IMPACT OF MYCORRHIZAL FUNGI AND NEMATODES ON GROWTH OF
ANDROPOGON GERARDII VIT., SOIL MICROBIAL COMPONENTS AND SOIL
AGGREGATION

by

PING HU

B.S., NANKAI UNIVERSITY, CHINA, 2004

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2008

Approved by:

Major Professor
Charles W. Rice

Abstract

Biotic interactions among mycorrhizal fungi, nematodes, plants and other microbial communities can have significant effects on the dynamics of C and nutrient cycling. The specific objectives of this study were (1) to evaluate the effects of grazing and mycorrhizal symbiosis on the allocation and storage of C, especially for plant above-and belowground biomass, (2) evaluate the biotic rhizosphere interactions and their role in C cycling, (3) determine the soil microbial community structure as a result of the plant-mycorrhizal symbiosis, and (4) determine the effect of mycorrhizal fungal abundance on soil aggregation. The soil for the experiment was sampled from the Ap horizon of a fine-silty, mixed, superactive, mesic Cumulic Hapludolls located at Konza Prairie Biological Station, Manhattan KS. The experiment was a three-way factorial in a complete randomized block design with four replications. The three factors were mycorrhizae (M), nematodes (N), and phosphorus (P). In a greenhouse study, 96 microcosms (52×32×40cm) were planted to *Andropogon gerardii* Vit. so that a third of the microcosms could be destructively sampled at the end of each growing season for three years.

Plant biomass was separated into aboveground, rhizomes, and roots. All components were dried and weighed at harvest. Mycorrhizal fungi and P increased plant aboveground biomass, while nematodes decreased plant aboveground biomass compared to non-inoculated controls. As expected, P increased plant root biomass, while mycorrhizal fungi increased plant rhizome biomass. Nematodes decreased both above- and belowground biomass.

Phospholipid and neutral lipid fatty acid (PLFA and NLFA) analysis were determined for both soil and roots. Water-stable aggregates were separated using a modified Yoder wet-sieving apparatus and analyzed for mass, total C and N, and the isotopic composition of C. There was a positive relationship between arbuscular mycorrhizal fungal abundance in the soil and the mass of the largest macroaggregates (>2000µm) after the 3rd year (r=0.67). The effect of roots on the macroaggregate (>2000µm) fraction was not apparent. Phosphorus significantly increased smaller macroaggregates (250-2000µm), along with significantly enhanced plant root biomass, which indirectly demonstrated the effect of roots on the formation of macroaggregates (250-2000µm). The addition of P induced more plant derived C into the aggregates than the non-P amended microcosms as suggested by the ^{13}C content of the aggregates. Our results confirmed

the importance of biotic and abiotic interactions among mycorrhizal fungi, nematodes, and phosphorus on plant growth and the resulting effect on the soil C cycle and soil aggregation.

Acknowledgements

Many thanks to my major advisor Dr. Charles W. Rice for leading me to the amazing world of soil microbiology and help me with my research and this thesis.

I want to thank Tim Todd for valuable advice for analyzing the data for this thesis. And thank Gail Wilson for helping me explain mycorrhizal related results.

Thanks to Paul White, who helped me a lot with my labwork during the past 2 years, which contributed to this thesis. Also, I appreciate the help from Habib, Prscilla, Cody, Karina Fabrizzi, and all the other students working in the soil microbial ecology lab who helped me with my research. Also I want to thank my friend Haifeng Liu for helping me organize the thesis.

We also want to thank the soil testing lab of Kansas State University and the support of this study from National Science Foundation (DEB-0236106).

Finally, I want to thank and dedicate my thesis to my parents: Panshan Hu, and Yiguang Wang.

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CHAPTER 1 - LITERATURE REVIEW

THE TERRESTRIAL CARBON CYCLE

Grasslands contain a major reservoir of the terrestrial ecosystem carbon. A minor change in carbon storage and flux in the grassland ecosystem could affect the concentration of atmospheric CO_2 and the resulting impact of global climate change. Many scientists have made significant contributions on the various aspects of the terrestrial C cycle. In relation to this thesis, there are three major foci that attract attention.

1. Carbon storage in grassland ecosystem, including storage in the plant and soil components.

Grassland ecosystems play a very important role in world C storage. It has been estimated that C stored in grasslands is about half of the amount stored by forests and equivalent to that stored in agricultural systems (White et al., 2000). Approximately 90% of the grassland C is in soil organic matter (SOM). The remaining 10% resides in plant biomass, most of that in the belowground portion (Reeder et al., 2001).

Globally, soils are considered to be the largest terrestrial C pool with twice the amount of C stored in the atmosphere and three times the amount of C in living plants (Schlesinger, 1995; Kimble and Stewart, 1995; Jobbagy and Jackson, 2000). Clearly, soil C dynamics significantly impact atmospheric CO_2. Soil C is the result of a balance of C inputs primarily derived from plant photosynthesis and deposited from litter and plant roots, and outputs as a result of microbial decomposition of organic matter, eluviations, and erosion (Entry and Emmingham, 1998). Soil C can be altered by changing soil water and temperature (Burke et al., 1989; Hontoria et al., 1999). The capacity of soil to store C is a function of ecosystem and soil characteristics, climate, and geomorphology (Jenny, 1941; Baldock and Skjemstad, 2000).

2. Soil biotic control in C cycling and microbial community feedbacks to climate change.

Microbial activities influence and are connected with soil organic carbon (SOC) dynamics in soil (Kandeler et al., 2005). Microbial biomass C estimates range from 500-3000μg g^{-1} in grassland soils (Ross et al., 1996; Kandeler et al., 2005). Unlike the diversity of SOC, microbial biomass C is relatively "fixed" in proportion of 0.9-6.0% of total organic C with a

mean value of 2-3%, which indicates a close relationship between microbial biomass C and soil available C for microbes (Kandeler et al., 2005).

It was reported that in most soils, microbial activities in "hot spots" (rhizosphere for example) represents most biological activities in soil (Beare et al., 1995), where fungi and bacteria play a key role in decomposing processes, microbial and faunal communities are competing with each other for C source. Carbon cycling has been recognized to be controlled by soil microbial communities (Ogram et al., 2006). Individual components of these microbial communities respond to environmental changes and also influence C cycling rates and pathways in return (Ogram et al., 2006). Soil microbial communities play a very important role in sequestration of C into biomass and soil, and release CO_2 from soil through decomposition and respiration.

Over the last decade, atmospheric CO_2 concentration has risen by 1.5 ppm per year (IPCC, 2001). Global warming has become a concern for terrestrial ecosystems. Researchers have studied the role of soil microbes in controlling plant response to elevated atmosphere CO_2 and their contribution to sequestration of C into soil (Niklaus et al., 2003). Elevated CO_2 stimulates plant photosynthesis resulting in enhanced root biomass and increased production of fine roots that enhance C turnover rate (Rogers et al., 1994). CO_2 enrichment alters soil microbial communities (Kandeler et al., 2005) through increased functional diversity and soil enzyme activity. Increases in soil moisture would also explain enhanced microbial activity under climate change. Körner et al (2000) observed higher soil water content under enriched CO_2 environment in a grassland study, resulting in greater soil microbial activity.

3．The effects of human activities, such as cultivation grazing and fire on the C cycle in grasslands.

A variety of human activities have the potential to directly influence the C cycle by changing the balance of C inputs and outputs. Cultivation of grasslands and the corresponding agricultural management practices can alter soil physiochemical properties and the composition and activity of the soil microbial community (Aguilar et al., 1988; Davidson and Ackerman, 1993; Jaiyeoba, 2003). Previous studies have shown that the conversion of grassland to cropland significantly reduces C inputs by decreasing litter and removing aboveground plant biomass at harvest (Huggin et al., 1998). Soil disturbance, such as plowing, harrowing and disking can

destroy aggregate structure (Elliott, 1986; Singh and Singh, 1996), increase soil respiration, and accelerate the mineralization of soil organic C (Buyanovsky et al., 1987; Li and Chen, 1998).

Grazing effects by aboveground herbivores on grassland C can be positive or negative depending on the intensity of grazing. Conant et al. (2001) suggested that proper grazing management can increase forage production and increase soil C. Even with reduced forage production by grazing, it may be possible to improve soil C (Conant et al., 2001). Fire has been shown to have the potential to influence carbon storage and dynamics by changing plant species diversity and dominance, plant tissue chemistry, productivity, SOM decomposition, and soil physical characteristics (Scholes and Archer, 1997; Peterson and Reich, 2001; Van Langevelde et al., 2003). Fire can result in increased above-and belowground plant productivity due to removal of litter and standing crop, and changes in nutrient distribution and availability (Raison, 1979; Rice and Owensby, 2000; Johnson and Matchett, 2001). Plant tissue chemistry, such as C to N ratio of shoots and roots, can be altered following fire (Ojima et al., 1994; Johnson and Matchett, 2001), leading to modification of SOM dynamics.

MYCORRHIZAE

Arbuscular mycorrhizal fungi (AMF) are a common group of soil fungi that belong to phylum Glomeromycota. They form haustoria-like structures called arbuscules and ovoid shaped organs that are rich in lipids called vesicles (Phavaphutanon, 1996). Arbuscular mycorrhizal fungi are often considered obligate symbionts of plants with approximately two-thirds of plant species capable of forming arbuscular mycorrhizal associations (Fitter and Moyersoen, 1996). The arbuscular mycorrhizal association is the most common type of mycorrhizal assocaition that dominates temperate and tropical grasslands, tropical forests, and desert communities (Read, 1991).

Previous research has suggested a lower level of root colonization for C_3 grasses compared with non-leguminous dicots, legumes, and C_4 grasses. C_3 grasses invest in an abundant, highly branched fine root system with many root hairs, which enable them to be less dependent on AMF for nutrient uptake and often exhibit lower levels of AMF colonization (Wilson and Hartnett, 1998). The morphology and architecture of roots is widely accepted to be an important factor in determining mycorrhizal responsiveness (Karanika et al., 2007). The native warm-season grass, big bluestem (*Andropogon gerardii* Vit.), is one of the dominant grasses of tallgrass prairie and has been found to be highly colonized by AMF under natural conditions, and are obligate mycotrophs in natural tallgrass prairie soil (Dhillion and Friese, 1994; Hartnett and Wilson, 2002).

Mycorrhizal plants usually have better growth compared with non-mycorrhizal plants. Plants with fine roots are less dependent on mycorrhizae for nutrient uptake (Hetrick and Wilson, 1988), but they may still derive benefits from the protection of pathogens (Newsham et al., 1995). The most important ecosystem function of mycorrhizae is to assist plants in the acquisition of mineral nutrients from soil (Dighton, 2003). Arbuscular mycorrhizal fungi benefit plants by improving plant phosphorus (P) uptake (Fitter, 1990), and most studies have mainly focused on the role of AMF in P uptake under controlled conditions (Newsham et al., 1995). In grassland ecosystems, N and P are limited in their inorganic forms. Arbuscular mycorrhizal fungi facilitate P uptake by increasing the rate of diffusion into plant roots, the P concentration at the root surface, and the rate of P dissociation from the surface of soil particles (Bolan, 1991). Minerals other than P such as Cu, Zn (Gildon and Tinker, 1983), and N also experience enhanced uptake.

It has been estimated that external hyphae of AMF contribute up to 80% of the P, 10% of the N, 10% of the K, 25% of the Zn, and 60% of the Cu absorbed by the plants (Li et al., 1991; Marschner and Dell, 1994).

Phosphorus fertilization is generally believed to depress AMF colonization. When plants are not nutrient limited, fungal colonization typically decreases, as plants invest most of their C to the development of aboveground biomass (Marschner et al., 1996). Nitrogen addition can have a positive effect on the symbiosis only when P remains a limiting factor for plant growth (Karanika et al., 2007). As Johnson et al. (2003) and Egerton-Waburton et al. (2007) proposed, the soil N:P ratio could be a reliable predictor for the effect of N enrichment on AM fungi.

To some extent, mycorrhizal fungi can be considered as "shunts" between the labile and non-labile pathways of catabolism (Trofymow and Coleman, 1982). The ability to transport C from the rhizosphere could potentially result in C sequestration (Treseder and Allen, 2000). Roots and associated mycorrhizae are the most important component of the global C flux through soil respiration (Treseder and Turner, 2007). The mycorrhizal contribution to C cycling is becoming of greater interest for its sensitivity to anthropogenic alterations of biogeochemical cycles (Staddon et al., 2003; Nilsson and Wallander, 2003). Arbuscular mycorrhizal fungi have been estimated to make up 20% to 30% of the soil microbial biomass in temperate grassland soils (Miller and Kling, 2000; Olsson and Wilhelmsson, 2000). The indirect effects of AMF on soil C flux may be of equal or greater importance than the direct effect of AMF on plant nutrition.

The important role of AMF in soil functioning and soil aggregate formation and stabilization has been of recent interest. In Miller and Jastrow's study (1992), the effect of AMF on soil aggregation consist of three processes: (1) hyphal growth into the soil matrix to form skeletal structure; (2) providing conditions of microaggreate formation; and (3) macroaggregates formation by roots and hyphae emeshing and binding of microaggregates. There are studies showing that AMF has a positive effect on soil aggregation and soil structure (Thomas et al., 1986; 1993). Arbuscular mycorrhizal fungi influence soil macroaggregate formation > 2000μm in diameter through both the physical entanglement by hyphae and glomalin secretions which provide the "glue" to bind soil particles. (Rillig et al., 2002). The discovery of glomalin (Treseder and Turner, 2007) has brought attention to its role on aggregate stability (Wright and Upadhyaya, 1998). This protein has a residence time of 6 to 42 years, and contributes up to 15% of the total stable soil organic carbon pool in grasslands (Miller and Kling, 2000). In addition,

organic matter trapped inside macroaggregates is thought to be less subject to degradation than that in the bulk soil thus contributing to sequestration of C in soil (Jones and Donnelly, 2004).

Arbuscular mycorrhizal fungi appear to be the most significant mediators of soil aggregation for several reasons (Rillig et al., 2002): (1) arbuscular mycorrhizal fungal hyphae represent a substantial and dominant component of soil microbial biomass (Miller et al., 1995; Rillig et al., 1999); (2) arbuscular mycorrhizal fungi are independent of carbon supply in the soil due to the ability to obtain carbon from the host plant (Smith and Read, 1997); and (3) arbuscular mycorrhizal fungal hyphae have a longer resistance time in soil than saprobic fungi due to preference of grazers on saprophytic hyphae (Klironomos and Kendrick, 1996; Rillig et al., 2002).

Arbuscular mycorrhizal fungi improve soil properties through their extended hyphal network (Bethlenfalvay and Schuepp, 1994). As an example, the improved soil aggregation associated with AMF can impact many other soil properties such as soil organic matter, bulk density, soil moisture, air capacity, and microbial activities.

NEMATODES

Nematodes are generally classified as the phylum-nematoda or nemata, unsegmented pseudocoelomates with typically thread-like shape (Abebe et al., 2008). Nematodes are the most abundant mesofauna in soil, with densities of 0.76 million per m^2 in a desert to 29 million per m^2 in a mixed deciduous forest (Bernard, 1992; Liang and Shi, 2000). In most grassland soils, the population density is about 3 to 4 million per m^2 (Yeates et al., 1997) which varies temporally due to soil physical and chemical conditions, and land management (Bardgett and Cook, 1998). The richness in taxonomy of soil nematodes has been reported to be as many as 75 "taxa" (Yeates et al., 1997) and 150 species (Hodda and Wanless, 1994). Kansas prairie soil has been reported to have up to 228 species (Yeates, 1998).

Many nematodes are "free-living" types found in the oceans, in freshwater habitats, and in soils. Plant-parasitic species form a smaller group. Plant-parasitic nematodes include ectoparasites, which cause no or little damage but some induce galls by feeding on root tips, and endoparasites, which cause extensive tissue destruction or localized damage (Hussey and Grundler, 1998). Current proposals for dividing free-living nematodes by feeding habit recognize seven groups: ingesters, bacterial feeders, carnivores, unicellular eukaryote feeders, and animal parasites (Moens, et al., 2004), some of which may fit in multiple types. For nematodes in tallgrass prairies, 16 to 41% are herbivores, 24 to 38% are microbivores, 5 to 20% are fungivores, and 26 to 40% are omnivores (Neher and Powers, 2005).

Nematodes are important participants in belowground nutrient and energy cycling. Both plant- and microbial-feeding nematodes can have a significant influence on the rate and direction of nutrient flux in the grassland ecosystems (Bardgett et al., 1999). They have been found to consume living plant material, fungi, bacteria, mites, insects, and each other (Guerena, 2006). Evidence suggests that 30 to 50% of the N present in crop plants is made available by the activity of bacteria-feeding nematodes (Ingham, 1996). Nematodes also have the greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination (Ploeg, 2001). Nematode grazing creates open wounds that provide entry for a wide variety of plant-pathogenic fungi and bacteria. These microbial infections are often more economically damaging than the direct effects of nematode grazing itself (Guerena, 2006).

Nematodes are important consumers of the belowground microbial biomass (Hunt et al., 1987) and are indicative of microbial turnover and flux of nutrients through the soil food web (Forge et al., 2005). Previous studies have shown that bacterial-feeding nematodes can increase N mineralization (Anderson et al., 1981; Griffiths, 1994). This group of nematodes is believed to accelerate bacterial turnover and thus increase SOM turnover (Griffith, 1994). Plant biomass growth and root development can be improved with more bacterial-feeding nematodes than usual (Mao et al., 2006, 2007). Microbial-feeding nematodes can promote nutrient cycling and plant growth in grasslands, while these effects have been reported to be strongly influenced by other nematode groups and other soil fauna (Bardgett et al., 1999).

When assessing the possible causal relationships among the plant-soil system, two functional groups of nematodes should be considered: plant-feeding nematodes and mycorrhizal hyphae feeding nematodes (Brussaard, 2001). Fungal-feeding nematodes can regulate fungal dynamics and decomposition processes (Verhoef and Brussaard, 1990). They use their stylet to suck the content of hyphae (Stanton, 1999) and digest both saprophytic and mycorrhizal fungi hyphae (Yeates, 1998). Plant-feeding nematodes are supported in grassland soils (Peterson, 1982; Porazinska et al., 2003) and have become a regulator of plant growth in these ecosystems (Smolik, 1974). Also, C and N dynamics can be altered by nematodes by influencing root exudation rates (Yeates et al., 1998; Yu et al., 2003). Facultative herbivores can also feed on fungi (Ayres et al., 2007). Moderate grazing on plants roots can increase plant productivity by increasing belowground carbon diversion, improving soil microbial activity, and increasing nutrient availability (Bardgett et al., 1999).

RHIZOSPHERE

The rhizosphere is one of the "hot spots" of biological activity in soil (Lavelle et al., 1992; Beare et al., 1995). It is the place where microorganisms, plant roots, and soil constituents interact (Lynch, 1990; Barea 2000). Biological interactions in the rhizosphere are a significant contributor to plant growth, ecosystem productivity and vegetation dynamics (Brussaard et al., 2001). The rhizosphere is a physical, chemical and biological environment that is clearly distinct from the bulk soil (Kennedy and Smith, 1995). The microbial population of the rhizosphere is significantly higher than that of bulk soil due to root exudation of organic carbon (Drever and Vance, 1994). The key issues involving rhizosphere formation and functioning include the supply of photosynthates and decay of plant tissue by the root associated microbiota, the supply of available nutrients to plants as derived from microbial activities, and microbial-induced changes in rooting pattern (Barea et al., 2002). The flux of C is critical for rhizosphere functioning (Toal et al., 2000).

The processes that occur in the rhizosphere play an important role in C sequestration and nutrient cycling in terrestrial ecosystems (Helal and Sauerbeck, 1989; Van Veen et al., 1991; Zak et al., 1996; Reich et al., 2006; Xu and Chen, 2006). The rhizosphere is one of the key fine-scale components in the global C cycle (Coleman et al., 1992). The rhizosphere influences plant and soil quality through positive feedbacks on plant adaptability to environmental stress, such as water and nutrient deficit, and soil-borne plant pathogens (Lynch, 1990; Bowen and Rovira, 1999). Arbuscular mycorrhizal (AM) fungi and nematodes are important members of the rhizosphere. There are many positive and negative interactions between AM fungi and soil microorganisms (Bonkowski et al., 2000; Jones et al., 2004; Johansson et al., 2004; Hodge, 2000). Some AM fungi can be inhibited while others may be stimulated by rhizosphere bacteria (Azcon, 1989). Soil bacteria can promote or inhibit AM fungi spore germination, while "mycorrhization helper bacteria" can increase root colonization (Fitter and Garbaye, 1994). On the other hand, AM fungi have also been reported to affect rhizosphere microorganisms either negatively or positively (Andrade et al., 1997; Amora-Lazcano et al., 1998). Arbuscular mycorrhizal fungi may alter the population composition and activity of soil microorganisms (Bansal and Mukerji, 1994; Wamberg et al., 2003), probably due to quantitative and qualitative

changes in root exudation in the rhizosphere (Hodge, 2000). Plant-feeding nematodes can affect microbial communities and activity by causing increased root exudation (Bardgett et al., 1999).

AM fungi and plant-and hyphal-feeding nematodes in rhizosphere have been considered to play major roles in biological interactions in the rhizosphere and mutually interact with apparent effects at plant and ecosystem levels (Brussaard et al., 2001). The ecological roles of AM fungi and nematodes cannot be evaluated alone, because of their different and often interacting effects on the rhizosphere microflora (Brussaard et al., 2001). For example, arbuscular mycorrhizal fungi may alter the effects of belowground grazers of plant roots (Bakhtiar et al., 2001). Nematodes and AM fungi seem to act in the opposing ways in association with root systems. Nematodes may physically disrupt root tissue and cause physiological alterations and impede the spread of internal mycelium by AM fungi. Conversely, mycorrhizal hyphae may reduce the number of entry points for nematodes and also cause physiological changes to reduce the susceptibility of host plants to nematodes (Fitter and Garbaye, 1994; Brussaard et al., 2001). Experiments have indicated that belowground herbivory by nematodes reduces biomass production of mycorrhizal C_4 grasses, and mycorrhizal conditions can improve above-and belowground biomass production (Hartnett and Wilson, 2002).

AGGREGATES

Soil structure is an essential and key factor to the soil and ecosystem functioning as it controls fluxes of water, gases, and nutrients (Rillig et al., 2002; Lichter et al., 2008). Soil aggregate stability can be a measure of soil structure (Six et al., 2004). Soil aggregates play a key role in dynamics of soil C due to their effects on energy and nutrient availability for microorganisms (Garcia-Oliva et al., 2004). Maintenance of aggregate stability prevents structural losses when the soil is subjected to mechanical stresses or climate influences (Denef et al., 2002). Microaggregates mainly form around persistent organic mater (humic materials) as clay particles incrust and protect organic matter from further decomposition and further stabilized by transient organic matter containing polysaccharides and mucigels (Bearden and Petersen, 2000). Macroaggregates hold microaggregates together in a "sticky string bag" with roots and mycorrhizal hyphae as major binding agents (Tisdall and Oades, 1982).

Aggregates physically protect soil organic C (SOC) by forming physical barriers between microbes and their enzymes and the substrates consequently microbial turnover (Elliott and Coleman, 1988). Previous studies indicate that macroaggregates physically protect soil organic C (Beare et al., 1995). Several factors influence soil aggregates, such as microbial extracellular polysaccharides (Roberson et al., 1995), glomalin (Wright et al., 1999), fungal hyphae (Tisdall, 1991), soil microbial biomass, plant roots, plant carbon and nitrogen inputs, and aromatic humics (Degens, 1997; Jastrow et al., 1998; Eviner and Chapin, 2002).

Microaggregates are more stable than macroaggregates, because they are associated with more persistent binding materials (Elliott, 1986). Research suggests that protection of SOC by microaggregates is greater than macroaggregates (Jastrow et al., 2007). However, macroaggregate turnover rate is very important for SOC stabilization (Plante and McGill, 2002). Particulate organic matter within macroaggregates represents a carbon pool with slow turnover rate due to physical protection from microbial decomposition. Stable macroaggregates contain more carbon and more relatively young carbon than in microaggregates (Six et al., 2000; Goh, 2004). Macroaggregates protect fresh carbon inputs from rapid mineralization (Plante and McGill, 2002). Stable macroaggregates can protect SOC from degradation thus increasing SOC content (Holeplass et al., 2004).

OBJECTIVES

The objectives of this study were to evaluate the effect of belowground biotic interaction among mycorrhizal fungi, nematodes, plants and soil microbial communities on the dynamics of nutrient cycling with emphasis on C. To be more specific, this project was to

1) evaluate the effects of grazing and mycorrhizal symbiosis on the allocation and storage of C, especially above-and belowground plant biomass;

2) determine interactions of mycorrhizae, plant roots, and nematodes as they affect C cycling, with a focused on:

assessing changes in soil microbial community structure, and

assessing the role of mycorrhizal fungi and roots on soil aggregation and subsequent impact on soil C storage.

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CHAPTER 2 - PLANT AND SOIL RESPONSES TO THE INTERACTION OF MYCORRHIZAL FUNGI, NEMATODES, AND PHOSPHORUS

LITERATURE REVIEW

There are many types of organisms living in the soil interacting with the plants and with each other so as to impact the soil characteristics and plant biomass quality and quantity. Among these organisms, arbuscular mycorrhizal fungi and nematodes have important roles in the prairie soil ecosystem. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts of plants and approximately two-thirds of modern plants can form arbuscular mycorrhizal associations (Fitter and Moyersoen, 1996). Read (1991) stated that the mycorrhizal association is the most ubiquitous and abundant form of terrestrial symbiosis and arbuscular mycorrhizae are considered the most common type of mycorrhizae which dominates grasslands, tropical forests, and desert communities. Previous research suggests a higher level of root colonization for C_4 grasses than C_3 grasses (Wilson and Hartnett, 1998). The morphology and architecture of roots is widely accepted to be an important factor in determining mycorrhizal responsiveness (Karanika et al., 2007). The native warm-season grass, big bluestem (*Andropogon gerardii* Vit.), is one of the dominant grasses of tallgrass prairie and has been found to be highly colonized by AMF under natural conditions, and are obligate mycotrophs in natural tallgrass prairie soil (Dhillion and Friese, 1994; Hartnett and Wilson, 2002).

