mechanisms that operate under field conditions. Previous studies were often conducted under controlled conditions using cultivated fungi of known genera to inoculate the pots. My research is one of the first to study the dynamics of indigenous fungi under field conditions. My results may help farmers utilize management practices that enhance the activity of natural AM fungi communities. Reduced tillage practices minimize the disturbance of the hyphae network in soil. As mycelium is an important component of the inoculum potential of AM fungi, is important to minimize disturbance of mycelium network. The frequency and intensity of the tillage practices also affect AM community composition and activity. By using reduced tillage practices (no-till) the farmer reduces both the intensity and frequency of disturbance and may improve the natural AM fungi activity. Furthermore, timing and quantity of N fertilizer applications may affect AM fungi activity. Future research is needed to evaluate the effect of N fertilization on AM fungi biomarkers concentration in agroecosystems.

The ability to access soil P varies among different plant species and genotypes within species and is affected by interactions between plants and soil microbes (Kaeppler et al. 2000). Genetic improvements occur in fully fertilized soils, and may result over time in a reduction in susceptibility to AM fungi symbiosis. Hybrid development does not focus on AM fungi interaction, being mainly oriented to yield potential, drought tolerance, insects, and herbicide resistance, and nutrient use efficiency. Kaeppler et al. (2000) tested two original parental lines and their derived lines and hypothesized that selections under conditions of chemical inputs selected against plant-mycorrhizal symbiosis. A second hypothesis is that selection has increased the inherent genetic ability to take up P through the root resulting in cultivars that are less dependant on the symbiosis. My research indicates that AM fungi improve P acquisition late in the corn season and thus geneticists should take plant-fungal interactions into consideration.

The benefits of a 'plant-symbiont' genetic selection are clearly for improvements in plant P nutrition during the reproductive stages of the crop, when more than 50% of this nutrient is absorbed. AM fungi may also improve resistance to pathogens by competing for nutrients and infection sites, interfering with pathogenicity factors, and inducing resistance in host plants (Punja and Utkhede 2003). Moreover, improvement of plant-water relationships in corn was shown by Subramanian et al. (1995). Mycorrhizas improved tolerance to moderate drought stress imposed at tasselling, and that response was hybrid dependant. Future research is needed to further describe and quantify the effect of AM fungi on pathogen resistance and plant-water relationships.

Finally, the use of ester-linked fatty acids resulted in a practical method to evaluate the dynamics of indigenous AM fungi in soils under field conditions. EL-

FAMEs resulted in a rapid, efficient, reproducible way to evaluate the AM fungal activity in soils. Furthermore, utilizing this method makes it possible to analyze large numbers of samples.

In addition to difficulties with the chloroform-extraction FAMEs methodology and the amount of time required per sample, the neutral and phospho-lipid C16:1cis11 fraction alone were not adequate to understand the dynamics of the fungi in soils. The glycolipid fraction may be of interest. However further studies are required to understand the function of the glycolipids in AM fungi, the concentration of this marker in each fungal structure, and the environmental factors that affect the concentration of this marker in agroecosystems.

Further work is needed to identify mechanisms controlling the temporal allocation of C from plant to AM fungi, and the mycorrhizal contribution to P uptake during the reproductive stages of corn. Similarly, further dynamics of these fungi in the soil have to be elucidated to evaluate the formation of specialized structures during the reproductive stages of corn.

References

Bossio, D.A., K.M. Scow, and K.J. Graham. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipids fatty acid profiles. Microb. Ecol. 36:1-12.

Gale, W. J., C. A. Cambardella, T. B. Bailey, 2000. Root-derived carbon and the formation and stabilization of aggregates. Soil Sci. Soc. Am. J. 64:201-207

Gliessman, S.R. The need for sustainable food production systems. p. 3-16 In: Eric Engles (Ed.) Agroecology: ecological processes in sustainable agriculture. Ann Arbor Press, Chelsea, MI.

Kaeppler, S. M., J.L. Parke, S.M. Mueller, L. Senior, C. Stuber, W.F. Tracy, 2000. Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. Crop Sci. 40:358-364.

Karlen, D.L., M. J. Mausbach, J.W. Doran, R.G. Cline, R. F. Harris, G. E. Schuman, 1997. Soil quality: Concept, rationale, and research needs. Soil Sci. Soc. Am. J. 61:4-10.

Punja, Z.K. and R.S. Utkhede, 2003. Using fungi and yeasts to manage vegetable crop diseases. Trends Biotech. 21:400-407.

Subramanian, K.S., C. Charest, L.M. Dwyer, and R.I. and Hamilton, 2003. Arbuscular mycorrhizas and water relations in maize under dreought stress at tasselling. New Phytol. 129:643-650.