An important ecosystem function of mycorrhizae is to assist in the acquisition of soil mineral nutrients (Dighton, 2003). Arbuscular mycorrhizal fungi are known to benefit plants by improving plant phosphorus (P) uptake (Fitter, 1990). In grassland ecosystems, nutrients like N and P are often limited in their inorganic forms. Arbuscular mycorrhizal fungi can facilitate P uptake by increasing 1) diffusion rate into plant roots; 2) P concentration at the root surface; and 3) the rate of P dissociation from the surface of soil particles (Bolan, 1991). Minerals other than P such as Cu, Zn, and N also experience enhanced uptake (Gildon and Tinker, 1983). It has been estimated that external hyphae of AMF can contribute up to 80% of the P, 10% of the N, 10% of

the K, 25% of the Zn, and 60% of the Cu absorbed by the plants (Li et al., 1991; Marschner and Dell, 1994).

While P fertilization is generally believed to depress AMF colonization, the addition of N on AMF colonization has had varied effects with neutral, positive, and negative responses were reported (Treseder and Cross, 2006), When plants are not nutrient limited, fungal growth decreases due to reduced dependency on mycorrhizal symbiosis and greater aboveground plant biomass C investment (Marschner et al., 1996). Nitrogen addition typically has a positive effect on the symbosis when P remains a limiting factor for plant growth (Karanika et al., 2007). As Johnson et al. (2003) and Egerton-Waburton et al. (2007) proposed, the soil N to P ratio could be a reliable predictor for the effect of N enrichment on AM fungal colonization.

The mycorrhizal assocaiation results in a significant flow of C into soil. Up to 20% of plant C can be used by AMF (Jakobsen and Rosendahl, 1990; Watkins et al. 1996). Approximately 20% to 30% of the soil microbial biomass in temperate grassland soils may be comprised of AM fungi (Miller and Kling, 2000; Olsson and Wilhelmsson, 2000). Allen and Allen (1986) estimated that AMF can contribute 1 mg C cm^{-3} to heterotrophic bacteria.   The ability of AMF to transport C from the rhizosphere to the soil matrix could potentially result in C sequestration (Treseder and Allen, 2000). The contribution of AMF to biogeochemical cycles also influence the residence time of nutrients by changing the nutrient concentration ratios of the vegetation, decrease mobility of nutrients by placing a greater proportion of nutrients in biomass, and increase reliance of the system on mineralization rather than on weathering (Miller and Jastrow, 1994).

In most grassland soils, the nematode population is about 3 to 4 million per m2 (Yeates et al., 1997) and varies due to soil physical and chemical conditions, and land management (Bardgett and Cook, 1998). Kansas prairie soil has been reported to have up to 228 species (Yeates, 1998). For nematodes in tallgrass prairies, 16 to 41% are herbivores, 24 to 38% are microbivores, 5 to 20% are fungivores, and 26 to 40% are omnivores (Neher and Powers, 2005).

Nematodes are important participants in belowground nutrient and energy cycling. Both plant- and microbial-feeding nematodes can have a significant influence on the rate and direction of nutrient flux in the grassland ecosystems (Bardgett and Chan., 1999). They have been found to consume living plant material, fungi, bacteria, mites, insects, and each other, and are themselves consumed by other organisms in the soil (Guerena, 2006). Evidence suggests that 30 to 50% of

the N present in crop plants is made available by the activity of bacteria-feeding nematodes (Ingham, 1996). Nematodes have the greatest negative effects on crop productivity when they attack roots of seedlings immediately after seed germination (Ploeg, 2001). Nematode grazing creates open wounds that provide entries to a wide variety of plant-pathogenic fungi and bacteria, which are often more economically damaging than the direct effects of nematode grazing itself (Guerena, 2006).

Nematodes are important consumers of the belowground microbial biomass (Hunt et al., 1987) and are indicative of microbial turnover and flux of nutrients through soil food webs (Forge et al., 2005). Previous studies have shown that bacterial-feeding nematodes can increase N mineralization (Anderson et al., 1981; Griffiths, 1994). This group of nematodes is believed to accelerate bacterial turnover and thus increase SOM turnover (Griffith, 1994). Experiments have shown enhanced plant biomass growth and developed root system with more bacterial-feeding nematodes than usual (Mao et al., 2006, 2007). Microbial-feeding nematodes can promote nutrient cycling and plant growth in grasslands, while these effects have been reported to be strongly influenced by other nematode groups and other soil fauna (Bardgett and Chan, 1999).

When assessing the possible causal relationships among the plant-soil system, two functional groups of nematodes should be considered: plant-feeding nematodes and mycorrhizal hyphae feeding nematodes (Brussaard, 2001). Facultative herbivores can also feed on fungi (Ayres et al., 2007). Fungal-feeding nematodes can regulate fungal dynamics and decomposition processes (Verhoef and Brussaard, 1990). They use their stylet to suck out the contents of hyphae (Stanton, 1998) and digest both saprophytic and mycorrhizal fungi hyphae (Yeates, 1998). Plant feeding nematodes are largely supported in grassland soils (Peterson, 1982; Porazinska et al., 2003) and they have become a major regulator of plant growth in these ecosystems (Smolik, 1974). Although these nematodes were considered to limit plant growth, moderate grazing on plant roots under certain conditions can increase plant productivity by increasing belowground carbon diversion, improving soil microbial activity, and increasing nutrient availability (Bardgett and McAlister, 1999).

Previously research has addressed the role of mycorrhizal symbiosis in grasslands and the influence of AM fungi in competitive interactions, plant-herbivore interactions, and community structure of plants (Hartnett and Wilson, 2002; Kula et al., 2005; Rice et al., 2004; Villarreal et al., 2006; Watson, 2005; Wilson, 2003; Wilson et al., 2001). Plant species responses to AM

fungal colonization are a key factor in C_4 grass dominance of tallgrass prairie (Hartnett and Wilson, 2002). Plant response to the interaction of belowground grazing by nematodes, aboveground grazing by ungulates, and AM fungal colonization indicate that mycorrhizal symbiosis offset plant biomass loss due to grazing (Kula et al., 2005; Wilson, 2003). Nematode grazing significantly reduced the total biomass of mycorrhizal plants of both aboveground grazed and ungrazed microcosms. Belowground grazing on soil C flux is not well known but Ingham and Detling (1986) suggested that root-feeding nematodes increased C allocation to the roots (Ingham and Detling, 1986). Others have documented increased transfer of plant C into the soil microbial biomass (Denton et al., 1999; Yeates et al., 1998).

AM fungi and plant-and hyphal-feeding nematodes have been considered to play major roles in biological interactions in the rhizosphere and mutually interact with apparent effects at plant and ecosystem levels (Brussaard et al., 2001). The ecological roles of AM fungi and nematodes cannot be evaluated alone, because of their different effects on the rhizosphere microflora (Brussaard et al., 2001).The relationship among AM fungi, nematodes, and plants are considered complex. Plant-feeding nematodes can affect microbial communities and activity by increasing root exudation (Bardgett et al., 1999) Arbuscular mycorrhizal fungi may alter the effects of belowground grazers of plant roots (Bakhtiar et al., 2001). Nematodes and AM fungi seem to act in the opposite ways in association with root systems. Nematodes may physically disrupt root tissue and cause physiological alterations and impede the spread of internal mycelium by AM fungi. Conversely, mycorrhizal hyphae may reduce the number of entry points for nematodes and also cause physiological changes to reduce the susceptibility of host plants to nematodes (Fitter and Garbaye, 1994; Brussaard et al., 2001). Experiments have indicated that belowground herbivory by nematodes reduces biomass production of mycorrhizal C_4 grasses, and mycorrhizal conditions can improve above-and belowground biomass production (Hartnett and Wilson, 2002).

This study integrates grazing (of both plants and microbes), arbuscular mycorrhizal symbiosis, the effects of grazing and mycorrhizal symbiosis on the allocation and storage of C and the feedback effects of changes in nutrient dynamics on the plant-fungal-grazer species interactions. We evaluated C dynamics in the *A. gerardii*-fungal-grazer system, addressed plant response to microbial interactions, explored biotic rhizosphere interactions and their role in C cycling, and analyzed soil microbial community structure.

MATERIALS AND METHODS

Experimental design

  The experiment was conducted as a three-way factorial arranged in a complete randomized block design with four replications. The three factors were mycorrhizae (M), nematodes (N), and phosphorus (P). The treatments were N, M, P, C (control), and their combinations (NM, NP, MP, and NMP). The greenhouse study was initiated in 2004 and was designed to last for 3 years. Ninety-six microcosms were set up and sampled at the end of each growing season, with 32 microcosms harvested at the end of each growing season. The first year results were reported by Watson (2005).

Soil

  The soil for the experiment was sampled in spring 2003 from the Ap horizon of a fine-silty, mixed, superactive, mesic Cumulic Hapludolls located at Konza Prairie Biological Station, Manhattan KS (N 39 06' 29.5'' W 96 36' 29.2''). The field had been dominated by C_3 crops for at least the last 15 years. The soil contained 1.6 g organic C kg^{-1}, 0.13g N kg^{-1}, 23 mg Bray-1 P kg^{-1}, 280 mg K kg^{-1}, 165 mg Mg kg^{-1}, and 5.8 mg Na kg^{-1}.

  To start the experiment, the soil was steam-pasteurized for 2h at 80^oC, passed through a sieve with 2 cm diameter openings, thoroughly mixed, put into plastic boxes (52×32×40 cm), and compacted by hand. Each microcosm contained 36 kg dry soil and the bulk density at the end of the first growing season averaged 0.72 g cm^{-3}.

Plants and growing conditions

  The dominant C_4 grass of the tallgrass prairie *Andropogon gerardii* Vit. was selected for this study. Plants were grown from seeds in trays filled with vermiculite until a height of 10-15 cm. Plants were then transplanted at a density of 140 plants per m^2 into the microcosms. Soil moisture was monitored and adjusted to 0.25 cm^3/cm^3 every other day with ThetaProbe soil moisture sensor (Delta-T Devices, Cambridge, England) inserted to a depth of 20 cm, and connected to a hand held ThetaMeter (Delta-T Devices, Cambridge, England). The aboveground biomass was clipped about 1 cm from the soil surface at the end of the growing season (late fall)

each year. Inorganic N was applied in the form of $(NH_4)_2SO_4$ at the rate of 15 mg N kg^{-1} dry soil at the beginning of the growing season of each year.

Treatments: Mycorrhizae (M), Nematodes (N), and Phophorus (P)

Mycorrhizal spores were isolated from tallgrass prairie soil collected from Konza Prairie Biological Station, Manhattan, KS. For isolation, a soil slurry was mixed in a blender, then wet sieved, decanted, centrifuged in a 20:40:60% sucrose density gradient (Daniels and Skipper, 1982). A 500 mL spore suspension was added to each microcosm in the top 25 kg soil to obtain a spore density of 30-40 spores per g dry soil. The spores isolated represented 10 members of *Glomus*, and one member each of the following genera: *Acaulospora*, *Entrosphora*, *Gigaspora*, and *Scutellospora*. The most abundant species included *Glomus heterosporum* (143 spores g^{-1} dry soil), *Glomus etunicatum* (111 spores g^{-1} dry soil), *Glomus intraradices* (90 spores g^{-1} dry soil), *Glomus macrocarpum* (56 spores g^{-1} dry soil), and *Glomus aggregatum* (46 spores g^{-1} dry soil).

Nematodes were obtained from soil under native vegetation of tallgrass prairie at Konza Prairie Biological Station, Manhattan, KS. The Christie-Perry technique (Christie and Perry, 1951) was used for the three-step isolation process. First, 1 kg soil was place into a 20 L bucket that contains 4 L water, and the slurry mixed with a household blender after 20-30 min. Second, the soil was passed through a 250 µm sieve and the material retained collected, wrapped in tissue paper, placed on a metal screen in a pot that filled with water, and put in dark over night. Third, water was carefully drained and the sediment which contained the nematodes was collected in a large Erlenmeyer flask and placed in a refrigerator at 4ºC. Approximately 50,000 nematodes were added into each microcosm. The functional composition of the nematode population was 65% fungivores, 15% microbivores, 10% herbivores and 10% omnivores-predators (Watson, 2005).

The phosphorus treatment consisted of a single application of superphosphate (0-20-0) at a rate of 90 g P kg^{-1} dry soil. The fertilizer was spread in a single layer at a depth of 10 cm in each microcosm.

Plant biomass

The aboveground biomass of each microcosm was clipped at approximately 1 cm from the soil surface. 32 microcosms were harvested each year at harvest. Cores (5 cm diameter $\times$ 15 cm long) were collected from each microcosm to recover roots and rhizomes. Roots and rhizomes were then separated and washed to remove soil in the 3rd growing season. For the 2nd growing season, rhizomes and roots were not separated. The above-and belowground biomasses were oven dried at 60° C for three days and weighed.

Soil microbial biomass C and N

The soil of each microcosm was subsampled from a square core soil sample of $15 \times 15 \times 15$ cm for soil microbial biomass C and N. The fumigation-incubation (FI) method (Jenkinson and Powlson, 1976) was used in this procedure. Briefly, two 25 g soil samples for each treatment were adjusted to a water content of 25 g/g and then incubated at 25° C for 7 days. After that, one of the two samples was fumigated with chloroform for 20h. Chloroform was then removed by vacumn and the samples were placed in mason jars and incubated for 10 days at 25° C. At the end of the incubation period, CO_2-C concentration was determined by gas chromatography (Shimadzu GC-8A, Kyoto, Japan) equipped with a thermal conductivity detector and a 2m Porapak (Q series) column at 70° C. The carrier gas was He at 14 mL min^{-1}. After gas sampling, 100 mL of 1 M KCl were added to the soil samples and placed on a rotary shaker for 1h at 300 rpm. The supernatant was then filtered through a Whatman #2 filter paper. The collected liquid phase was analyzed for inorganic N by the Agronomy Soil Testing Lab at Kansas State University. Nitrate was determined with cadmium reduction/ colorimetry and ammonium by indophenol colorimetric reaction. Both analyses were performed on a Rapid Flow Analyzer (Model RFA300, Alpkem Corporation, Clackamas, OR, USA). The calculations were determined by Voroney and Paul's equation (1984) as follows:

Microbial Biomass C = $(C_f - C_{unf})/ 0.41$.

Where: C_f = CO_2-C evolved from the fumigated samples.
C_{unf} = CO_2-C evolved from the unfumigated samples.

PLFA-NLFA

 Phospholipid and neutral lipid fatty acids (PLFA and NLFA) analysis were determined for both soil and roots at the end of the growing season with the Balkwill's method (1998). Soil was sampled with a hand probe (JMC Soil Smaplers, Newton, IA, USA) following the 2nd growing season, and subsampled from a $15 \times 15 \times 15$ cm core following the 3rd growing season. Roots were sampled from 2 cores (5 cm diameter $\times$ 15 cm long). Both the soil and roots were freeze-dried and ground into a powder. For each microcosm, 5 g of soil and 30 mg of roots were weighed for this procedure. Lipids were extracted with a single phase of chloroform, methanol, and phosphate buffer solution (5:10:4) and extracted for 3 hours. The extracted material was washed in preconditioned silica gel disposal extraction columns (J.T. Baker, Phillipsburg, NJ, USA) by chloroform, acetone, and methanol respectively to get the neutral lipids, glycolipids, and phospholipids separated. Neutral and phospholipids were then reacted with alkaline methanol to cleave the fatty acid from the glycerol molecule resulting in fatty acid methyl esters. These samples were analyzed by gas chromatography (HP 6890, Agilent Incorporated, Palo Alto, CA, USA). A 25 m Ultra-2 (J&W Scientific, Agilent Technologies, Palo Alto, CA, USA) column was used with He as the carrier gas at a flow rate of 1 mL min^{-1}. Temperature was programmed as $80\,^{\circ}\text{C}$ as an initial point, increasing to $155\,^{\circ}\text{C}$ at the speed of $20\,^{\circ}\text{C}$ min^{-1} and then gradually increase to $270\,^{\circ}\text{C}$ at the rate of $5\,^{\circ}\text{C}$ min^{-1}. Peaks represent the concentrations of fatty acid components by comparing each individual peak area with the internal standard (19:0) peak area.

 The fatty acids were denoted as **A:B**, where **A** is the total number of carbons of the chain and **B** is the number of double bonds present. ω represent the position of the double bond from the α end of the fatty acid. The prefixes **a** and **i** refer to anteiso and iso branching, and the suffixes **c** and **t** indicate cis and trans conformations. Methyl groups were denoted by **aMe**, where **a** represent for the position of the methyl group. Total ion areas were transformed to ng using 19:0 as the internal standard and individual fatty acids were analyzed in terms of nmol g^{-1} dry soil and mole percentage. The index using in this study and the microbe group they represent are as follows: Linoleic acid (18:2 ω 6), and 18:1 ω 9c represent general fungi, 16:1 ω 5c in NLFA represent for AMF. The sum of i15:0, i16:0 and i17:0, and a17:0 indicates gram-positive bacteria, while the sum of 16:1 ω 5 in PLFA, cy19:0, and 18:1 ω 7c represent gram-negative

bacteria. 10Me16:0, 10Me17:0, and 10Me18:0 indicate actinomycetes (Hogberg et al., 2007; Bradley et al., 2007).

Root colonization

        Roots of *Andropogon gerardii* Vit. were subsampled at the end of the growing season. They were washed free of soil, stained with trypan blue following Koske and Gemma's (1989) method, and measured for percentage of root length colonized by AM fungi following magnified gridline intersect method (Johnson et al., 2003).

Nematode populations

        Nematodes were sampled from 0-15 cm depth with a 2 cm diameter hand probe (JMC Soil Smaplers, Newton, IA, USA) and identified to genus and assigned to functional groups following Yeates et al. (1993) including herbivores, fungivores, and microbivores.

Soil CO_2 flux and isotopic composition

        Surface CO_2 flux was measured once a week with a LI-8100 Automated Soil CO_2 Flux System (LI-COR Inc., Lincoln, Nebraska) equipped with a 9.8 cm diameter survey chamber. Soil temperature was measured at a depth of 15 cm with an auxiliary sensor connected to LI-8100 at the same time as the CO_2 flux measurements. PVC soil collars (9.8 cm in diameter and 7.4 cm in length) were inserted at the beginning of the growing season. The measurement length was 2 min. and the interval time between microcosms was 30 seconds. All measurements were taken around noon on sunny days.

        To measure the isotopic composition of the CO_2-C, spinal needles (12 cm long) were installed in all microcosms. Plastic syringes of 10 mL were connected to the needle and wrapped with aluminum foil. When sampling, the syringes were pumped three times and then 10 mL of gas were taken and injected into preconditioned 10ml Vacutainers tubes. The tubes were conditioned by subjecting to a 4-step process: 3 min vacuum, 1 min flush with He, 1 min vacuum, 1 min flush with He, and finally 3 min of vacuum. The isotopic composition was determined at the Stable Isotope Mass Spectrometry Laboratory of the Division of Biology at Kansas State University with a ThermoFinnigan Delta Plus mass spectrometer coupled to a ThermoFinnigan GasBenchll (Waltham, MA).

Statistical analysis

Data across the three years were analyzed as a factorial experiment by Proc Mixed (SAS Institute Inc., 2002, Cary, NC, USA). Data for individual years was analyzed by SAS Proc GLM (SAS Institute Inc., 2001). Results from May and September were analyzed individually. Differences were considered significant at $p \leq 0.05$ unless otherwise stated.

RESULTS

Plant Growth

Plant biomass after the first growing season was reported by Watson (2005). The second and third year growth (Tables 2.1, 2.2) and the analysis of variance (Tables 2.3, 2.4) are reported in this thesis. Abovground biomass after the 2nd growing season responded to an interaction between M and P where both produced a positive effect on aboveground biomass but was not different than the combination of M and P (Fig. 2.1). At the end of the 3rd growing season, there was a significant three-way interaction between N, M and P for aboveground biomass (Table 2.4). Nematodes significantly decreased aboveground biomass, while all other treatments did not significantly affect aboveground biomass compared to the control (Fig. 2.2). It appears that for plant biomass, M and P played a significant positive role in the 2nd year while nematode depressed plant growth for the 3rd year.

At the end of the 2nd growing season, phosphorus significantly increased belowground plant biomass by 209% compared with the non-P treated microcosms (Fig. 2.3). At the end of the 3rd growing season, belowground biomass increased 112% for the mycorrhizal treatment (Fig. 2.4). There was a significant two-way interaction between N and P for belowground plant biomass, where NP significantly decreased overall biomass compared to the control with P (Fig. 2.5). Rhizome and root biomass responded differently to the treatments. There were two-way interactions for root biomass in the N+P and N+M treatment (Table 2.4). Nematodes decreased root biomass in the presence of phosphorus (Fig. 2.6) and in the absence of M (Fig. 2.7). Nematodes also significantly decreased rhizome biomass by 41% and P decreased rhizome biomass by 35% (Fig. 2.8 and Fig. 2.9). The mycorrhizal treatment significantly increased rhizome biomass by 73% (Fig. 2.10), which accounted for the enhanced total belowground biomass in the mycorrhizal treatment. There was a significant three-way interaction in root to shoot ratio among N, M and P where the combination of N and P produced a higher root:shoot ratio than N or P alone, while adding M to NP treatment, the root: shoot ratio decreased suggesting a compensasory growth while M reduced the need for roots (Fig. 2.11). Phosphorus alone reduced root to shoot ratio compared with the control, but this effect disappeared in the presence of nematodes (Fig. 2.11).

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After the 2nd growing season, there was a three-way significant interaction for root colonization by AM fungi (Table 2.5). The M treatment increased root colonization as expected since the mycorrhizal incolum was added to these treaments. The M alone treatment had the highest level of colonization, and the addition of N and P both reduced that level (Fig. 2.12). After the 3rd growing season, nematodes continuously decreased mycorrhizal colonization compared to the other treatments (Fig. 2.13). The non-mycorrhizal treatments were colonized by AMF which suggested contamination during the project. We assume that this happened in May of the 3rd year, when another project of ^{15}N injection in these microcosms resulted in transfer and contamination of spores to the control microcosms.

Soil Responses

Microbial biomass C was measured in the 2nd and 3rd growing seasons. After the 2nd growing season, there was a three-way significant interaction for soil microbial biomass C (Table 2.6), where both P and M increased soil microbial biomass C (Fig. 2.14). At the end of the 3rd growing season, there were no significant treatment effects for MBC (Fig. A.23). Microbial biomass N (MBN) and inorganic N were not different among treatments (Fig. A.24).

Microbial biomass as indicated by total PLFA was positively affected by P (Fig. 2.15) after the 2nd growing season. This was similar to the response measured by the fumigation technique. The composition of the microbial community was affected by P addition as P increased the abundance of general bacteria, gram positive bacteria, actinomycetes, and fungal abundance (Fig. 2.16-2.19). The M treatment also had a positive effect on general bacteria abundance but not other members of the microbial community (Fig. 2.20). Arbuscular mycorrhizal fungal abundance as indicated by NLFA was not affected by the treatments (Table 2.7). This is in contrast to root colonization where P decreased colonization even in the prescence of mycorrhizal fungi. This suggests that AMF was present in the soil but was unable to adequately colonize the roots in the presence of P. Addition of AM fungi had a negative effect on relative abundance of general fungi (Fig. 2.21).

At the end of the 3rd growing season, there was a weak two-way interaction ($p<0.1$) between M and P for total PLFA (Table 2.8), where either M or P supported larger microbial biomass than the control (Fig. 2.22). The ratio between NLFA and PLFA, which is an indicator of stress of the microbial community, showed a two-way interaction between N and M, N alone

reduced the ratio compared to the other treatment combinations (Fig. 2.23). Another two-way interaction was present between M and P, where M associated with P significantly increased the NLFA to PLFA ratio compared to P alone (Fig. 2.24), possibly suggesting more easily available carbon source in P associated mycorrhizal treatments. Fungal biomass at the end of the 3rd growing season was estimated by the total of 18:1 ω 9c and 18:2 ω 6,9c fatty acids derived from neutral lipids (NLFAs) (Table 2.9). Mycorrhizae significantly increased general fungal abundance (Fig. 2.25). The relative abundance of general fungi was not significantly different among treatments (Table 2.10), suggesting higher abundance of other microbial groups in the M treated microcosms. Arbuscular mycorrhizal fungi (AMF) abundance was estimated with the NLFA derived 16:1 ω 5c (Table 2.9). Similar to the results of total fungal abundance, there was a significant two-way interaction between M and P, where the combination of M and P resulted in greater AMF than with P alone (Fig. 2.26) indicating a positive response of P amended soil for AMF abundance. This result indicates that although AMF was inhibited by P addition, M treatments still resulted in significantly higher AMF abundance in soil compared to non-mycorrhizal treatments. The relative abundance of AM fungi was not significantly different among treatments. Total bacteria abundance was estimated with combined indicators of gram-positive bacteria, gram-negative bacteria, and actinomycetes. No significant treatment effect was observed (Table 2.11). The mycorrhizal treatment significantly increased the relative abundance of gram-negative bacteria by 28% (Fig. 2.27), while the mycorrhizal treatment decreased the relative abundance of general bacteria and gram-positive bacteria by 20% and 11%, respectively (Fig. 2.28 and Fig. 2.29). The nutritional status of the bacterial community was examined with the ratio of saturated to total monounsaturated fatty acid. The mycorrhizal treatments significantly increased the bacteria nutritional stress by 135% (Fig. 2.30), indicating a significant competition between mycorrhizae and bacterial community. The fungal to bacterial biomass ratio at the end of the 3rd growing season was calculated by using general fungal abundance in NLFA and total bacteria abundance in PLFA (Table 2.12). A two-way interaction was detected between M and P, where the combination of M and P produced higher fungal to bacterial biomass ratios than the control, M, and P treatments (Fig. 2.31). The relative abundance ratio between fungi and total bacteria was not significantly different (Table 2.13).

If we compare fatty acid results of 2nd and 3rd year, the 3rd year resulted in more alteration in soil microbial communities due to treatments, while for the 2nd year, significant results were confined to the P effects.

CO$_2$ flux

For results of year 3 in 2006, no significant results were detected throughout the growing season (Figure 2.32).The hypothesis was that nematodes can enhance nutrient cycling in the soil and emit more CO$_2$ to the atmosphere. But our results for CO$_2$ flux were not significantly affected by nematodes. This lack of response may be due to multiple factors. Larger plant biomass of M and P treatments may increase C inputs into soil that enlarged the active C pool and result in lower loss of CO$_2$ that balanced the effects that the nematodes played in enhancing CO$_2$ loss from soil. The isotopic C analysis for belowground CO$_2$ flux in May, June, and August did not show significant results among treatments.

DISCUSSION

Microbial interactions and plant response

At the end of the first year, mycorrhizal plants produced three times more plant biomass than nonmycorrhizal plants and plants without P amendment (Watson, 2005). This growth response continued into the 2nd growing season, which also had greater plant biomass in mycorrhizal plants than nonmycorrhizal plants. However P negated the effect of the mycorrhizal treatment. At low to moderate soil P, plants benefit from the mycorrhizal symbiosis (Smith and Read, 1997). Plants colonized with mycorrhizal fungi were found to grow very poorly under extremely low P levels (Schweiger et al., 2007), while at high P levels, mycorrhizal inoculation was were found to have no positive effects on plant growth or P absorption. In this study, treatments significantly affected root biomass. Phosphorus consistently increased root biomass after 3 years (Fig. 2.6). Adler et al. (1984) reported that plant root biomass accumulate faster with P addition, and Hossain et al. (2006) also found a more highly developed root system with longer root length in groundnut with increasing P level in the soil. Alternatively, big bluestem inoculated with mycorrhizae had greater rhizome production regardless of whether or not plants were fertilized with low levels of P (Hetrick et al., 1990). This result was supported by the result from the 3rd growing season, where mycorrhizal plants resulted in higher rhizome biomass than the nonmycorrhizal plants (Fig. 2.10), indicating that mycorrhizal symbiosis may allow the plant to store C and other nutrients for re-growth.

In years 1 and 2, there was a large reduction in root colonization with added P which is consistent with previous studies (Smith and Read, 1997), indicating P addition can impede the mycorrhizal symbiosis. Our results in year 3 did not show these effects, which may be partly explained by contamination of AM fungi resulting in root colonization in the nonmycorrhizal microcosms. Watson (2005) reported that root colonization was highly related to AM fungal abundance in soil and in roots determined by NLFA analysis in the first year. However there was no relationship between root colonization and AM fungal abundance in my study.

In year 1 of this study, microbivores were the dominant group of the nematode community (Watson, 2005). This was probably due to the time needed to establish root biomass because by the 3rd growing season, plant-feeding nematodes were dominant (Fig. 2.33-2.34). In year 1, nematodes did not significantly affect plant growth (Watson, 2005), but after 3 years,

nematodes decreased both above- and belowground biomass. Different nematodes in terms of feeding strategies have different influence on plants. Bacterial-feeding nematodes have been reported to increased plant growth (Ingham et al., 1985). They were also found to have a significant effect on early development of plant roots (Mao et al., 2006). Studies showed that bacterial-feeding nematodes may help plants develop a more highly branched root system with longer and finer roots (Mao et al., 2007). Fungal-feeding nematodes have been reported to reduce plant yield (Giannakis and Sanders, 1990), but in our study, this group composed of a very small proportion of the total nematode population across the 3 years. However, plant-feeding nematodes were found to decrease plant biomass in a greenhouse study by Hartnett and Wilson (2002). Root biomass in year 3 was significantly affected by an interaction between nematodes and mycorrhizae, where plant-feeding nematodes reduced root biomass when mycorrhizae were not present (Fig. 2.7). It is likely that the mycorrhizal symbiosis may compensate for the grazing by nematodes (Rabatin and Stinner, 1988; Fitter and Garbaye, 1994).

Microbial interactions and soil microbe community

Brussaard et al. (2001) pointed out that the most important interactions in terms of effects on plant response and soil microbe dynamics are those between AM fungi and nematodes. Marschner et al. (2003) suggested that the effect of mycorrhizal colonization on rhizosphere microbial community is plant mediated, probably involving changes in root exudation. A series of experiment considering the effects of low level herbivory by root-feeding nematodes demonstrated positive effects on soil community due to the "leakage" of nutrients from damaged root tissue, which can provide extra C and other nutrients for other soil microbes (Yeates et al., 1998; Bardgett et al., 1999). However, there was no affect of nematodes on the microbial community across the 3 years of this study. Moreover, a negative effect on soil microbial biomass was found during the 2nd year (Fig. 2.14). This may due to limited C input from plants grazed by nematodes, which provide a C source for microbes in the soil.

Phosphorus significantly increased soil microbial biomass as estimated by total PLFA in the first two growing seasons (Fig. 2.15, and Fig. 2.35). There was a trend for P to increase microbial biomass in the nonmycorrhizal treatments in year 3, but this was not significant (Fig. 2.22). The actinomycete population in the first two years was also found to be significantly higher in P treated soils. These results suggested that P addition to low P level soils can increase

the microbial population which may be due to the improvement in plant root biomass and thus more available C inputs into the soil. Mycorrhizal fungi and P addition tended to enhance soil microbial biomass. This may relate to enhanced C input from plants, as P and M treatments exhibited higher plant biomass.

The AM fungal biomarker was positively affected by the mycorrhizal inoculation (Fig. 2.26). AM fungi abundance in roots was not in agreement with the colonization data. In the 2nd year, AM fungi were found in non mycorrhizal treated microcosms as indicated by NLFA in both roots and soil, while no root colonization was observed indicating NLFA may be more sensitive to a small change in AM fungi than estimates of root colonization. General fungal biomass was increased by the mycorrhizal treatment (Fig. 2.25). This fungal response should be due to AM fungal abundance instead of saprophytic fungi because saprophytic fungi were reported to be suppressed by AM fungi (Olsson et al., 1998; McAllister et al., 1994).

Bacterial abundance was not consistently affected by the mycorrhizal treatment. Other studies have reported variable effects of AM fungi on bacteria (Van Aarle et al., 2003; Amora-Lazcano et al., 1998; Olsson et al., 1996). However, there could be species-specific bacterial response, where some species are enhanced and others inhibited (Wamberg et al., 2003).

Our results showed a consistent increase of fungal to bacterial ratio in the P treatments along with an increase in microbial biomass as indicated by total PLFA and root biomass (Fig. 2.31). In other studies, root-feeding nematodes did not affect the ratio (Bardgett et al., 1999), although a decrease in fungal to bacterial ratio occurred in the root zone (Bardgett et al., 1998; Mawdsley and Bardgett, 1997). Watson (2005) hypothesized that as C limitation is overcome with root C inputs other nutrients may become limiting and fungi may have a competitive advantage under nutrient limitations due to their ability to explore soil spaces by external hyphae.

Our results confirmed the role of the mycorrhizal symbiosis in C allocation in a plant-mycorrhizal-soil system in which mycorrhizae resulted in more C allocated to belowground rhizomes for storage rather than roots. This would be an important strategy for the plants. Mycorrhizal colonization would allow the plants to acquire nutrients through the hyphae and thus allocate more resources on the rhizomes for regrowth. Nematodes suppressed plant biomass. In addition, rhizome biomass was depressed in the presence of nematodes, suggesting that more C was allocated to root regrowth. Phosphorus induced more plant root biomass and larger microbial communities due to enhanced C inputs from plants. Phosphorus addition impeded

mycorrhizal colonization, but AMF abundance in soil was still significantly higher than non-mycorrhizal ones, as well as fungal to bacterial ratio.

Due to the contamination of AM fungi in the 3rd year, the results related to the M effects need to be considered with caution. The contamination of AM fungi did affect some of the results such as aboveground plant biomass. But there was still significant accumalted effect of mycorrhizal treatments from the previous 2 years related to plant belowground biomass and soil microbial communities.

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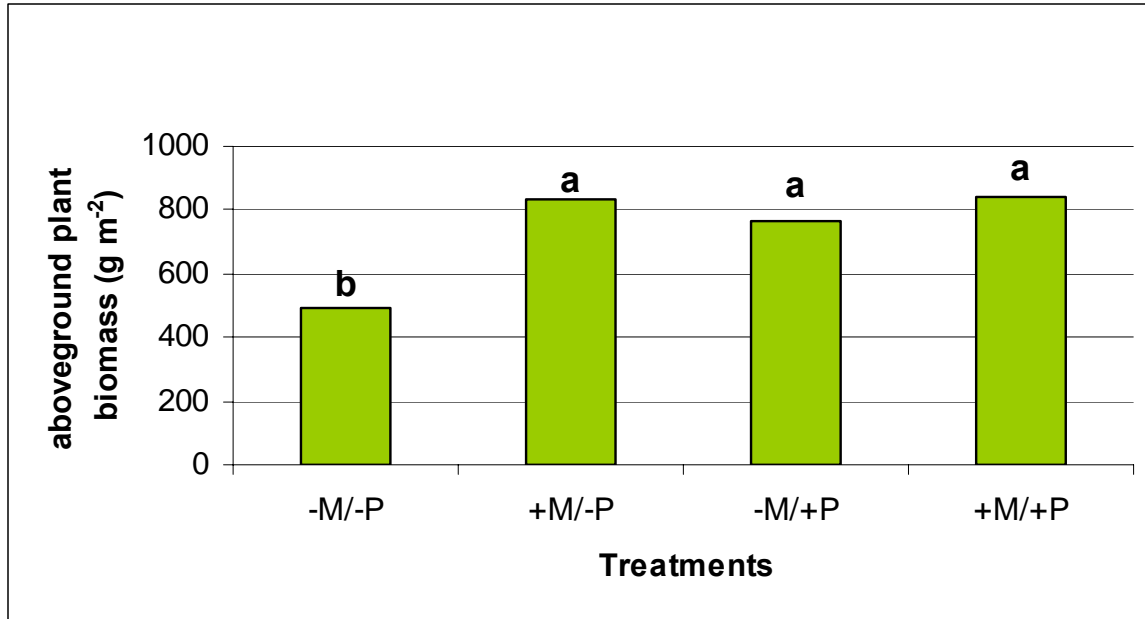
Figure 2.1. Aboveground biomass of *Andropogon gerardii* Vit. at the end of the 2nd growing season as affected by mycorrhizal fungi (M) and phosphorus (P) treatments. With (+M) or without (-M) mycorrhizae and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).
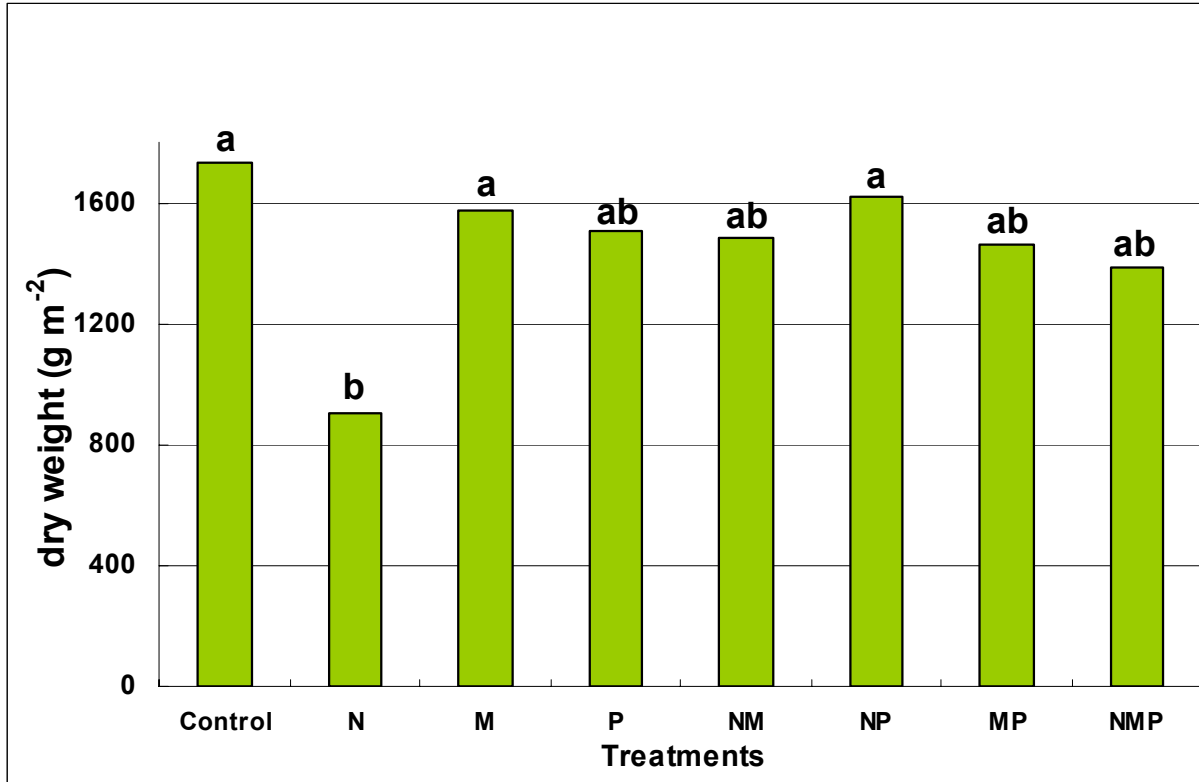
Figure 2.2. Aboveground plant biomass of *Andropogon gerardii* Vit. at the end of the 3rd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05).
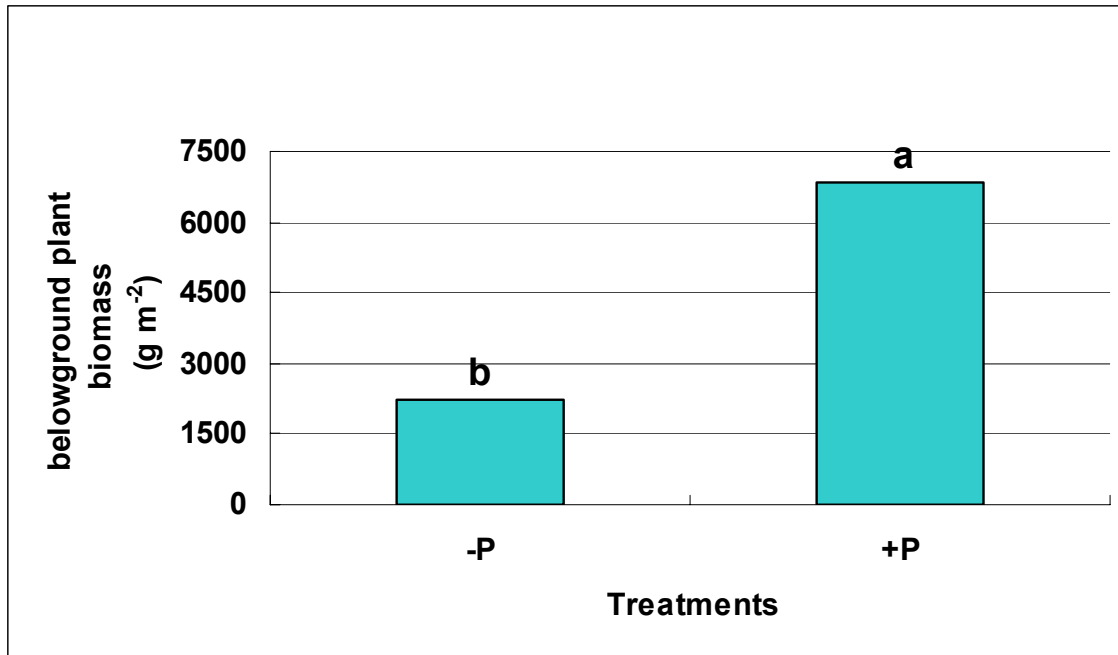
Figure 2.3. Belowground plant biomass of *Andropogon gerardii* Vit. at the end of the 2nd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
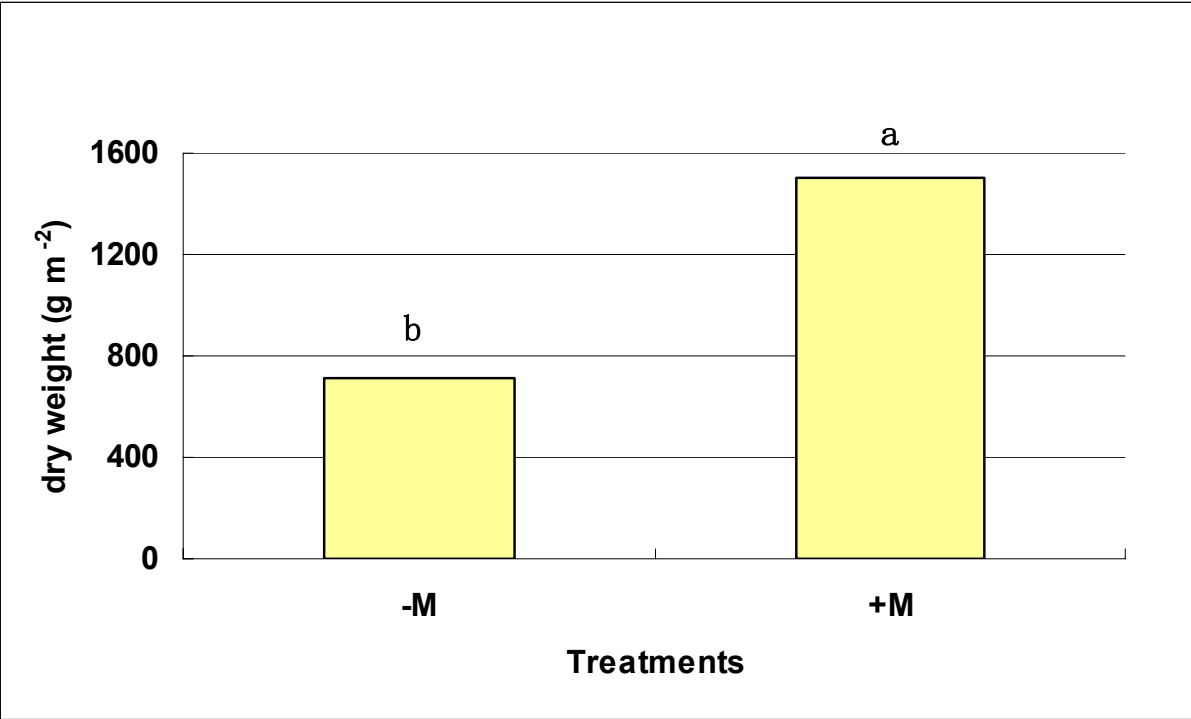
Figure 2.4. Belowground plant biomass of *Andropogon gerardii* Vit. at the end of the 3rd growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).

Figure 2.5. Belowground plant biomass of *Andropogon gerardii* Vit. at the end of the 3rd growing season. With (+N) or without (-N) nematodes and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).

Figure 2.6. Root biomass of *Andropogon gerardii* Vit. at the end of the 3rd growing season. With (+N) or without (-N) nematodes and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).

Figure 2.7. Root biomass of *Andropogon gerardii* Vit. at the end of the 3rd growing season. With (+N) or without (-N) nematodes and with (+M) or without (-M) mycorrhizae. Different letters indicate significant difference (P<0.05).
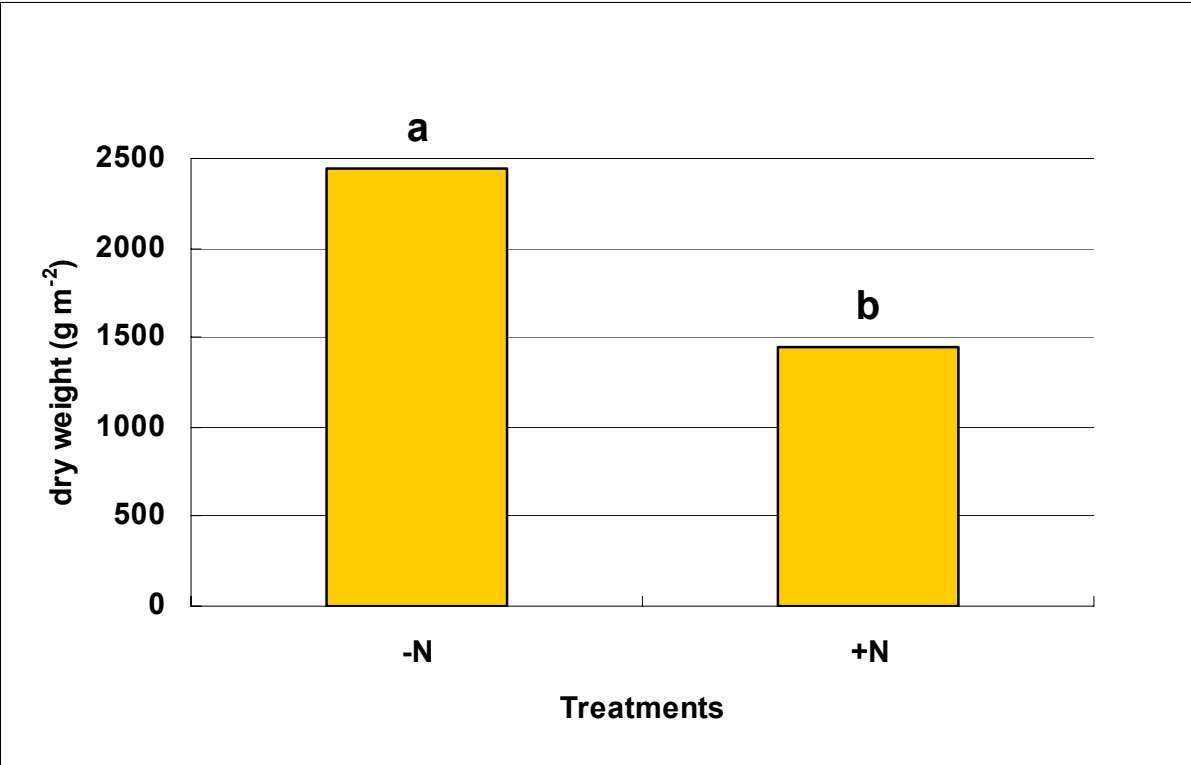
Figure 2.8. Rhizome biomass of *Andropogon gerardii* Vit. at the end of the 3rd growing season. Without (-N) or with (+N) nematodes. Different letters indicate significant difference (P<0.05).
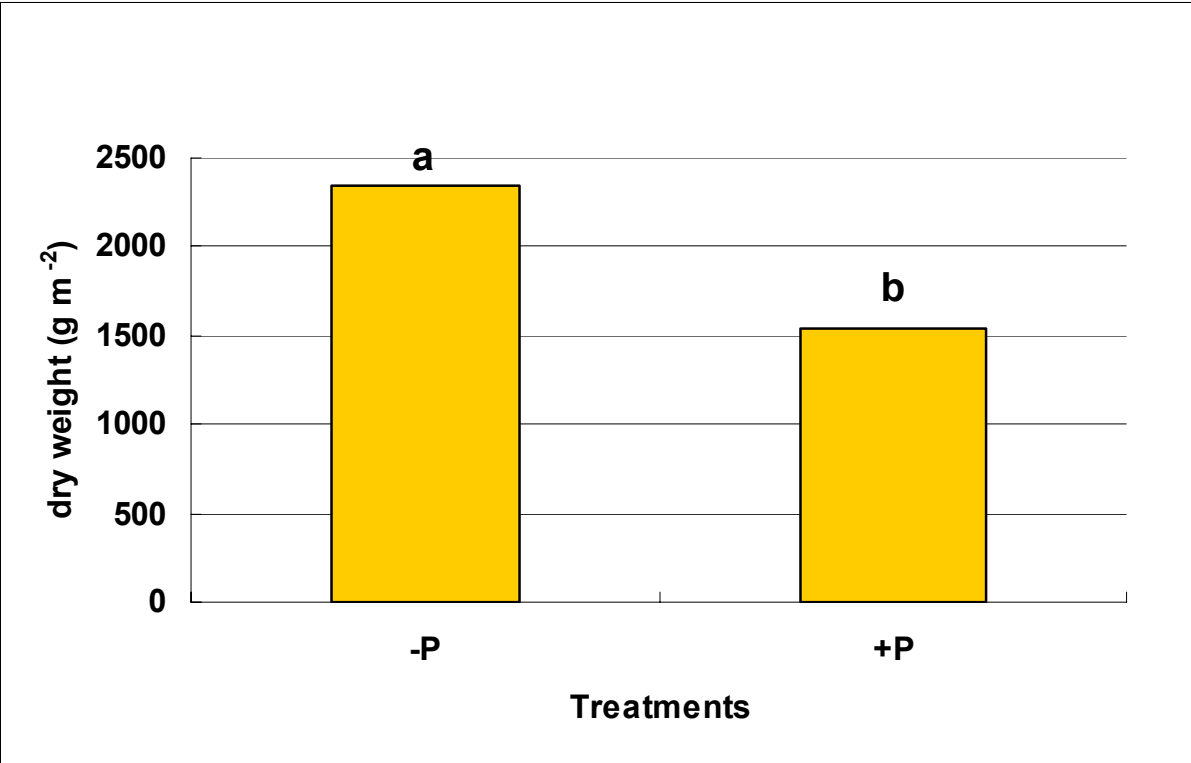
Figure 2.9. Rhizome biomass of *Andropogon gerardii* Vit. at the end of the 3rd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
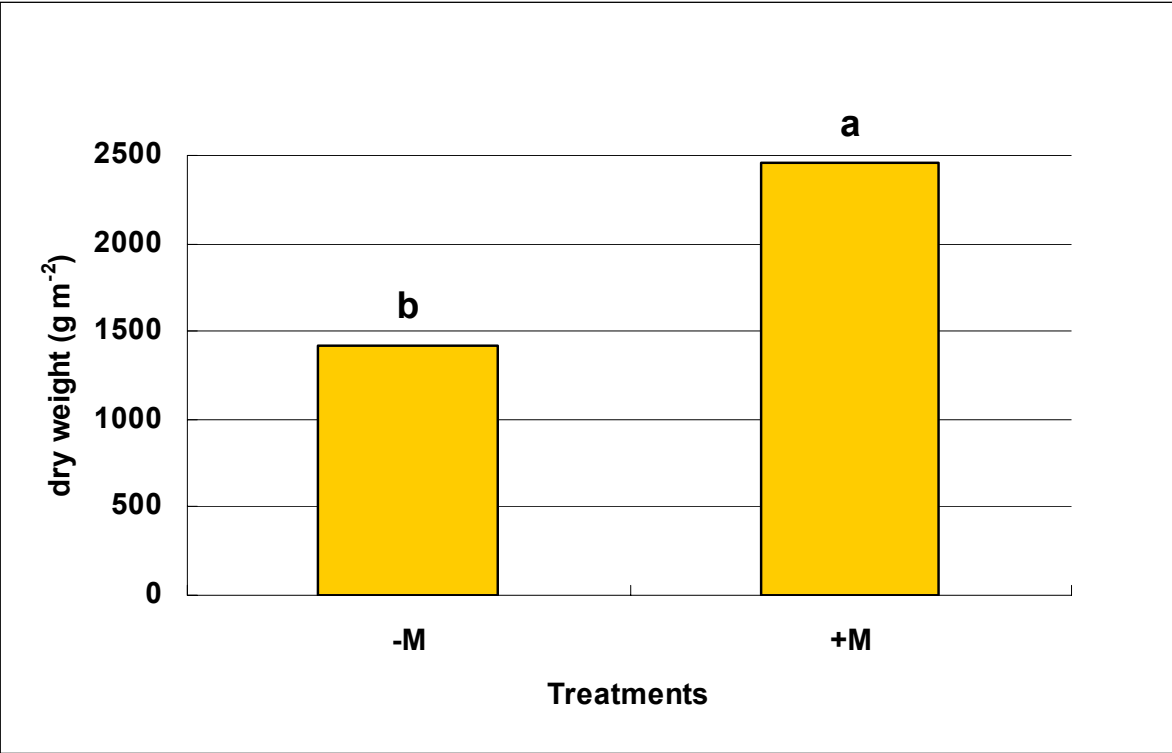
Figure 2.10. Rhizome biomass of *Andropogon gerardii* Vit. at the end of the 3rd growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).
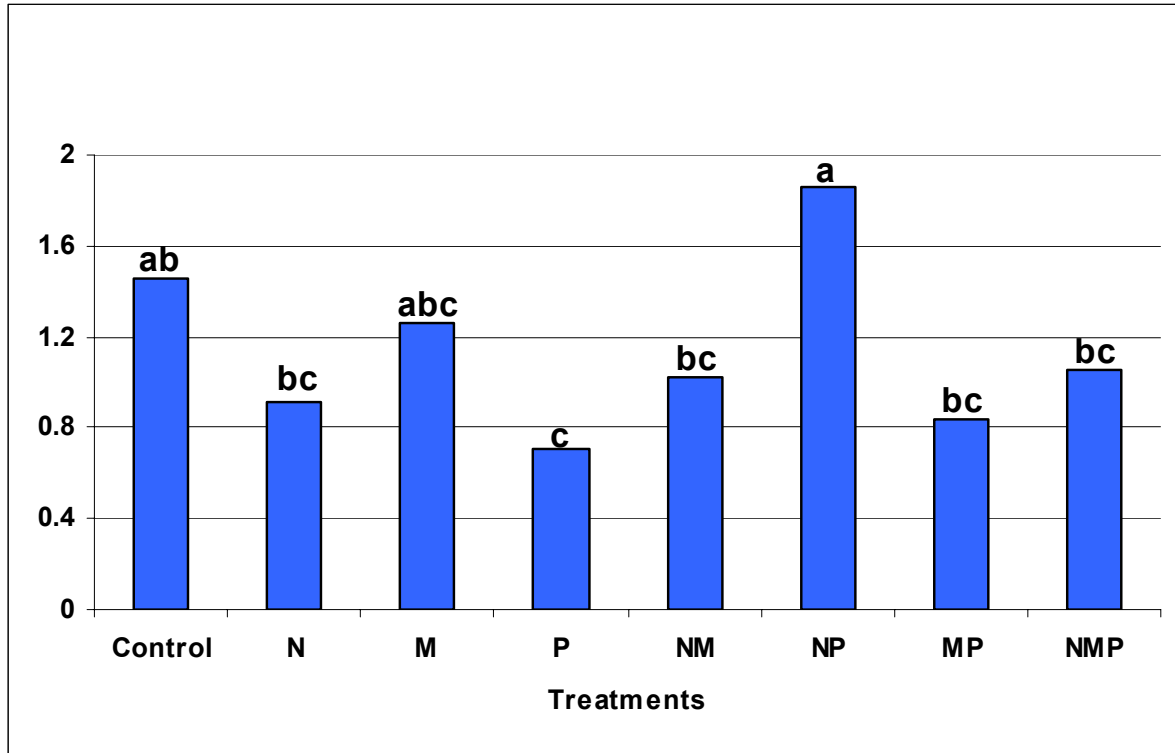
Figure 2.11. Root to shoot ratio of *Andropogon gerardii* Vit. at the end of the 3rd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05).
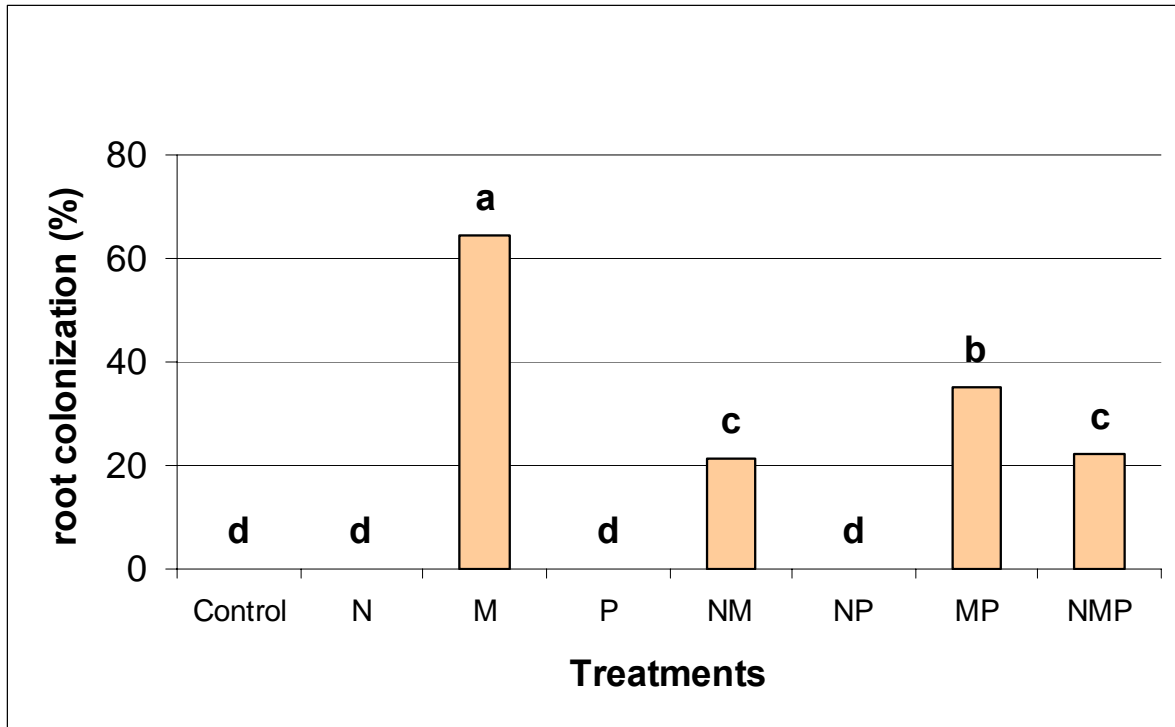
Figure 2.12. Root colonization of *Andropogon gerardii* Vit. by AM fungi at the end of the 2nd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05)
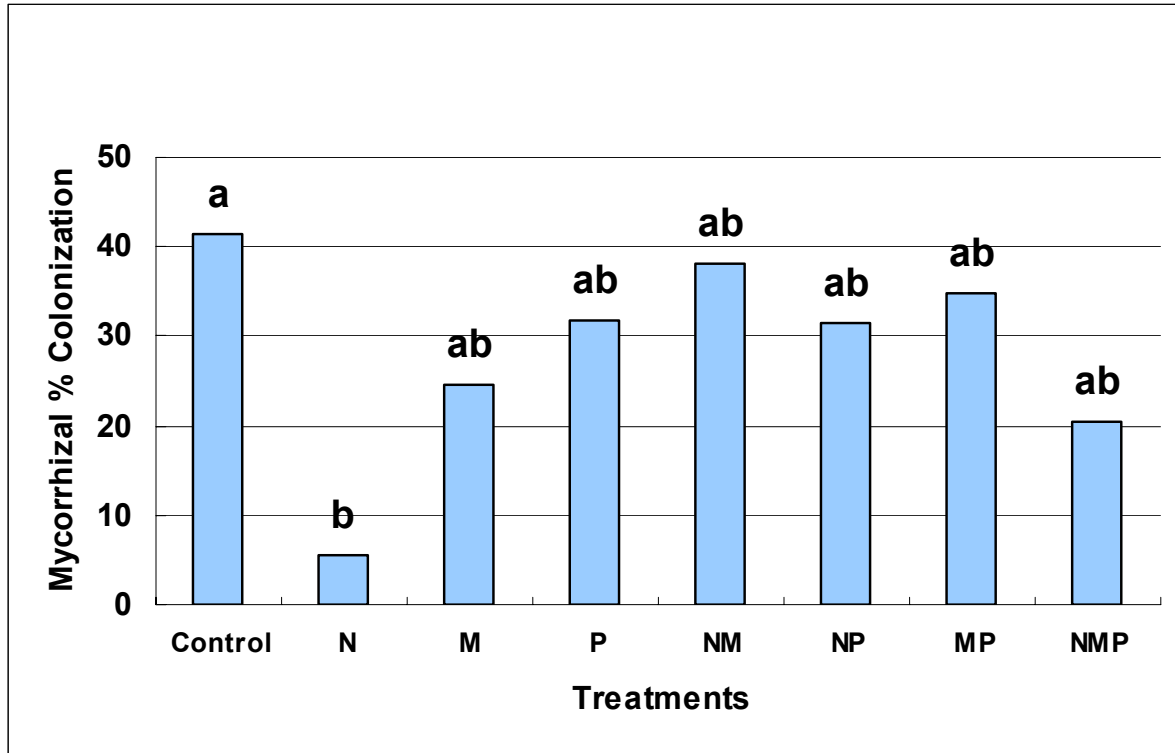
Figure 2.13. Root colonization of *Andropogon gerardii* Vit. by AM fungi at the end of the 3rd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05).
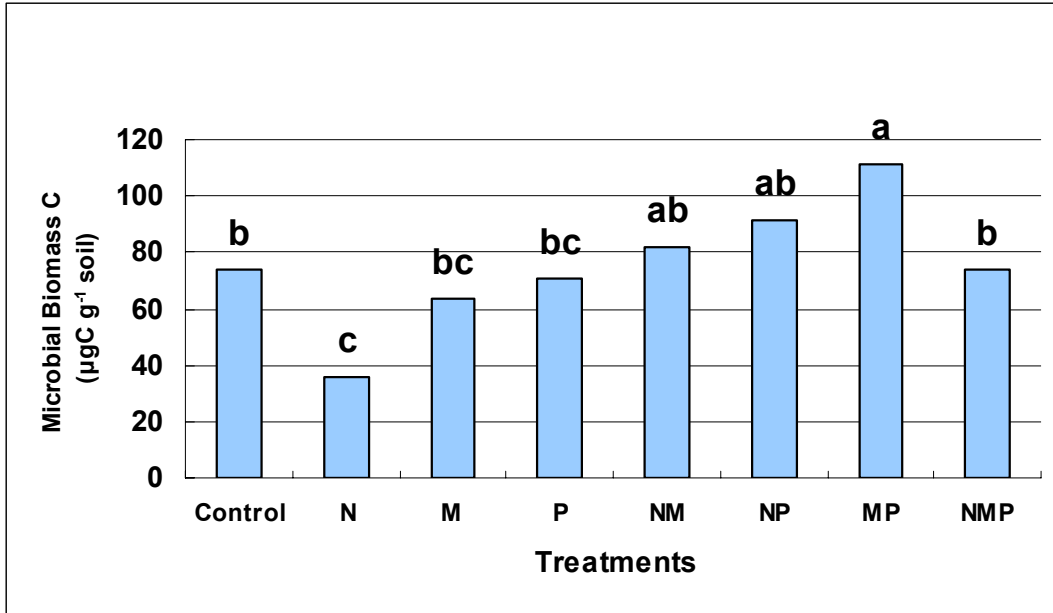
Figure 2.14. Single effect on microbial biomass carbon of *Andropogon gerardii* Vit. at the end of the 2nd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05).
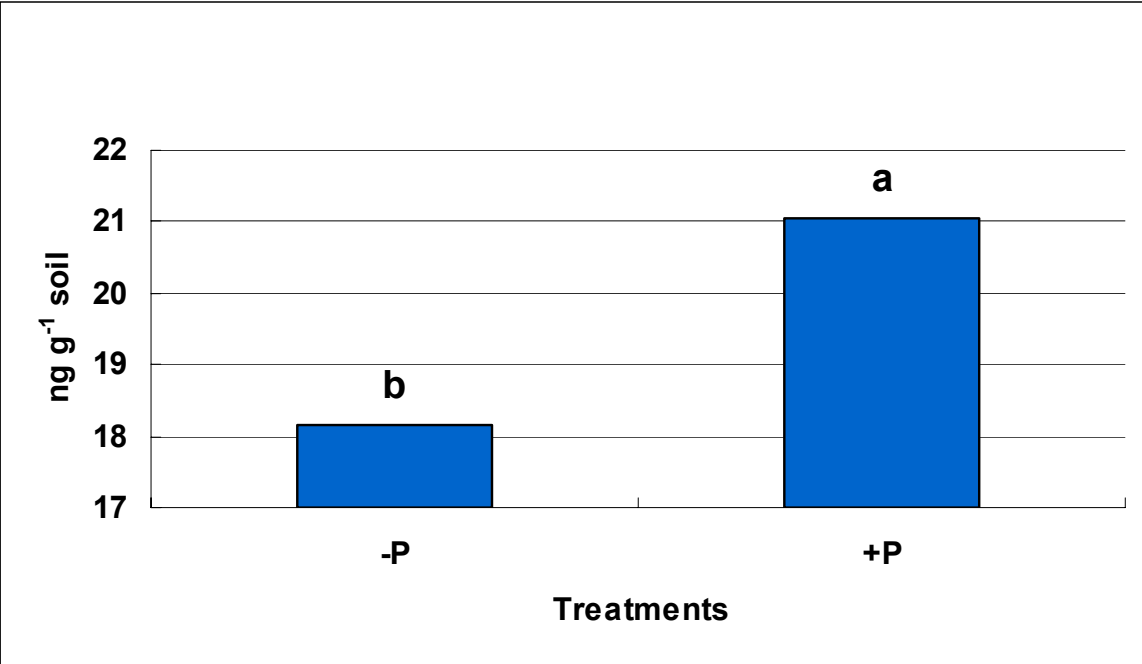
Figure 2.15. Microbial biomass indicated by total PLFA in soil at the end of the 2nd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
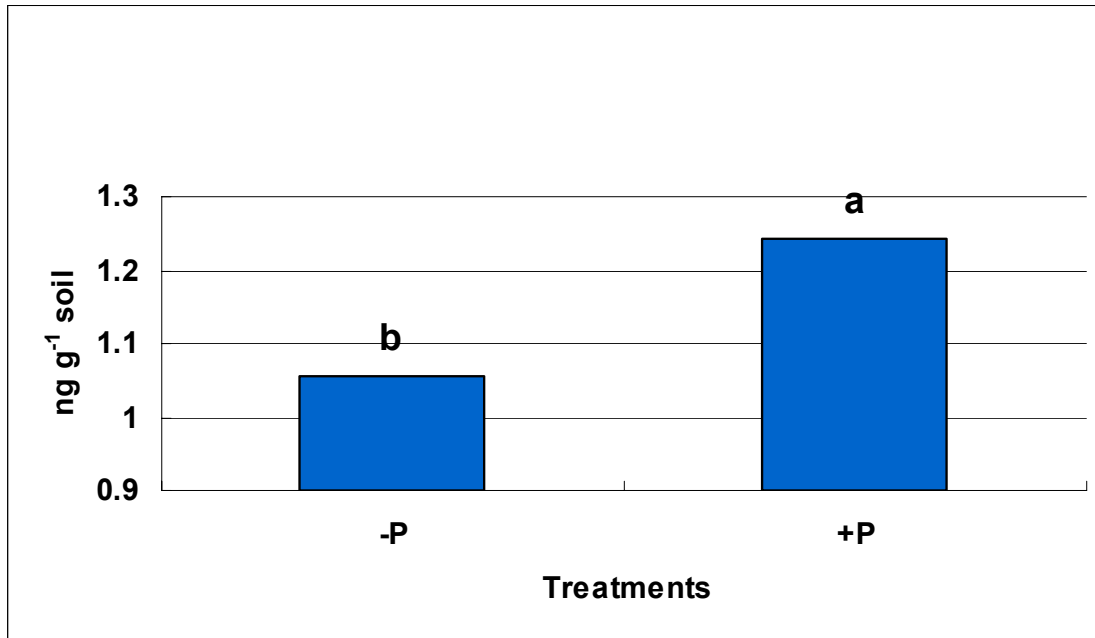
Figure 2.16. Abundance of general bacteria in the soil indicated by PLFA at the end of the 2nd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
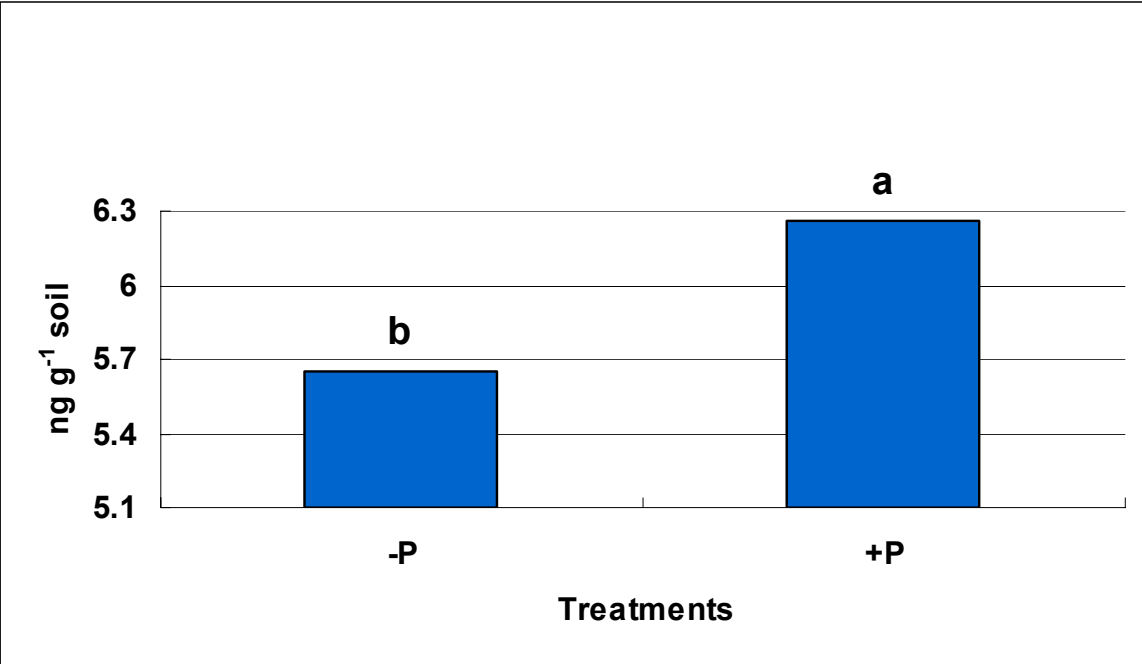
Figure 2.17. Abundance of gram-positive bacteria in soil indicated by PLFA at the end of the 2nd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
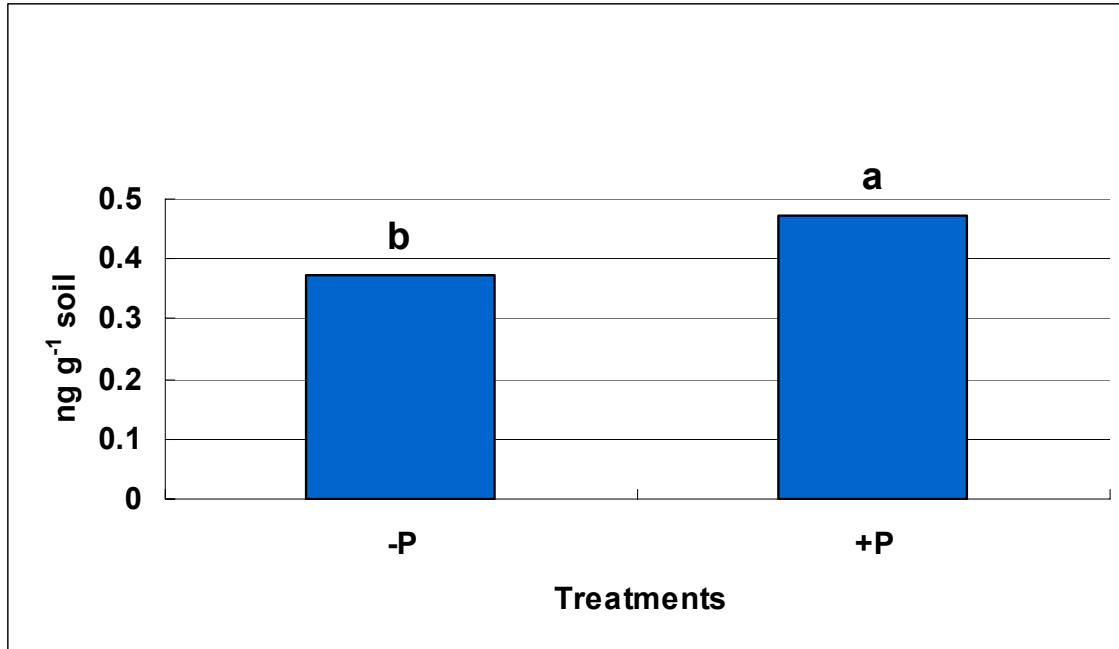
Figure 2.18. Abundance of actinomycetes in soil indicated by PLFA at the end of the 2nd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
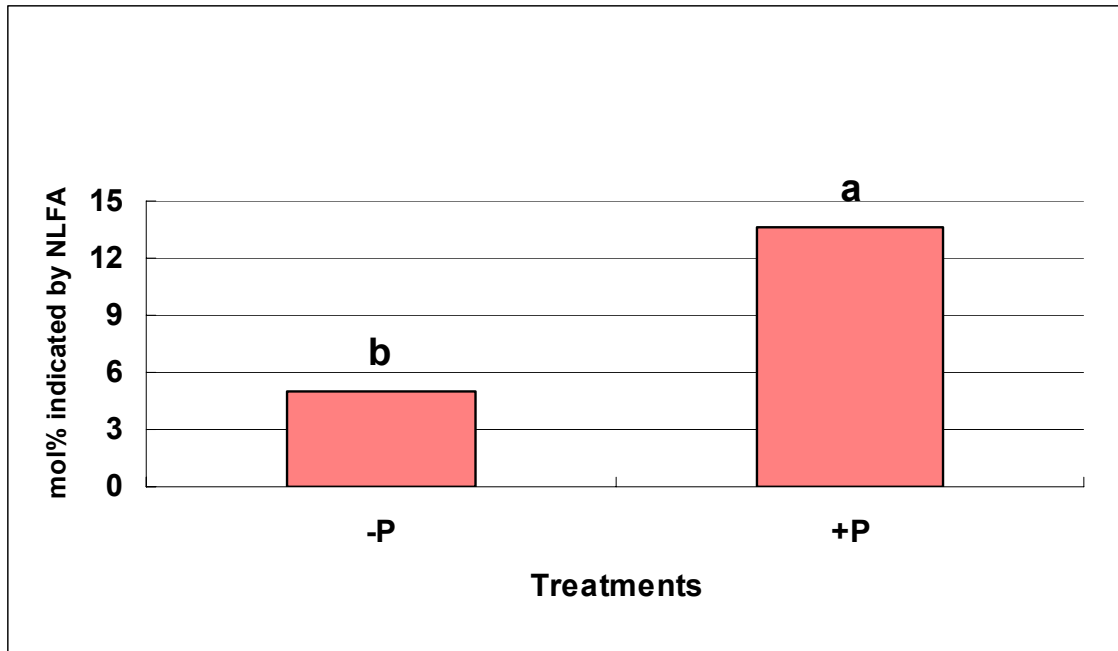
Figure 2.19. Relative abundance of general fungi in soil indicated by 18:1 ω 9 and 18:2 ω 6 in NLFA at the end of the 2nd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
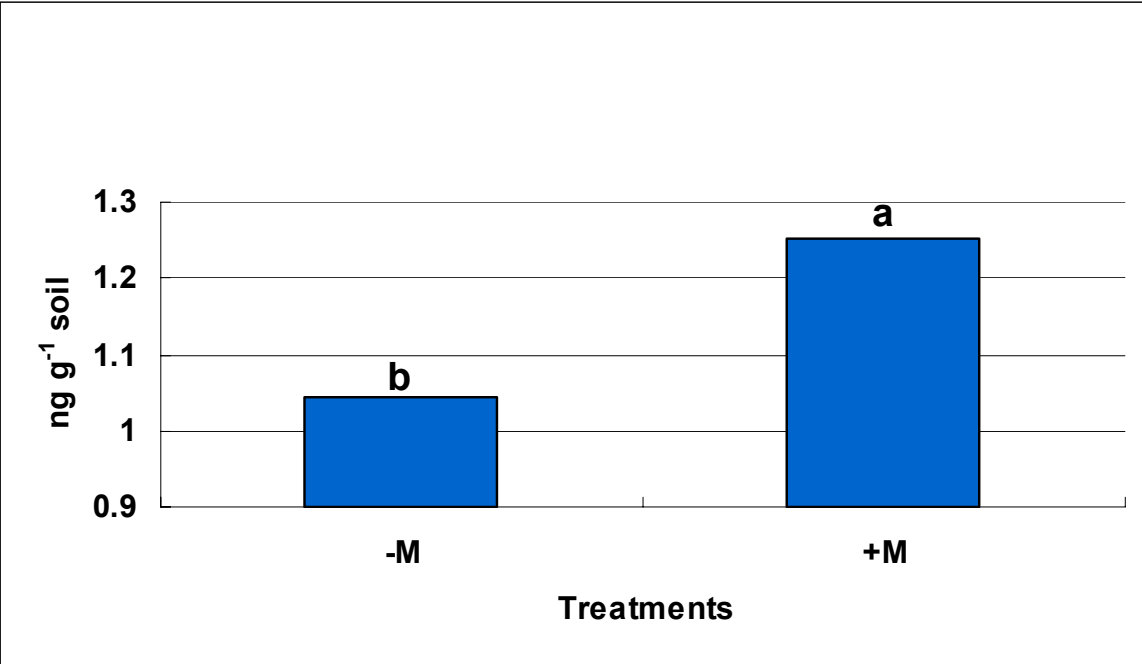
Figure 2.20. Abundance of general bacteria in soil indicated by PLFA at the end of the 2nd growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).
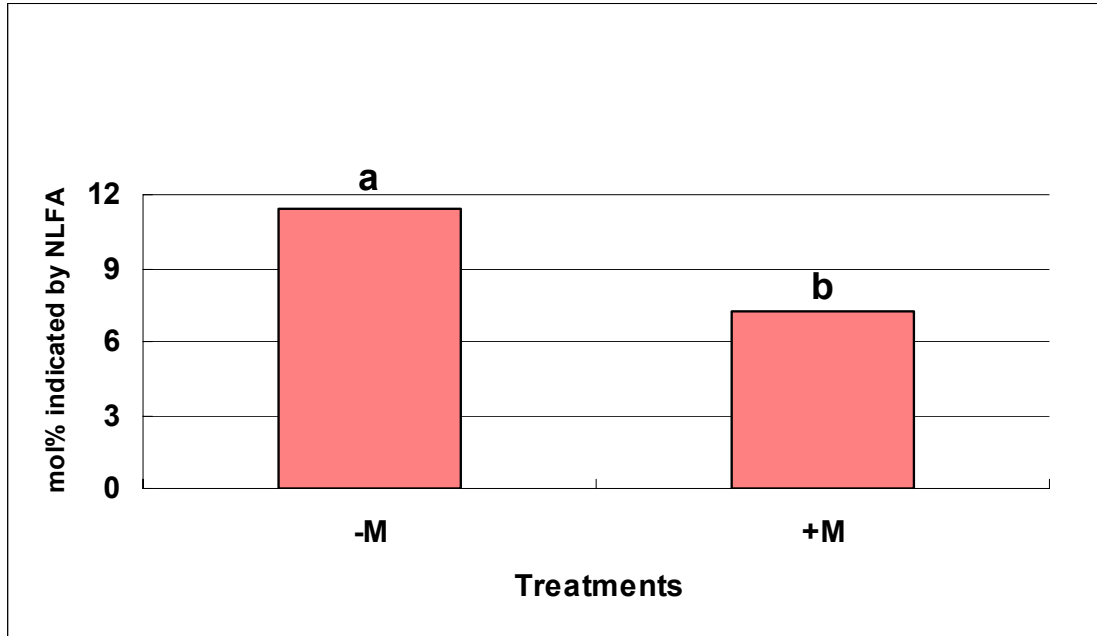
Figure 2.21. Relative abundance of general fungi in soil indicated by 18:1 ω 9 and 18:2 ω 6 in NLFA at the end of the 2nd growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).
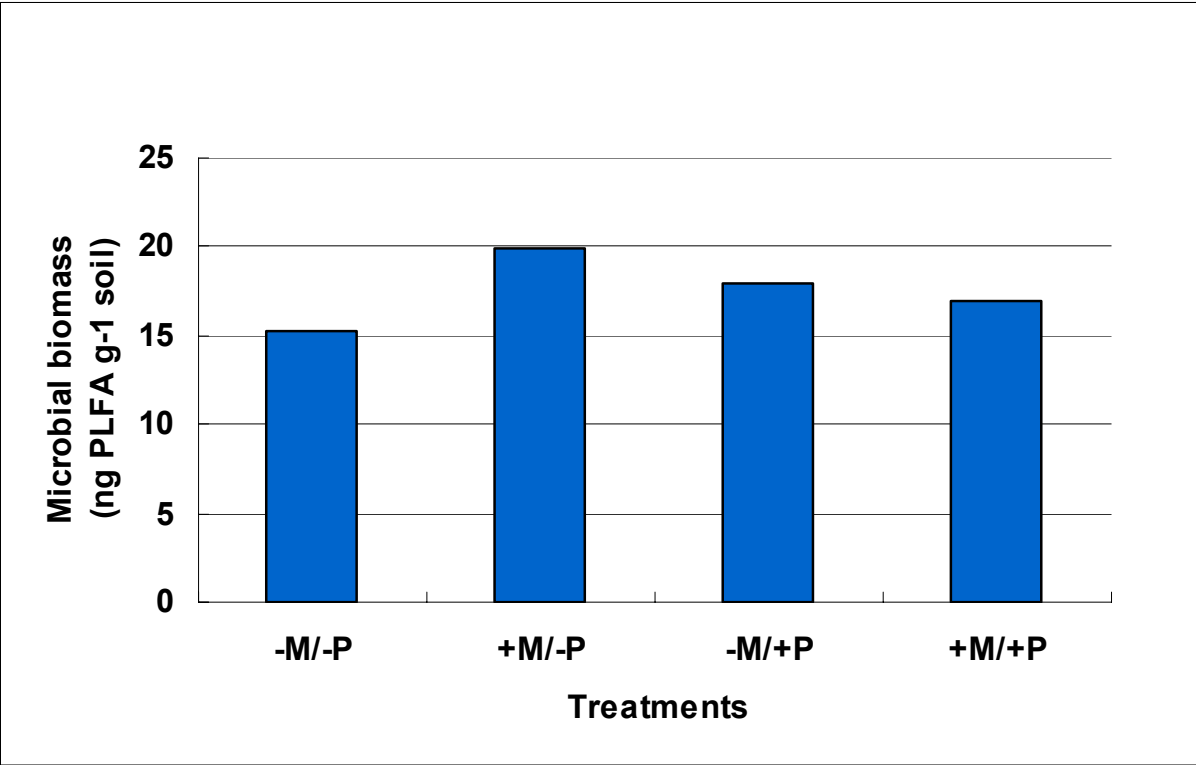
Figure 2.22. Microbial biomass indicated by total PLFA in the soil at the beginning of the 3rd growing season. With (+M) or without (-M) mycorrhizae and with (+P) or without (-P) phosphorus.
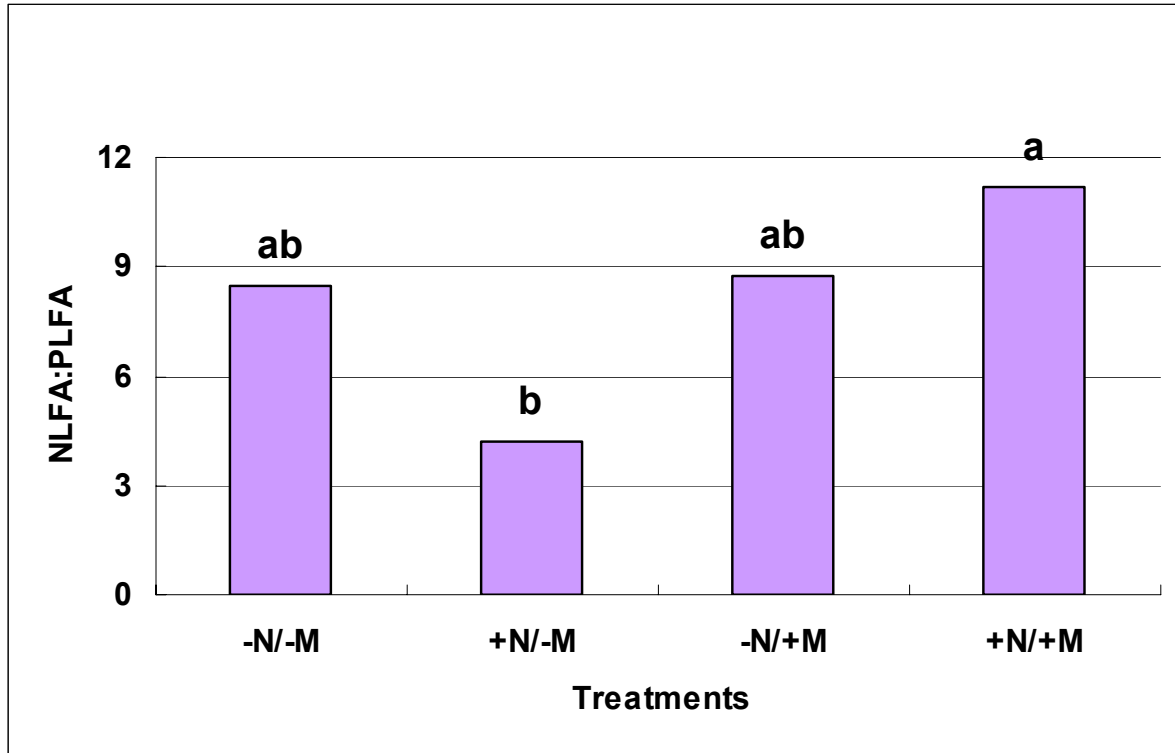
Figure 2.23. NLFA to PLFA ratio in soil at the end of the 3rd growing season. With (+M) or without (-M) mycorrhizae and with (+N) or without (-N) nematodes. Different letters indicate significant difference (P<0.05).
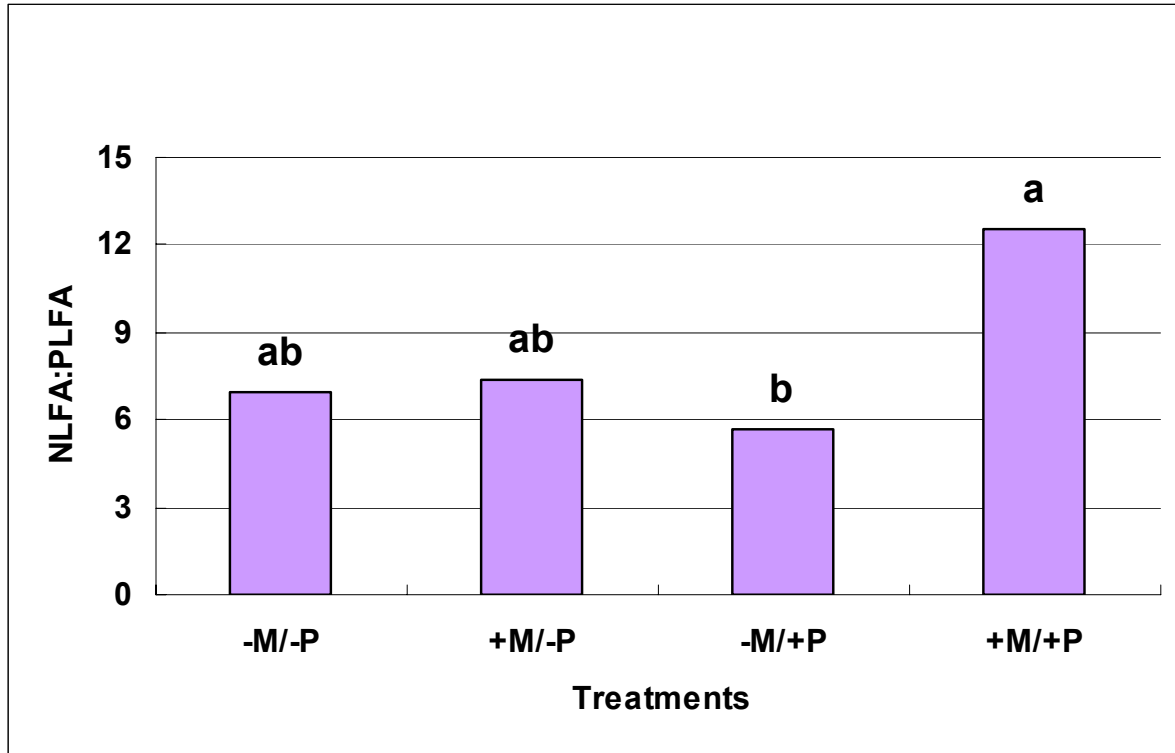
Figure 2.24. NLFA to PLFA ratio in soil at the end of the 3rd growing season. With (+M) or without (-M) mycorrhizae and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).
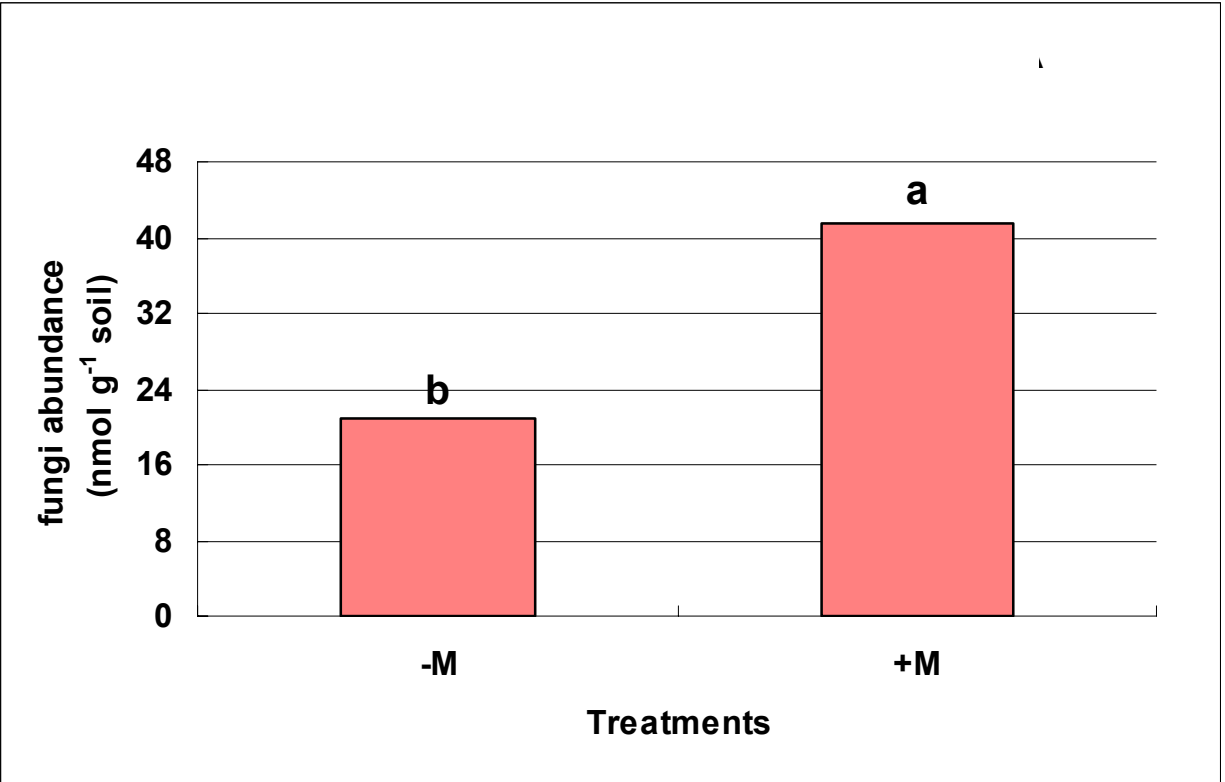
Figure 2.25. General fungal abundance in soil indicated by 18:1 ω 9 and 18:2 ω 6 in NLFA at the end of the 3rd growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).
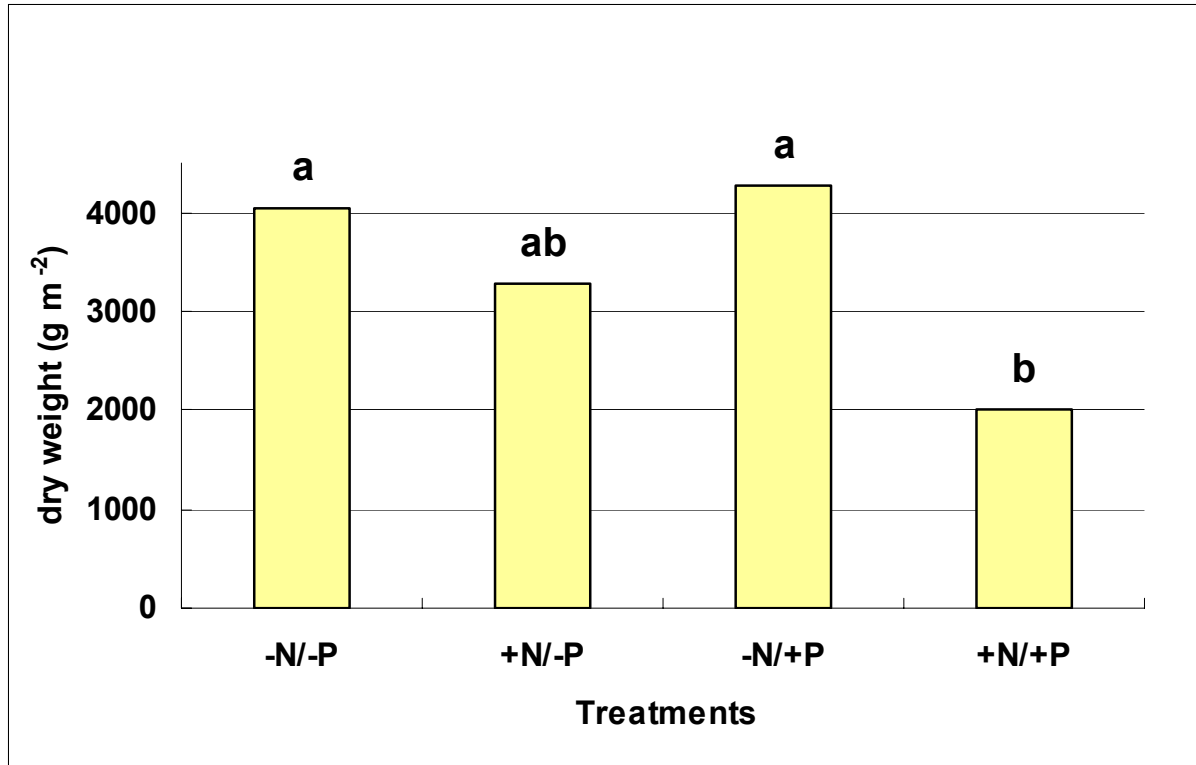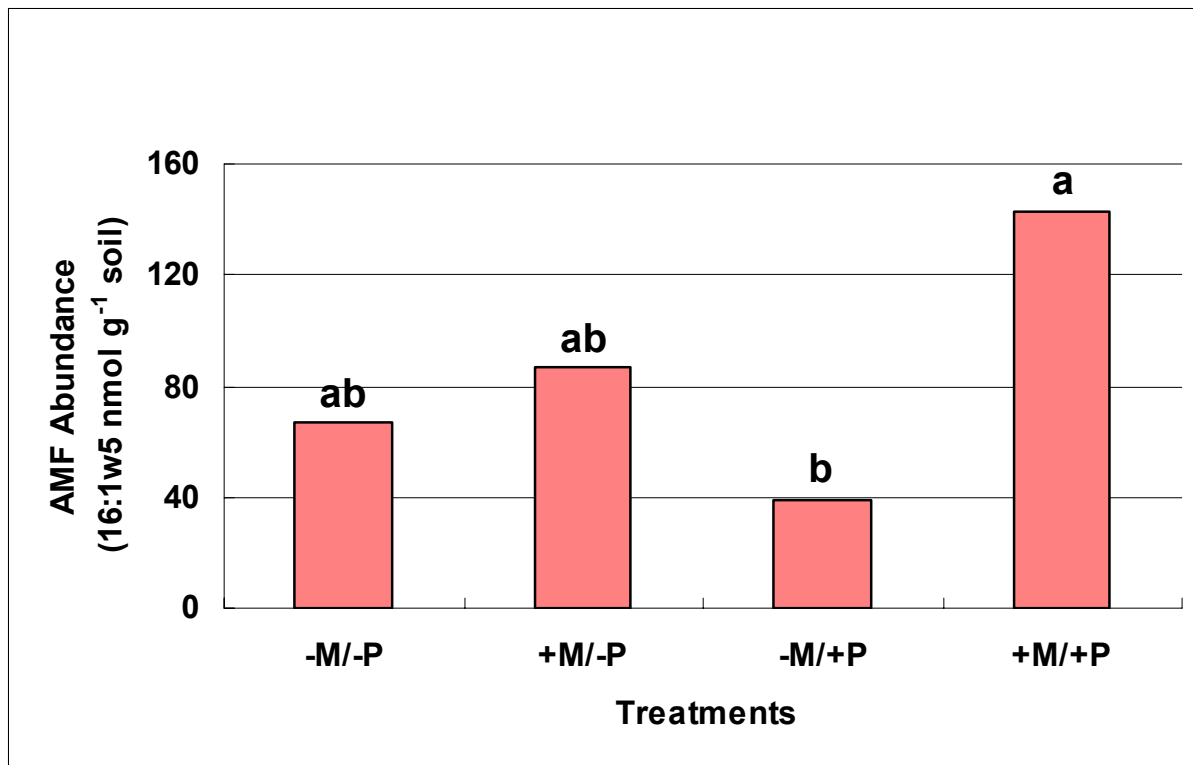
Figure 2.26. AM fungal abundance in soil indicated by 16:1 ω 5 in NLFA at the end of the 3rd growing season. With (+M) or without (-M) mycorrhizae and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).
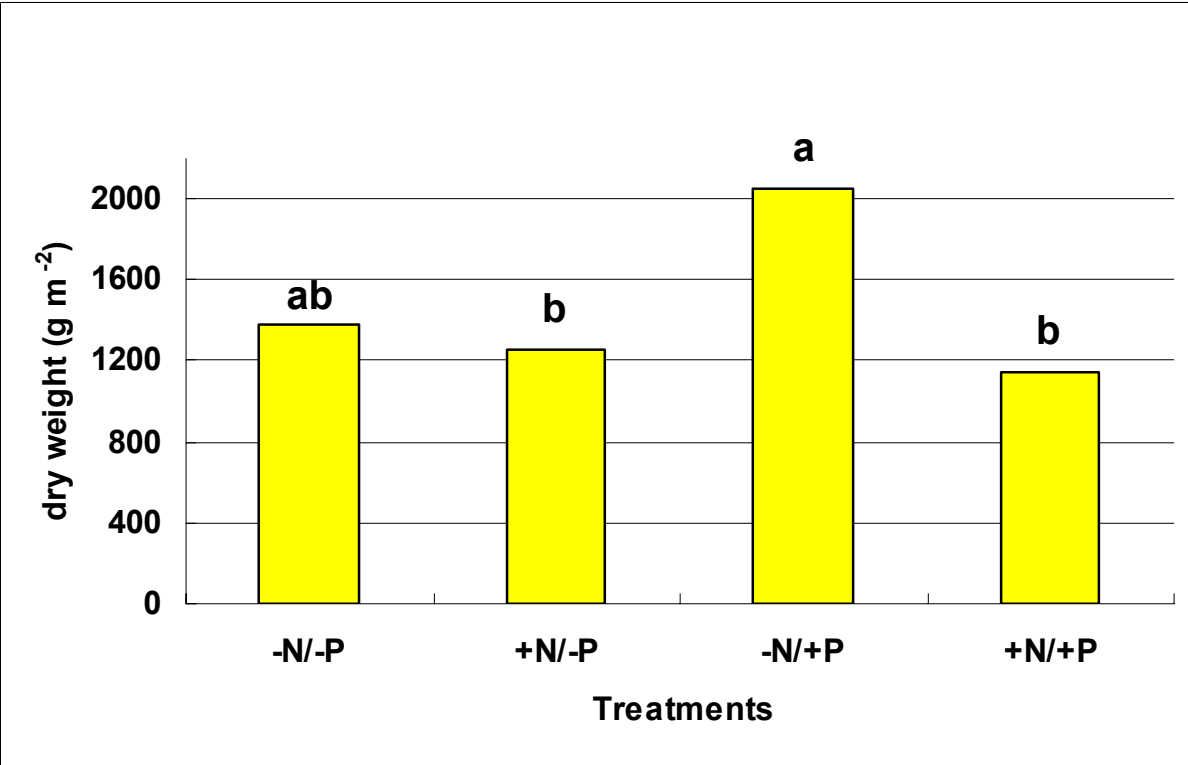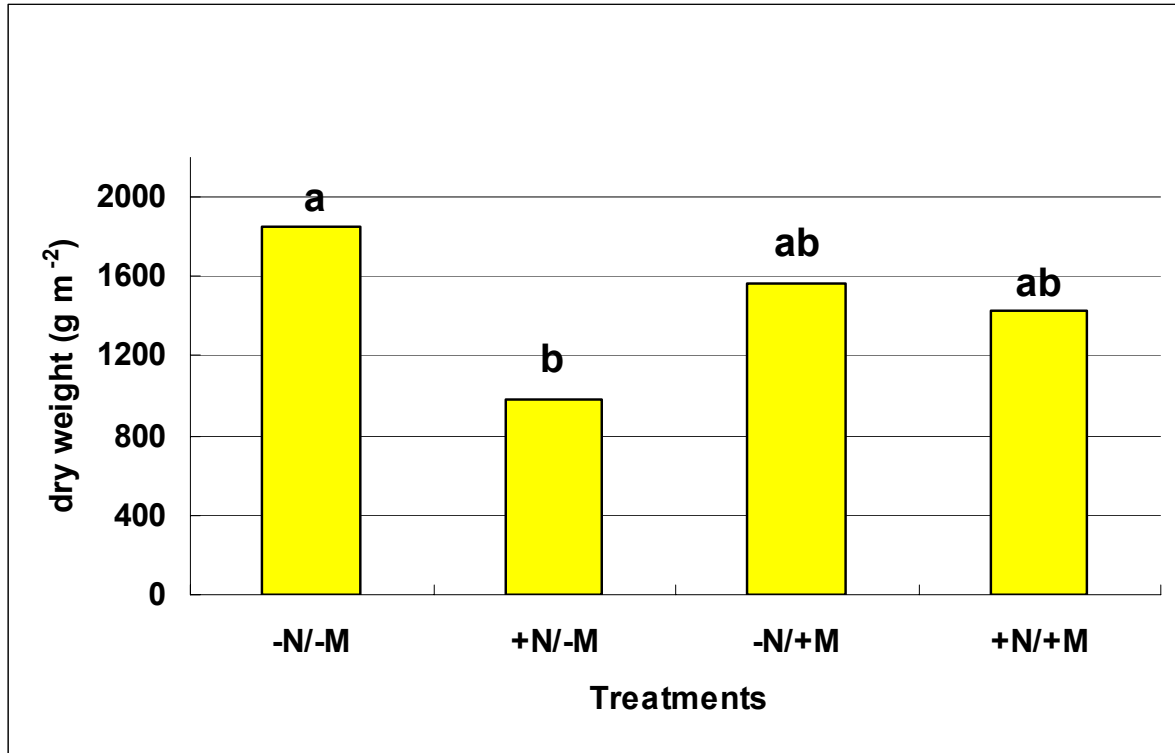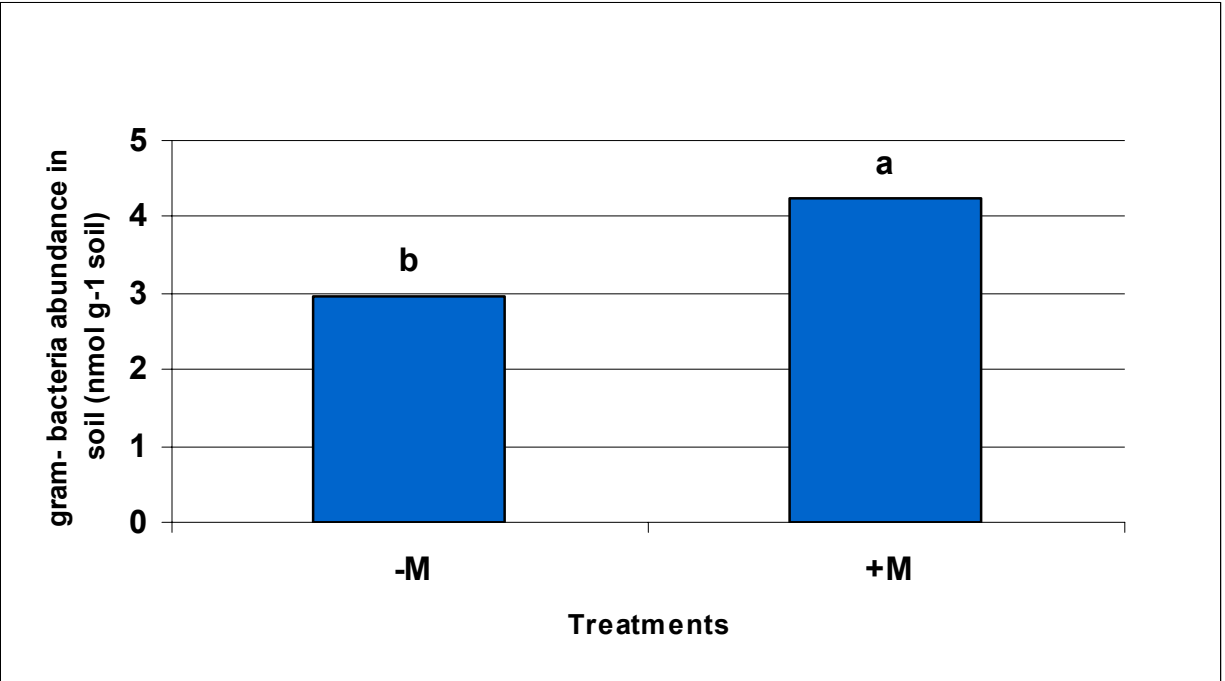
Figure 2.27. Abundance of gram-negative bacteria in soil indicated by PLFA at the end of the 3rd growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).
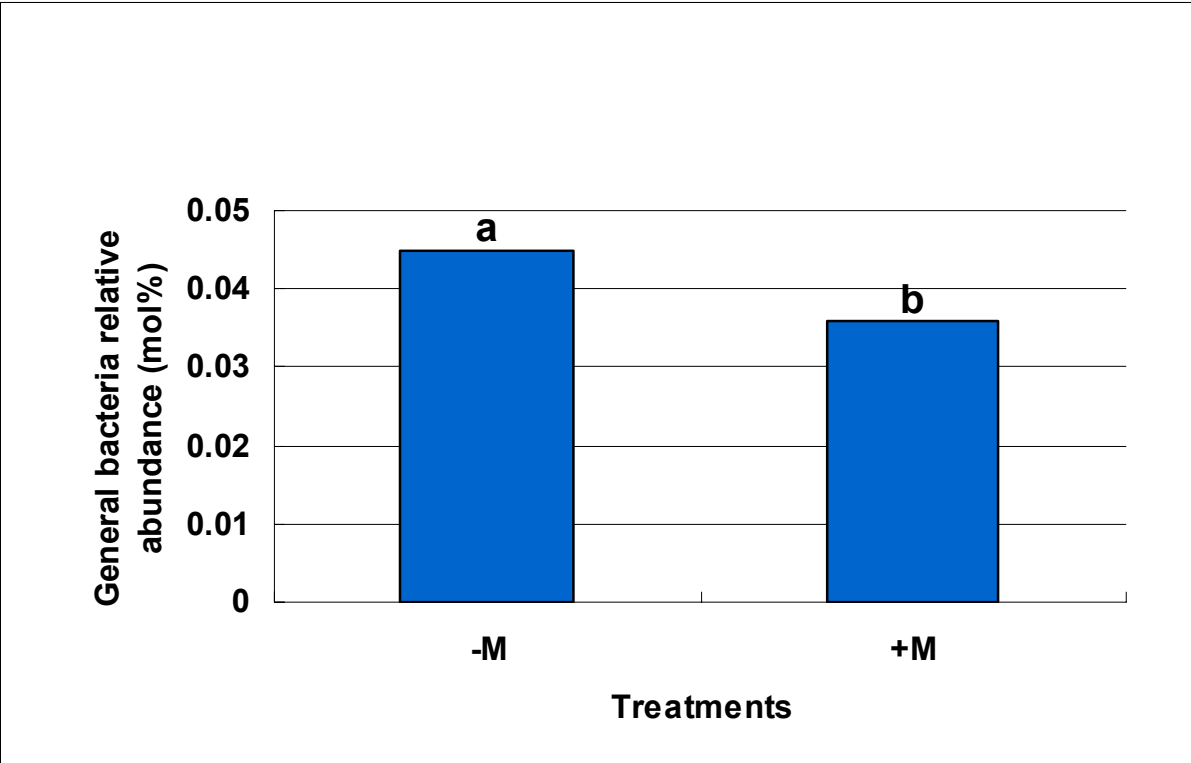
Figure 2.28. Relative abundance of general bacteria in soil indicated by PLFA at the end of the 3ʳᵈ growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).
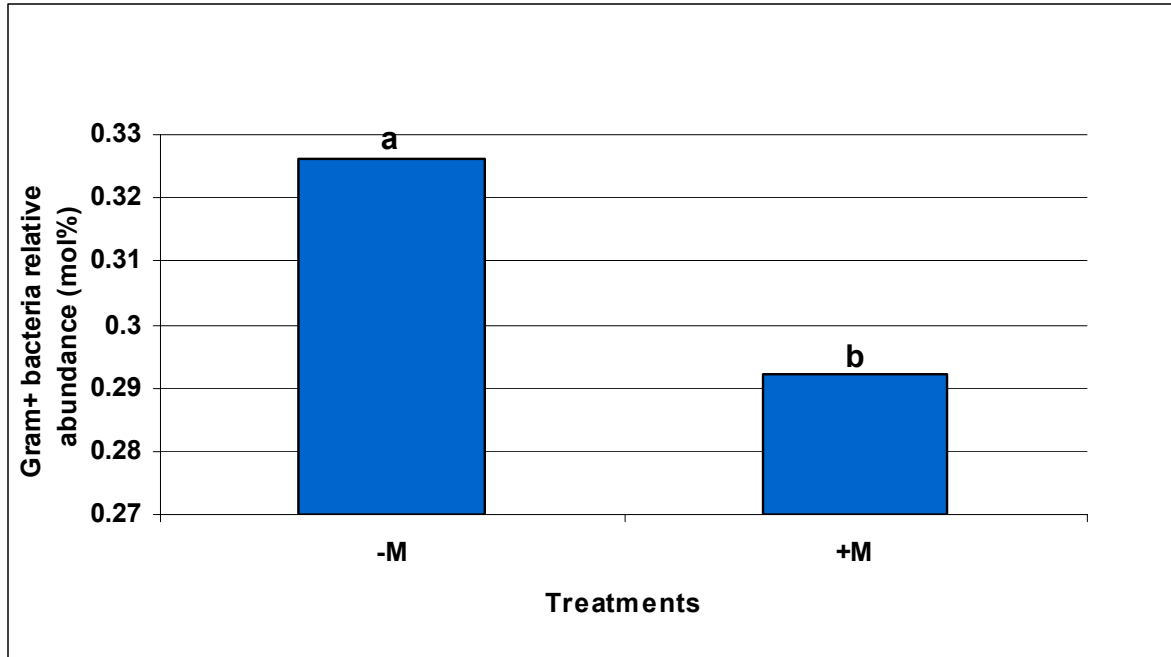
Figure 2.29. Relative abundance of gram-positive bacteria in soil indicated by PLFA at the end of the 3rd growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).
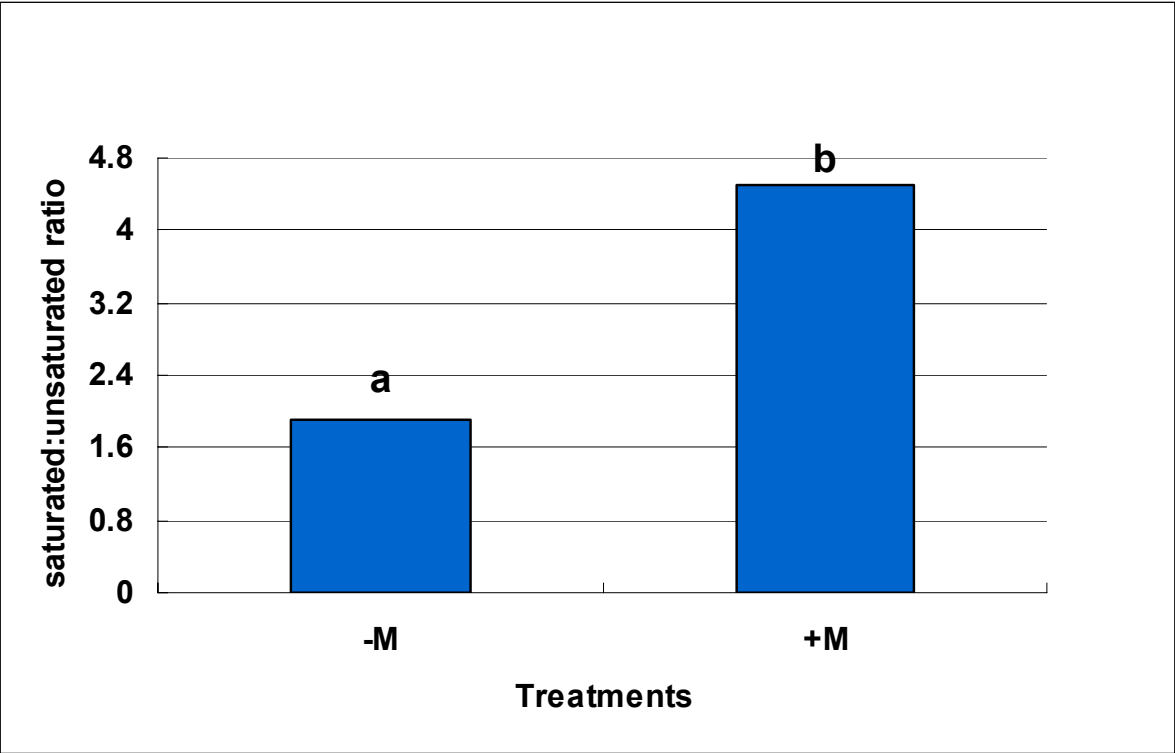
Figure 2.30. Bacteria stress in soil indicated by ration of saturated to unsaturated PLFA at the end of the 3rd growing season. Without (-M) or with (+M) mycorrhizae. Different letters indicate significant difference (P<0.05).
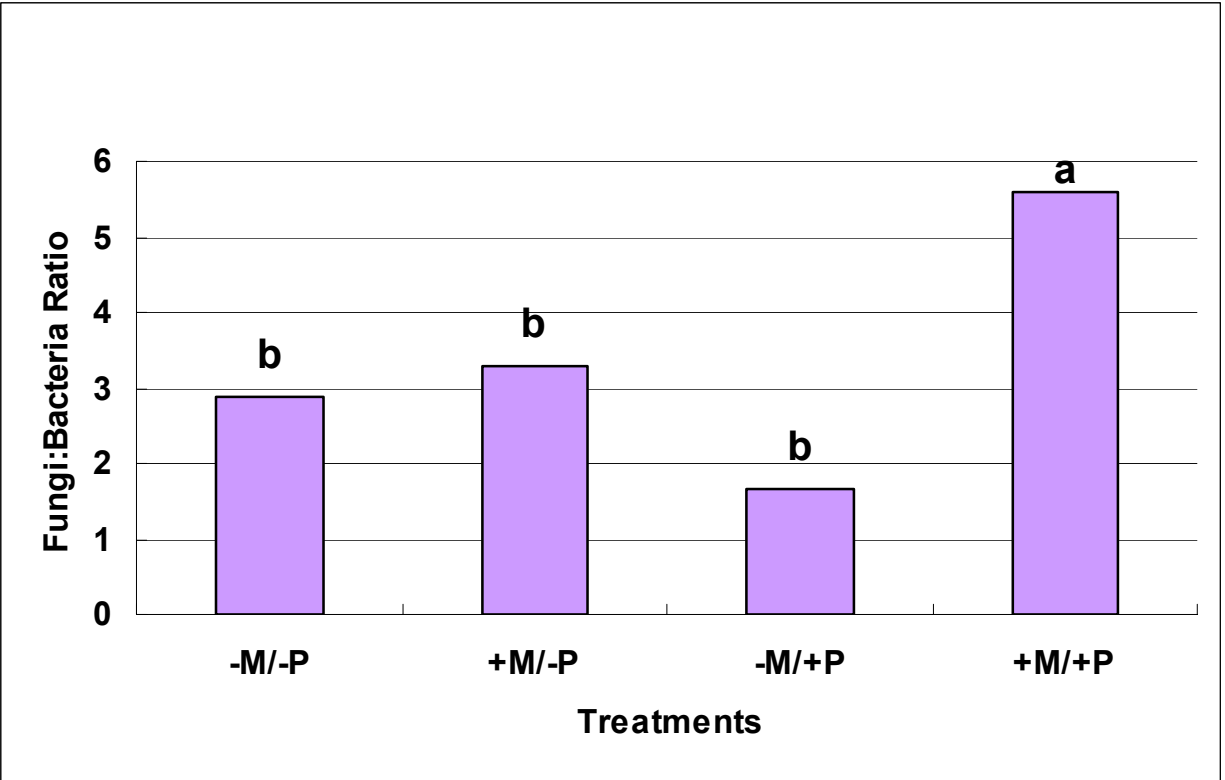
**Figure 2.31. Fungi to bacterial ratio in soil indicated by NLFA for fungi and PLFA for bacteria at the end of the 3rd growing season. With (+M) or without (-M) mycorrhizae and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).**
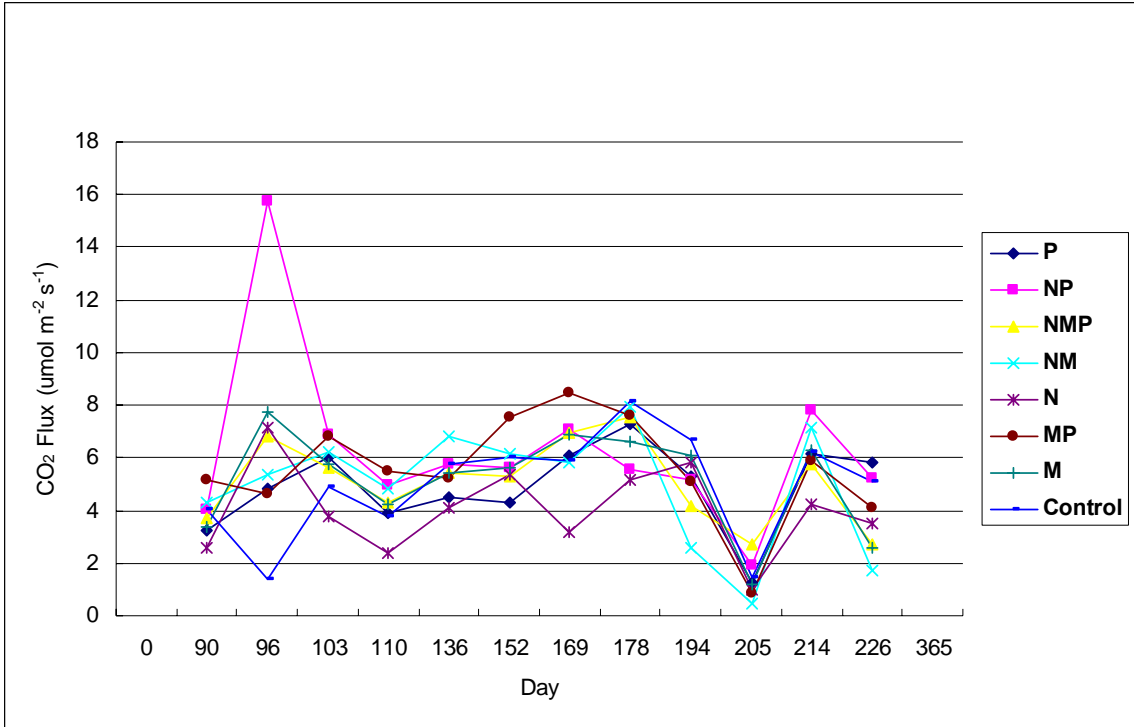
Figure 2.32. CO_2 flux in the soil through the 3rd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P).

2005

2006
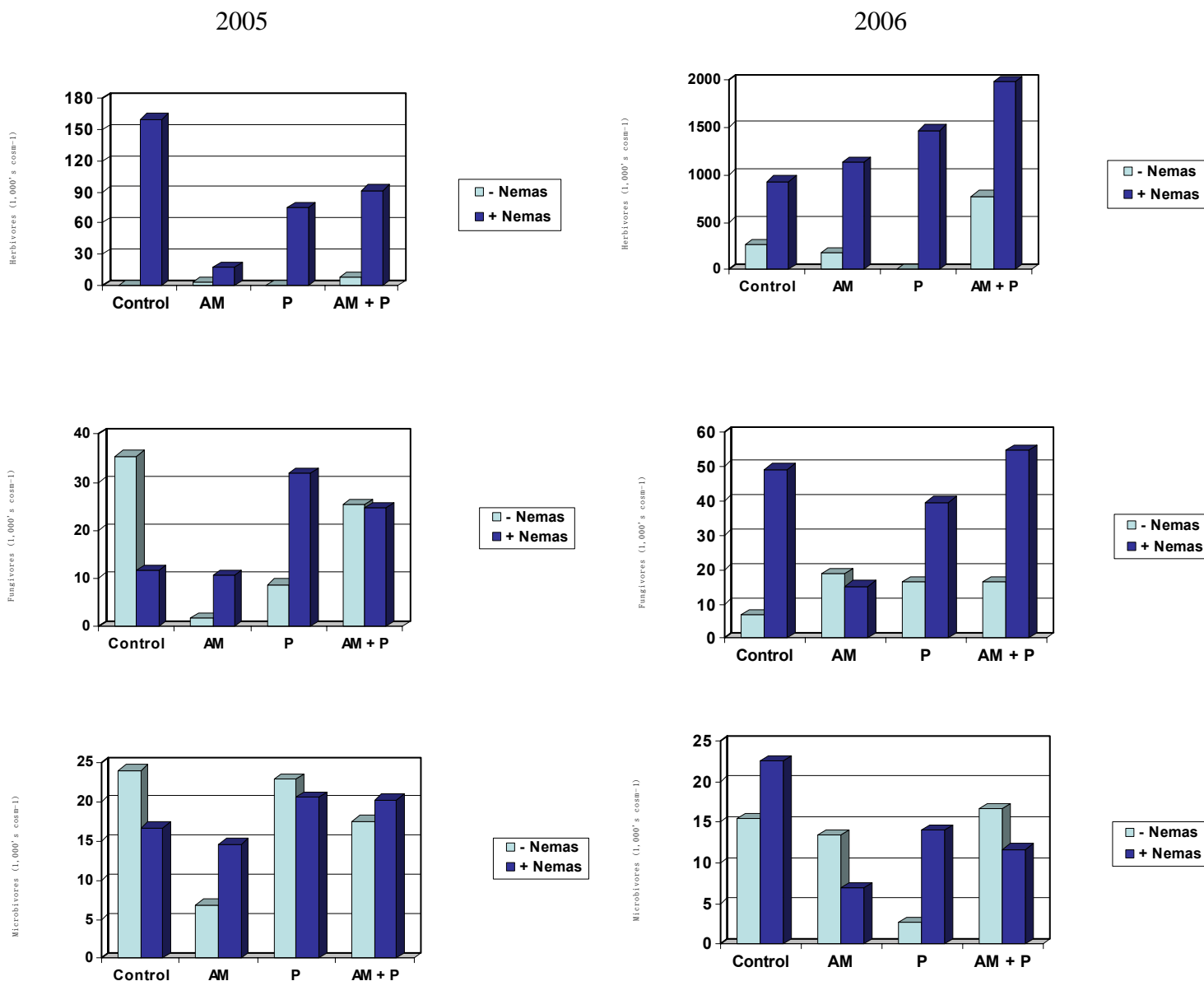
Figure 2.33. Nematodes communities at the end of the 1ˢᵗ growing season. With (+Nemas) or without (-Nemas) nematodes in treatments of control, AM fungal inoculation (AM), P addition (P), and combination of both AM fungal inoculation and P addition (AM+P). Figure 2.34. Nematodes communities at the end of the 2ⁿᵈ growing season. with (+Nemas) or without (-Nemas) nematodes in treatments of control, AM fungal inoculation (AM), P addition (P), and combination of both AM fungal inoculation and P addition (AM+P).
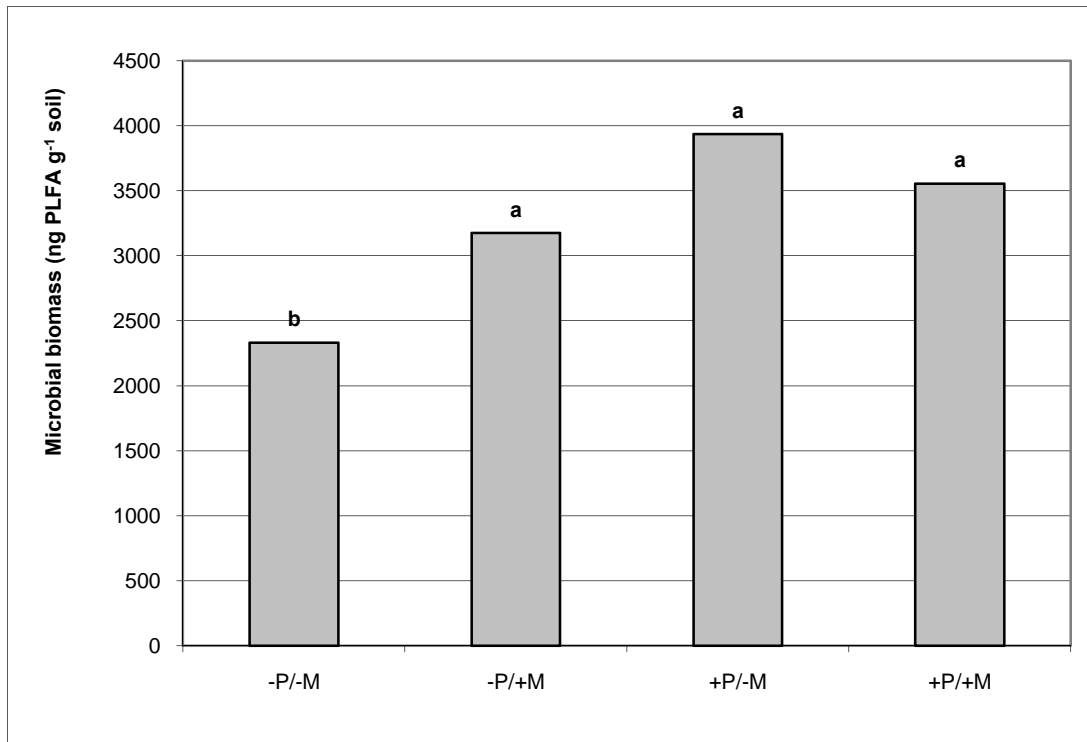
Figure 2.35. Microbial biomass indicated by total PLFA in the soil at the end of the 1st growing season as reported byWatson (2005). With (+M) or without (-M) mycorrhizae and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.1).

Table 2.1. Above-and belowground plant growth after the 2nd growing season for *Andropogon gerardii* Vit. as affected by mycorrhizae, nematodes, and P.

Treatments	Aboveground		Belowground	
	Mean (g m^{-2})	SD	Mean (g m^{-2})	SD
Control	573.6	190.1	2175.4	3439.4
N	411.2	175.8	1200.1	536.5
M	903.1	54.0	2908.0	2715.9
P	752.0	79.2	4523.5	1219.1
NM	765.0	86.0	2551.9	772.5
NP	775.5	75.3	9173.1	4010.7
MP	858.4	174.5	5737.6	2246.4
NMP	816.9	406.6	7856.4	4457.6

N=nematodes, M=mycorrhizae, P=Phosphorus.

SD=standard deviation.

Table 2.2. Above-and belowground plant biomass, roots, rhizomes, and root to shoots ratio of *Andropogon gerardii* Vit. in microcosms at the end of the 3rd growing season.

Treatments	Aboveground		Belowground		Roots		Rhizomes		Root:Shoot	
	Mean (g m^{-2})	SD	Mean (g m^{-2})	SD	Mean(g m^{-2})	SD	Mean (g m^{-2})	SD	Mean	SD
Control	1735.5	91.3	3348.6	1275.2	1389.1	721.6	1959.5	611.3	0.8	0.4
N	904.6	639.9	2698.0	1861.5	1003.3	653.6	1694.7	1212.1	1.2	0.2
M	1575.3	179.7	4727.5	1156.9	1362.4	376.6	3365.2	1266.2	0.9	0.3
P	1504.3	257.8	4176.2	1655.4	2321.1	939.3	1855.1	1020.6	1.2	0.5
NM	1484.5	205.3	3878.3	778.8	1516.4	354.6	2361.9	1045.2	1.0	0.3
NP	1622.2	253.0	1133.2	191.9	948.6	287.4	184.6	107.8	1.0	0.1
MP	1462.1	98.2	4359.6	559.4	1780.0	298.8	2579.6	670.1	0.6	0.2
NMP	1382.0	63.2	2859.7	1030.7	1340.7	243.9	1519.0	894.3	1.5	0.6

N=nematodes, M=mycorrhizae, P=Phosphorus.

SD=standard deviation.

Table 2.3. Analysis of variance of above-and belowground plant biomass at the end of the 2nd growing season.

Effects	Aboveground	Belowground
	P value	
N	0.0851	0.1820
M	<0.0001	0.6209
P	0.0049	<0.0001
N*M	0.8203	0.6332
N*P	0.1242	0.0517
M*P	0.0059	0.5854
N*M*P	0.6193	0.4336

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.4. Analysis of variance of above-and belowground plant biomass, roots, and rhizomes in microcosms at the end of the 3rd growing season.

Effects	Aboveground	Belowground	Roots	Rhizomes	Root:Shoot
			P value		
N	0.0364	0.0014	0.0131	0.0054	0.3448
M	0.7337	0.0133	0.6623	0.0042	0.2315
P	0.5037	0.2163	0.1554	0.0206	0.7446
N*M	0.1868	0.4293	0.0654	0.9225	0.3175
N*P	0.0242	0.0813	0.0493	0.2743	0.0018
M*P	0.0911	0.7013	0.4133	0.9915	0.3440
N*M*P	0.0272	0.3081	0.6109	0.3128	0.0538

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.5. Analysis of variance of AM fungal abundance and root colonization of *Andropogon gerardii* Vit. at the end of the 2nd growing season.

Effects	Colonization	AM Fungi
	P Value	
N	<0.0001	0.2521
M	<0.0001	0.6364
P	0.0010	0.1407
N*M	<0.0001	0.6066
N*P	0.0005	0.1403
M*P	0.0010	0.5711
N*M*P	0.0005	0.6736

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.6. Analysis of variance of soil microbial biomass C at the end of the 2nd growing season as measured by the fumigation-incubation technique.

| Effects | Microbial Biomass C |
	P Value
N	0.3006
M	0.1040
P	0.0146
N*M	0.9654
N*P	0.9086
M*P	0.6990
N*M*P	0.0031

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.7. Analysis of variance of AMF and general fungal biomass as estimated from NLFA derived fatty acid indicators at the end of the 2nd growing season.

Effects	AM Fungi	General Fungi
	P value	
N	0.4400	0.7371
M	0.4190	0.9463
P	0.1243	0.0881
N*M	0.4372	0.8157
N*P	0.1879	0.1682
M*P	0.1544	0.4813
N*M*P	0.5905	0.3251

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.8. Analysis of variance of total NLFA and PLFA and NLFA/PLFA ratio at the end of the 3rd growing season.

Effects	NLFA	PLFA	NLFA/ PLFA
		P value	
N	0.6400	0.9146	0.6333
M	0.0361	0.2990	0.0612
P	0.4171	0.9495	0.3093
N*M	0.1527	0.6284	0.0828
N*P	0.3659	0.2281	0.4028
M*P	0.2236	0.0994	0.0981
N*M*P	0.3608	0.2807	0.3804

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.9. AMF and general fungal abundance as estimated from NLFA derived fatty acid indicators at the end of the 3rd growing season.

Treatments	AM Fungi	General Fungi
	nmol g^{-1} soil	
Control	110.9	35.7
N	23.7	16.4
M	69.2	34.5
P	20.3	6.9
NM	92.0	35.7
NP	56.6	24.9
MP	129.4	43.8
NMP	156.0	52.4

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.10. Analysis of variance of relative general fungal biomass as estimated from NLFA derived fatty acid indicators at the end of the 3rd growing season.

	General Fungi
Effects	***P* value**
N	0.5454
M	0.5060
P	0.9081
N*M	0.9632
N*P	0.2294
M*P	0.5221
N*M*P	0.3971

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.11. Analysis of variance of bacterial biomass as estimated from PLFA derived fatty acid indicators at the end of the 3rd growing season.

Effects	Gram+	Gram-	Actinomycetes	General Bacteria	Total Bacteria
			P value		
N	0.4841	0.5484	0.7450	0.7230	0.9553
M	0.8158	0.0632	0.9878	0.6867	0.2590
P	0.5052	0.3145	0.4301	0.3902	0.7767
N*M	0.8905	0.2660	0.9197	0.2395	0.5129
N*P	0.0982	0.4331	0.1253	0.2953	0.1694
M*P	0.1176	0.2879	0.1161	0.0922	0.1302
N*M*P	0.4017	0.4355	0.4661	0.5514	0.3498

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.12. AMF and general fungi to total bacterial abundance ratios as estimated from NLFA derived fatty acid indicators for fungi and PLFA derived fatty acid indicators for bacteria at the end of the 3rd growing season.

Treatments	AMF:Bacteria	Fungi:Bacteria
Control	12.4	3.9
N	2.4	1.8
M	6.0	3.0
P	2.4	2.4
NM	8.5	3.6
NP	5.3	6.1
MP	13.9	4.6
NMP	18.8	6.6

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 2.13. Analysis of variance of AMF, and general fungi to total bacterial relative abundance ratio as estimated from NLFA derived fatty acid indicators for fungi and PLFA derived fatty acid indicators for bacteria at the end of the 3rd growing season.

Effects	AMF:Bacteria	Fungi:Bacteria
	P Value	
N	0.6544	0.5264
M	0.0052	0.6709
P	0.4498	0.8111
N*M	0.4202	0.8941
N*P	0.0460	0.1990
M*P	0.6811	0.5147
N*M*P	0.0096	0.3962

N=nematodes, M=mycorrhizae, P=Phosphorus.

CHAPTER 3 - THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI AND PLANT ROOTS ON SOIL AGGREGATES

LITERATURE REVIEW

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts of plants and approximately two-thirds of modern plants can form arbuscular mycorrhizal associations (Fitter and Moyersoen, 1996). Read (1991) stated that the mycorrhizal association is the most ubiquitous and abundant form of terrestrial symbiosis and arbuscular mycorrhizae are considered the most common type of mycorrhizae which dominates grasslands, tropical forests, and desert communities. The native warm-season C_4 grass, big bluestem (*Andropogon gerardii* Vit.) is one of the key grasses of tallgrass prairie and has been found to highly colonize with AMF under natural environments (Hetrick and Bloom, 1983; Dhillion and Friese, 1994).

The most important ecosystem function of mycorrhizae is believed to be acquisition of soil mineral nutrients (Dighton, 2003). Arbuscular mycorrhizal fungi are known to benefit plants mainly by improving plant phosphorus (P) uptake (Fitter, 1990; Newsham et al., 1995). However, the effect of AMF on soil C flux may be of equal importance to the effect on plants and ecosystems. The ability of AMF to transport C away from the rhizosphere could potentially result in C sequestration (Treseder and Allen, 2000). The mycorrhizal symbiosis can consume up to 20% of plant C (Jakobsen and Rosendahl, 1990, Watkins et al., 1996). It is estimated that AMF makes up 20% to 30% of the soil microbial biomass in temperate grassland soils (Miller and Kling, 2000; Olsson and Wilhelmsson, 2000). In addition, arbuscular mycorrhizal fungi have been implicated in the formation of macroaggregates (>250 µm) in soil, thus improving soil structure.

Soil structure is an essential and key factor to soil and ecosystem functioning as it controls fluxes of water, gases, and nutrients (Rillig et al., 2002; Lichter et al., 2008). Soil structure is often expressed as the degree of aggregate stability (Six et al., 2004). Maintenance of aggregate stability prevents structural losses when soil is subjected to mechanical stresses or climate influences (Denef et al., 2002). Soil aggregates play a key role in the dynamics of soil carbon due to their effects on energy and nutrient availability for microorganisms (Garcia-Oliva

et al., 2004). The importance of organic matter on soil structure has been well recognized (Chaney and Swift, 1984), and there is a strong relationship between SOM and soil structure stability. Soil aggregation is thought to protect C rich detritus from microbial degradation, an increase in aggregate stability could prove to be important in increased sequestration of C (Miller and Jastrow, 2000; Six et al., 1998, 2000). Aggregates physically protect SOM by forming physical barriers between microbes and enzymes and their substrates and controlling food web interactions and consequently microbial turnover (Elliott and Coleman, 1988; Beare et al., 1994).

The role of AMF in soil function and soil aggregate formation and stabilization has stimulated research in this area (Miller and Jastrow, 1990; Oades and Waters, 1991; Bearden and Petersen, 2000). Mycorrhizal fungi stabilizes aggregates through both the activities of hyphae and secretions of a glycoprotein, "glomalin" (Rillig et al., 2002). Arbuscular mycorrhizal fungi, in particular, influence the formation soil macroaggregates (> 2mm in diameter). Miller and Jastrow suggested that the effect of AMF on soil aggregation consists of three processes: (1) growth of hyphae into soil matrix to form skeletal structure; (2) providing conditions for microaggreate formation; and (3) macroaggregates formation by roots and hyphae emeshing and binding of microaggregates.

Microaggregates (<250 µm) form around persistent organic mater (humic materials) as clay particles incrust and protect organic matter from further decomposition and these microaggregates are stabilized by transient organic matter containing polysaccharides and mucigels (Bearden and Petersen, 2000; Lehmann et al., 2007). Microaggregates are believed to contain recalcitrant SOM that is physically protected from decomposition by mineral soil particles (Cambardella and Elliott, 1994; Six et al., 2000). Macroaggregates that contributes 40% of total SOC (Fransler et al., 2005) bind microaggregates by microbial exudates, fungal hyphae, and labile organic matter (Tisdall and Oades, 1982). Turnover of macroaggregates is important for SOC stabilization. Particulate organic matter within macroaggregates represents a carbon pool with a slow turnover rate due to physical protection from microbial decomposition (Plante and McGill, 2002). Stable macroaggregates protect SOC from degrading resulting in increased SOC content in soil (Holeplass et al., 2004).

Belowground grazing on C flow is not clear. Root-feeding nematodes can increase C allocation to the roots (Ingham and Detling, 1986), and increase transfer of C to the microbial biomass (Denton et al., 1999; Yeates et al., 1998). Since both mycorrhizal fungi and root

herbivores depend on translocated C, competitive interactions between fungi and root herbivores are likely to alter C allocation (Ingham, 1988). Soil fauna (e.g. protozoans, nematodes, and microarthropods) also can graze on fungal hyphae (Bakhtiar et al., 2001; Harris and Boerner, 1990; Ingham, 1988). Johnson et al. (2005) reported disruption of C flow through AM fungal networks due to hyphal grazing by collembolan. Bacterial grazing by soil fauna can affect C allocation (Ingham et al. 1985).

The objectives of this study is to
1) determine the role of arbuscular mycorrhizal fungi and roots on aggregate formation
2) to determine if C and N is enhanced in soil aggregates
3) to determine the interaction of mycorrhizae and nematodes on the symbiosis of *Andropogon gerardii* Vit. and the resultant feedbacks on soil aggregation.

MATERIALS AND METHODS

Experimental design

The experiment was a three-way factorial in a complete randomized block design with four replications. The three factors were mycorrhizae (M), nematodes (N), and phosphorus (P). The treatments were N, M, P, C (control), and their combinations (NM, NP, MP, and NMP). The greenhouse study was was designed to last for 3 years. Ninety-six microcosms were set up and one set of 32 microcosms were sampled at the end of each growing season. The first year results were reported by Watson (2005).

Soil

The soil for the experiment was collected from the Ap horizon of a soil classified as a fine-silty, mixed, superactive, mesic Cumulic Hapludolls soil at Konza Prairie Biological Station, Manhattan KS (N 39 06' 29.5'' W 96 36' 29.2''). The field had been in C_3 crops for at least the last 15 years. The soil contained 1.6 g organic C kg^{-1}, 0.13 g N kg^{-1}, 23 mg Bray-1 P kg^{-1}, 280 mg K kg^{-1}, 165 mg Mg kg^{-1}, and 5.8 mg Na kg^{-1}.

To start the experiment, the soil was steam pasteurized for 2h at 80° C, passed through a sieve with 2 cm diameter openings, thoroughly mixed, put into plastic boxes (52×32×40 cm), and compacted by hand. Each microcosm contained 36 kg dry soil and the bulk density at the end of the first growing season averaged 0.72 g cm^{-3}.

Plants and growing conditions

The dominant C_4 grass of the tallgrass prairie, *Andropogon gerardii* Vit., was the plant seleted for this study. Plants were grown from seeds in trays filled with vermiculite until a height of 10-15 cm. Plants were then transplanted at a density of 140 plants per m^2 into the microcosms. Soil moisture was monitored and adjusted to 0.25 cm^3/cm^3 every other day with ThetaProbe soil moisture sensor (Delta-T Devices, Cambridge, England) inserted to a depth of 20 cm, and connected to a hand held ThetaMeter (Delta-T Devices, Cambridge, England). The aboveground biomass was clipped about 1 cm from the soil surface at the end of the growing season (late fall) each year. Inorganic N was applied in the form of $(NH_4)_2SO_4$ at the rate of 15 mg N kg^{-1} dry soil at the beginning of the growing season of each year.

Treatments: Mycorrhizae (M), Nematodes (N), and Phophorus (P)

Mycorrhizal fungal spores were isolated from tallgrass prairie at Konza Prairie Biological Station, Manhattan, KS. For isolation, a soil slurry was mixed in a blender, then wet sieved, decanted, centrifuged in a 20:40:60% sucrose density gradient (Daniels and Skipper, 1982), and finally suspended into 16 L distilled water. A 500mL spore suspension was added to each microcosm in the top 25 kg soil to obtain a spore density of 30-40 spores per g dry soil. The spores isolated represented 10 members of *Glomus*, and one member each of the following genera: *Acaulospora*, *Entrosphora*, *Gigaspora*, and *Scutellospora*. The most abundant species included *Glomus heterosporum* (143 spores g^{-1} dry soil), *Glomus etunicatum* (111 spores g^{-1} dry soil), *Glomus intraradices* (90 spores g^{-1} dry soil), *Glomus macrocarpum* (56 spores g^{-1} dry soil), and *Glomus aggregatum* (46 spores g^{-1} dry soil).

Nematodes were obtained from soil under native vegetation of tallgrass prairie at Konza Prairie Biological Station, Manhattan, KS. The Christie-Perry technique (Christie and Perry, 1951) was used for the three-step isolation process. First, 1 kg soil was place into a 20 L bucket that contains 4 L water, and the slurry mixed with a household blender after 20-30 min. Second, the soil was passed through a 250 µm sieve and the material retained collected, wrapped in tissue paper, placed on a metal screen in a pot that filled with water, and put in dark over night. Third, water was carefully drained and the sediment which contained the nematodes was collected in a large Erlenmeyer flask and placed in a refrigerator at 4° C. Approximately 50,000 nematodes were added into each microcosm. Most of the nematodes were herbivores; microbivores, omnivores and fungivores contributed a small proportion of the total.

The phosphorus treatment consisted of a single application of superphosphate (0-20-0) at a rate of 90 g P kg^{-1} dry soil. The fertilizer was spread in a single layer at a depth of 10 cm in each microcosm.

Soil sampling

Two cores (5 cm diameter ×15 cm long) were sampled for AM fungi in roots, and soil aggregates and AM fungal abundance in soil were sampled from a square core of 15×15×15 cm. The soil was washed off the roots and a subsample of was placed into diluvials, and frozen for NLFA-PLFA analysis later. The remaining roots were oven dried at 60°C for 3 days and

weighed. A subsample of soil was frozen for NLFA-PLFA analysis. Another subsample of 150 g soil was collected, put into paper bags, and air dried for aggregate analysis.

Soil aggregation, C & N content and isotopic composition

Water-stable aggregates were separated using a modified Yoder wet-sieving apparatus (Yoder, 1936). The apparatus was modified and designed to handle stacked sieves (12.7 cm diameter) and to allow for complete recovery of all particle fractions from each treatment (n=4), >2000 µm, 250 to 2000 µm, 53 to 250 µm, and 20 to 53 µm diameter. Macroaggregates were defined as >2000 µm and 250 to 2000 µm size fractions; microaggregates were defined as 53 to 250 µm and 20 to 53 µm size fractions. Sieves with mesh opening $\cong$ 250 µm diameter were contained on the oscillation cylinders. The amount of soil used was $\cong 0.4$ g of air-dried soil cm^{-2} of sieve area. Two 50 g subsamples of air dried soil from the greenhouse microcosms were placed on the top sieve of each nest (for the first growing season, 100 g of soil subsamples were collected and the results of aggregate weights were divided by a factor of 2 to compare with the second and third growing season). Soils were evenly distributed over the surface of the top of the nested sieves. The nest was set at the lowest point when the oscillation cylinders were filled with distilled water to the level of the soil samples on the top. To repel bubbles created at the sieve surface when adding water to the cylinders, the apparatus was turned on for a few seconds. The soils were then submerged in water for 10 min before the start of the wet-sieving. The apparatus has specifications of oscillation time (10 min), stroke length (4 cm), and a frequency of 30-cycle min^{-1} was held constant.The soil that was retained on each of the four sieves was collected and allowed to settle. The supernatant water of all fractions was drained together with all floating organic matter. The soil was then air-dried, weighed, ground into powder by mortar and pestle, and weighed into tin capsules for C and N contents. Total C and N contents of aggregates and the bulk soil were determined by direct combustion using a Carlo Erba C/N Analyzer (Carlo Erba Instruments, Milano, Italy). Isotopic composition of each aggregate fraction was determined by Europa Scientific ANCA-SL isotope-ratio mass spectrometer (PDZ Europa, Northwich, UK).

PLFA-NLFA

Phospholipid and neutral lipid fatty acids (PLFA and NLFA) analysis were determined for both soil and roots at the end of the growing season with the Balkwill's method (1998). Soil

was subsampled from a big core of $15 \times 15 \times 15$ cm for the 3rd growing season, and sampled with a hand probe (JMC Soil Smaplers, Newton, IA, USA) for the 2nd growing season. Roots were sampled from 2 cores (5 cm diameter $\times$ 15 cm long). Both the soil and roots were freeze-dried and ground into a powder. For each microcosm, 5 g of soil and 30 mg of roots were weighed for this procedure. These samples were analyzed by gas chromatography (HP 6890, Agilent Incorporated, Palo Alto, CA, USA). A 25 m Ultra-2 (J&W Scientific, Agilent Technologies, Palo Alto, CA, USA) column was used with He as the carrier gas at a flow rate of 1 mL min^{-1}. The program of temperature was set up as 80°C as an initial point and increase to 155 ^{o}C at the speed of 20 ^{o}C min^{-1} and then gradually increase to 270 ^{o}C at the rate of 5 ^{o}C min^{-1}. Peaks represent the concentrations of fatty acid components by comparing each individual peak area with the internal standard (19:0) peak area.

The index using in this study and the microbe group they represent are as follows: Linoleic acid (18:2 ω 6), and 18:1 ω 9c represent saprophytic fungi, 16:1 ω 5c in NLFA represent for AMF. The sum of i15:0, i16:0 and i17:0, and a17:0 indicates gram-positive bacteria, while the sum of 16:1 ω 5 in PLFA, cy19:0, and 18:1 ω 7c represent gram-negative bacteria. 10Me16:0, 10Me17:0, and 10Me18:0 indicate actinomycetes (Hogberg et al., 2007; Bradley et al., 2007).

Root colonization

Roots of *Andropogon gerardii* Vit. were removed in the end of the growing season. They were washed free of soil, stained with trypan blue following Koske and Gemma's (1989) method, and measured for percentage of root length colonized by AM fungi following magnified gridline intersect method (Johnson et al., 2003).

Nematode populations

Nematodes were sampled from 0-15 cm depth with a 2 cm diameter hand probe (JMC Soil Smaplers, Newton, IA, USA) and identified to genus and assighned to functional groups following Yeates et al. (1993) including herbivores, fungivores, and microbivores.

Statistical analysis

Data across the three years were analyzed as a factorial experiment by Proc Mixed (SAS Institute Inc., 2002, Cary, NC, USA). Data for individual years was analyzed by SAS Proc GLM

(SAS Institute Inc., 2001). Differences were considered significant at p≤0.05 unless otherwise stated.

RESULTS

Aggregate Distribution

The average recovery of the aggregate distribution was 90.3% indicating that the <20 µm fraction as a small proportion of the soil aggregate sizes. After the second growing season, significant treatment effects were evident in the aggregate sizes <2000 µm (Table 3.1-3.2). For the smaller macroaggregate fraction (250-2000 µm) there was a significant three-way interaction among N, M, and P (Fig. 3.1). Interestingly, the N treatment enhanced this fraction in the presence of M or P individually, but not when combined with both M and P. This may be explained by more root exudes in the N treatments to facilitate C turnover and aggregate formation by the microbial community. When M was added to the soil where P was not a limiting nutrient for plant growth, greater plant biomass and AM fungi tended to compensate for the negative impact of nematodes on soil macroaggregate formation (Fig. 3.1). For the microaggregates (53-250 µm), there was a significant two-way interaction between N and M (Table 3.2). Nematodes reduced this fraction in the absence of M (Fig. 3.2). For the smaller microaggregate fraction (20-53 µm), nematodes also decreased the microaggregate fraction (Fig. 3.3). This negative effect of nematodes may due to more large aggregates (Fig. 3.4).

After the third growing season significant differences in aggregate distribution was confined to macroaggregates >2000 µm in diameter (Table 3.3-3.4). Mycorrhizal fungi increased macroaggregates >2000 µm. The M treatment increased the mass of macroaggregates more than three times relative to the other treatments. The average recovery percentage of this year was 95.6%.

By examining the changes in aggregate distribution over the course of the experiment, we observed significant changes in distribution (Fig.3.4). There were more macroaggregates present in the soil across all 3 years. In the first two years, there were small changes in aggregate distribution (Tables 3.1, 3.5). After the 3rd growing season, macroaggregates >2000 µm had increased significantly compared to the first two growing seasons. This time line suggests time is needed for the formation of macroaggregates >2000 µm (Fig. 3.5), supporting the hypothesis of the role of mycorrhizae in development of aggregates.

Total C and Nitrogen

Total C and nitrogen were measured on each aggregate fraction. After the 2nd growing season, nematodes significantly increased the C and nitrogen content of the microaggregates (20-53 µm and 53-250µm) (Table 3.6-3.7, Fig. 3.6-3.9). However, there was no significant effect of any treatment on the total C and N of the macroaggregate fraction.

After the 3rd growing season, total C and nitrogen in different aggregate fractions resulted in a three-way significant interaction among N, M, and P (Table 3.8-3.9) of both total C and nitrogen in the smaller macroaggregates (250-2000 µm).The combination of N and M resulted in a significantly higher carbon and nitrogen content than N and M alone. The combination of all factors of N, M, and P was not significantly different than either the control or the single effects. Other two-factor combinations (NP and MP) were not significant (Fig.3.10 and Fig. 3.11). For the microaggregate fraction (53-250 µm), there was a two-way significant interaction between N and M (Table 3.8-3.9) as well as a significant single effect of P (Table 3.8-3.9). Mycorrhizae enhanced the C and nitrogen content of this aggregate fraction when nematodes were absent in the soil, while P increased the C and nitrogen content regardless whether N or M were present in the soil (Fig. 3.12 and Fig. 3.13).

[13]C analysis showed a two-way significant interaction between M and P for the largest macroaggregate fraction (>2000 µm) (Table 3.10). Phosphorus had a significantly less negative value when mycorrhizal fungi were absent indicating more C_4 derived C from big bluestem in the macroaggregates. The mycorrhizal treatment induced a more negative value when P was added to the soil (Fig. 3.14), again suggesting greater C flow from the plant to the aggregates. There was a significant effect of P addition on the smaller macroaggregate fraction (250-2000 µm) (Table 3.10), where P induced a less negative value (Fig. 3.15) probably as a result of more plant production and C input into the soil.

AMF and Root Colonization

After the 2nd growing seaon, there was a three-way significant interaction among N, M, and P in root colonization percent (Table 3.11), where the combination of NM, MP, and NMP showed significantly greater root colonization than the control (Fig. 3.16). Mycorrhizae alone resulted in significantly more root colonization than the combination with the other two factors of N and P. Mycorrhizal fungi combined with P resulted in greater colonization than when nematodes were present (Fig. 3.16). It appears that both N and P had a negative effect on AM

fungal root colonization. However, throughout the three growing seasons, arbuscular mycorrhizal fungal abundance in the roots as estimated by 16:1 ω 5c NLFA showed no significant difference among treatments (Table 3.12).

After the 3rd growing season, root colonization by AM fungi was not significant between treatments due to contamination of nonmycorrhizal treatments (Table 3.12, Fig. 3.17). Arbuscular mycorrhizal fungi (AMF) abundance in soil at the end of the 3rd growing season was estimated with the NLFA derived 16:1 ω 5c (Table 3.13). There was a significant two-way interaction between M and P, where the combination of M and P resulted in greater AMF than with P alone (Fig. 3.18) indicating a positive response of P amended soil for AMF abundance. The relative abundance of AM fungi in the soil matrix was not significantly different among treatments.

Since there were varying levels of AM fungal abundance in the soil due to contamination, we examined the relationship between AM fungal abundance in the soil and the mass of macroaggregates. There was a significant positive linear relationship between AM fungal abundance and macroaggregates >2000µm with an r value of 0.67 (Fig. 3.19). This result supports the role of mycorrhizal fungi in macroaggregate formation.

DISCUSSION

The mechanisms involved in aggregate stabilization are reported to be based on the enmeshment of soil particles by fungal hyphae, particularly mycorrhizal hyphae, roots, and exudation of polysaccharides (Bearden and Peterson, 2000). In our study, P increased root biomass but did not increase macroaggregates (>2000 µm). Only the mycorrhizal treatment significantly increased levels of largest macroaggregates (>2000 µm) after three years. This result was consistent with Jastrow et al. (1998), who demonstrated that AM fungal hyphae provided the most important direct effect on soil aggregation of all soil factors. Microcosms with AM fungi increased macroaggregates (>2000 µm) 3.5 times more than the nonmycorrhizal treatments (Fig. 3.5). The previous 2 years, there were no significant differences in macroaggregates between treatments indicating there was a minimal time required for macroaggregates to form (Fig. 3.5).

The lack of correlation with root biomass and the significant correlation with mycorrhizal fungal abundance in the soil suggest that mycorrhizal fungi significantly contribute to macroaggregate formation. Plant roots are reported to be important in binding agents at the scale of macroaggregates (Thomas et al., 1993; Six et al., 2004). Six et al. (2004) reported that roots affected aggregation through penetration, altered soil water regime, root exudation, dead root decomposition, and root entanglement. Bearden and Petersen (2000) concluded in their study that the formation of aggregates between 1 and 2mm was associated with hyphal length and not with root growth, roots and hyphae were involved in the formation of aggregates >2000 µm. Our results, however, support the role of mycorrhizae on macroaggregate (>2000 µm) formation, where AM fungi abundance showed a positive linear relationship with macroaggregate mass. The effect of roots on this fraction was not apparent. The soil and plant used in Bearden and Peterson's study was quite different from ours. They used semi-arid Indian vertisol with *Sorghum bicolor* (L.) as the hosted plant species. In our study, there was a significant interaction between mycorrhizae and P in the smaller macroaggregate fraction (250-2000 µm), where P significantly increased this fraction of aggregates, when AM fungi were present in the soil. This supports our earlier discussion in chapter II, where P significantly enhanced plant root biomass.

Arbuscular mycorrhizal fungi were found to improve soil aggregation through both physical and chemical bindings (Jastrow and Miller, 1991; Oades and Waters, 1991). Miller and

Jastrow (1992) proposed that hyphae of AM fungi may affect soil aggregation directly by providing the skeletal structure which can physically hold soil particles together, by entangling hyphae as a source of aggregate binding agents, and by enmeshing soil microaggregates into macroaggregates. It is also possible that the increase of stable aggregates resulting from mycorrhizae can be attributed to the proliferation of fungal hyphae in rhizosphere soil (Roldan et al., 1994; Jeffries and Barea, 2001).

Suppression of AM fungi was reported to result in a significant decrease in extradical hyphal networks which lead to a breakdown of water stable macroaggregates (Wilson, 2003). Root colonization was found not to be correlated to aggregate distribution in our study. Root colonization was highly related to AM fungal abundance in soil and in roots in year 1 (Watson, 2005). For the second and third year, there was no obvious relationship between root colonization and AM fungal abundance. Moreover, Rice et al. (2004) observed a positive relationship between soil C and hyphal networks. Thus, it is the fungal networks in the soil, not in the roots, that are involved in the aggregate formation.

The ^{13}C content of the macroaggregates showed an interaction between mycorrhizae and P, where mycorrhizal treatments induced more soil derived C into the macroaggregates than the nonmycorrhizal treatments when P was added to the soil. Our hypothesis was that mycorrhizae would induce more plant-derived C into the macroaggregates. The results do not support the hypothesis of direct transport of plant C into aggregates. However, the effect of P produced greater growth (Chapter 2) thus produced more photosynthate for plant C input into the soil. The ^{13}C results confirmed that P addition induced more plant derived C into the aggregates than the non-P amended microcosms (Fig. 3.15). This is consistent with more root biomass resulting in greater mass of macroaggregates (250-2000 µm).

The effect of general fungi and bacteria on macroaggregate formation may be excluded in our study. General bacteria in the soil did not change among treatments, and general fungal abundance indicated by NLFA was not related to macroaggregates.

Based on our results, mycorrhizal fungi had a positive effect on soil macroaggregate formation. The contaminated controls in the 3rd growing season had a much higher level of colonization and AMF abundance than the M treatment that may have contributed to the higher level of macroaggregate formation (Fig. 3.17, Table 3.13). In spite of this, the M treatment had statistically higher levels of aggregation than the non-M treatments (Fig. 3.5) One of the most

important results in our study was the linear relationship between AM fungal abundance in soil and mass of soil largest macroaggregate >2000 µm. Phosphorus played a significant role in plant root development and contributed to soil smaller sized macroaggregates (2000-250 µm).

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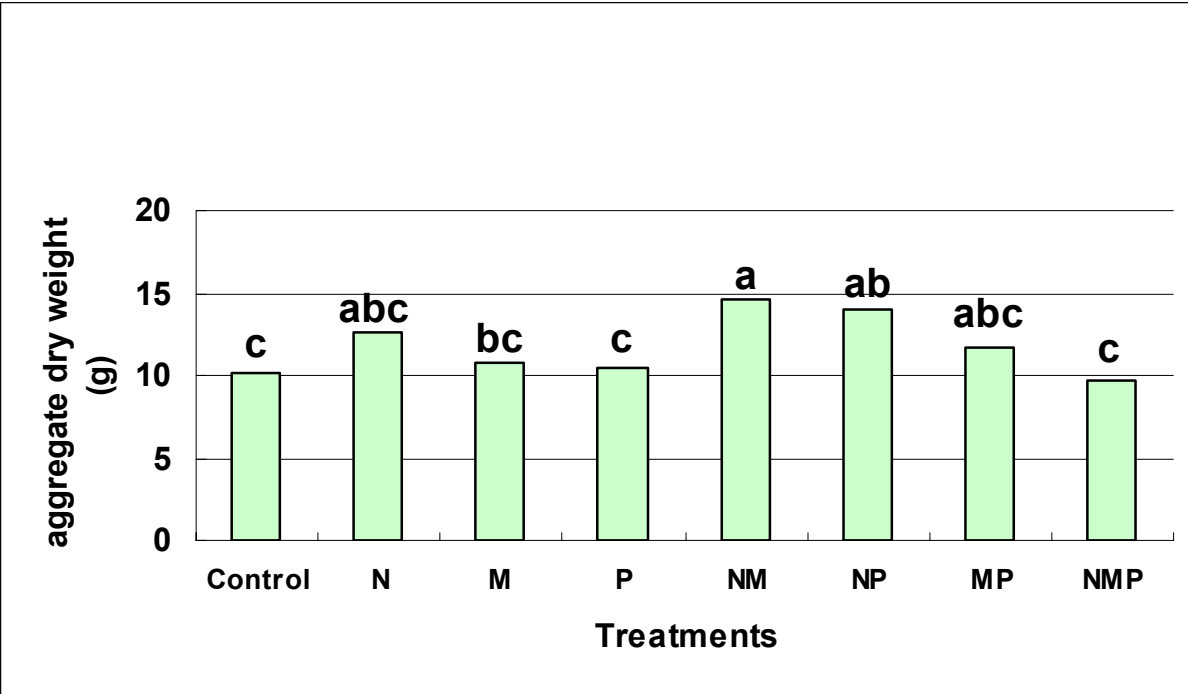
Figure 3.1. Soil macroaggregates (2000-250 μm) at the end of the 2nd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05).
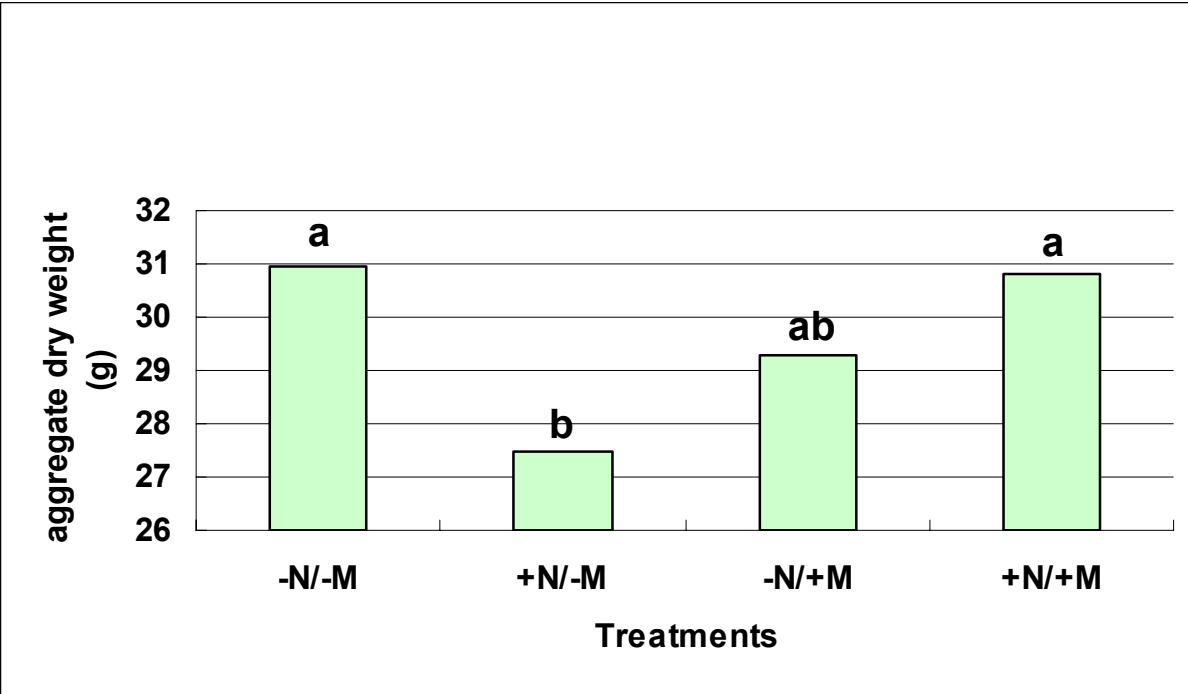
Figure 3.2. Soil microaggregates (250-53μm) at the end of the 2nd growing season. With (+N) or without (-N) nematodes and with (+M) or without (-M) mycorrhizae . Different letters indicate significant difference (P<0.05).
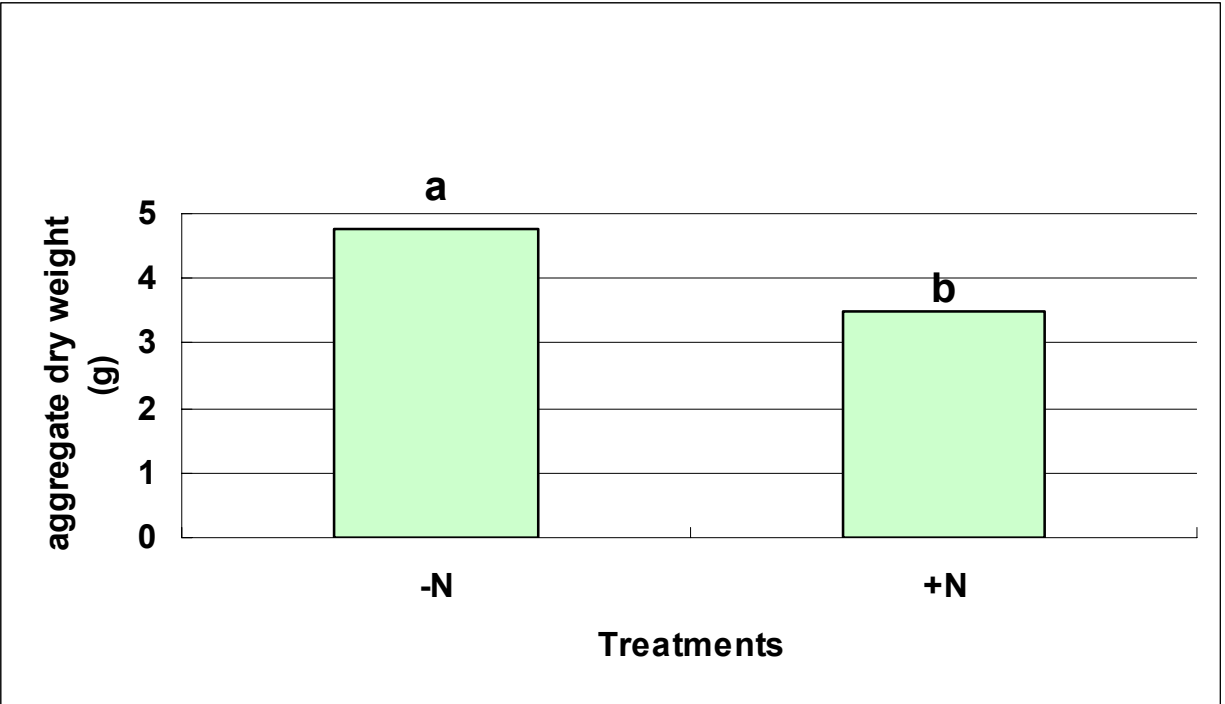
Figure 3.3. Soil microaggregates (53-20μm) at the end of the 2nd growing season. Without (-N) or with (+N) nematodes. Different letters indicate significant difference (P<0.05).
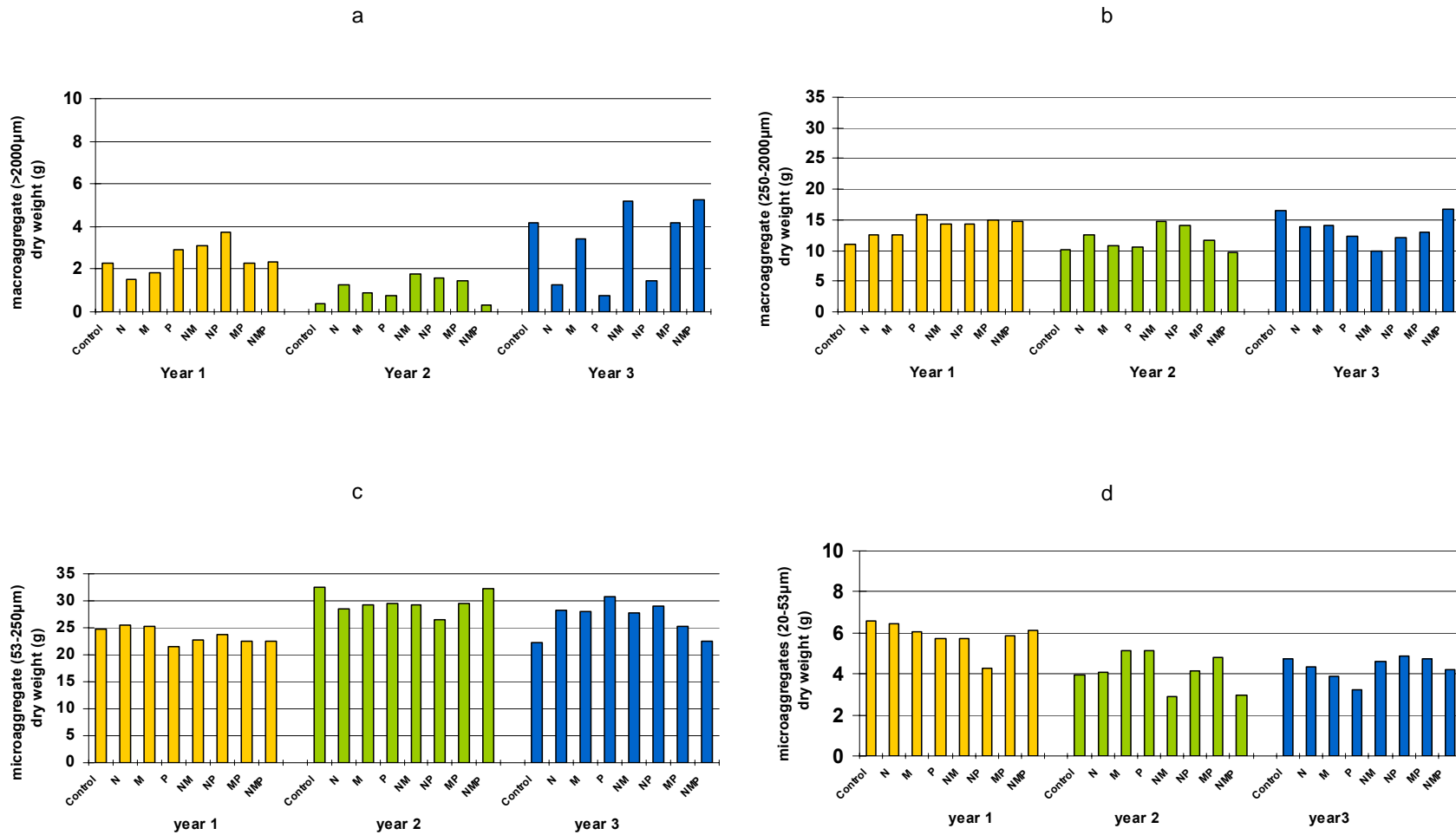
a

b

c

d

Figure 3.4. Soil aggregate distribution at the end of the growing season from the 1st, 2nd, and the 3rd year. a) macroaggregates>2000 µm; b) macroaggregates 2000-250 µm; c) microaggregates 250-53 µm; d) microaggregates 53-20 µm. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Notice that the y axis varies with aggregate size.
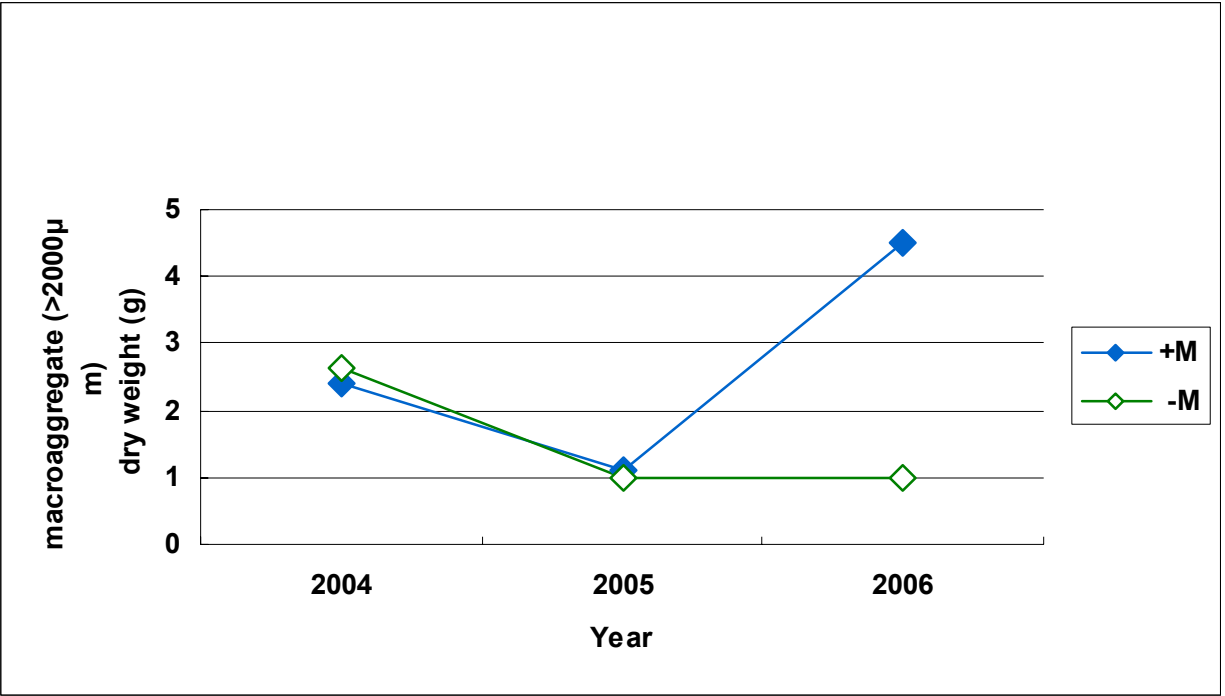
Figure 3.5. Macroaggregates (>2000 ㎛) in soil at the end of the growing season from the 1st to the 3rd year. With (+M) or without (-M) mycorrrhizae. At year 3 the difference between +M and –M was significant at P level of 0.05.
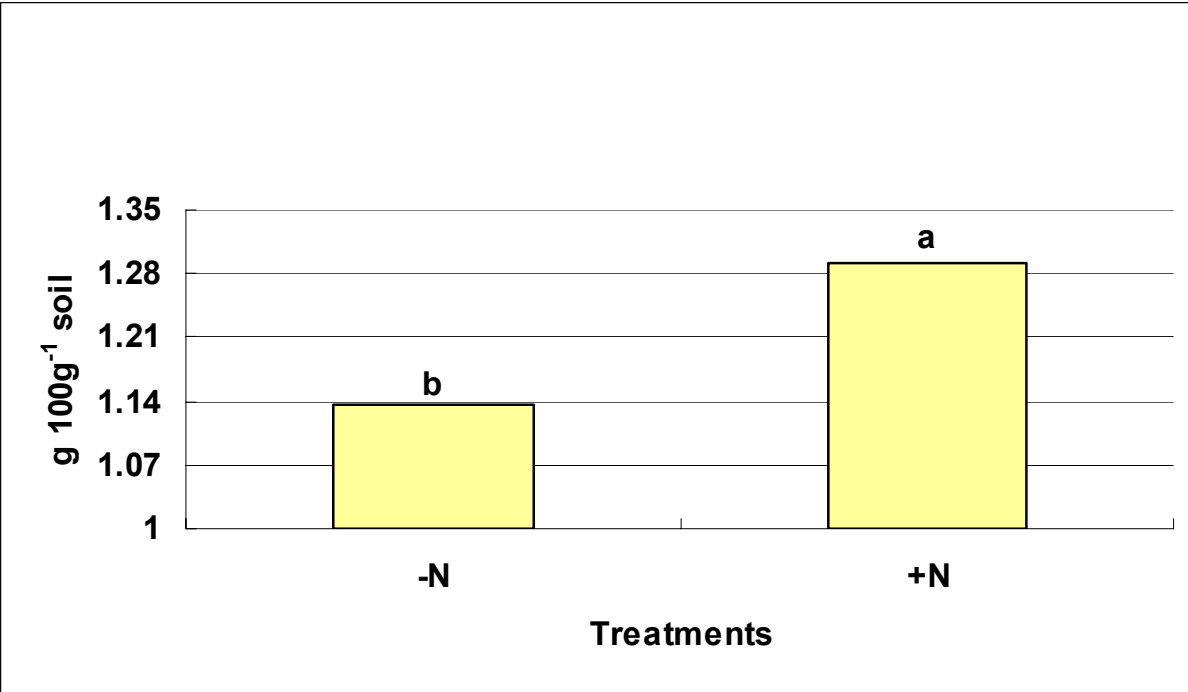
Figure 3.6. Total C in soil microaggregates (250-53 μm) at the end of the 2nd growing season. Without (-N) or with (+N) nematodes. Different letters indicate significant difference (P<0.05).
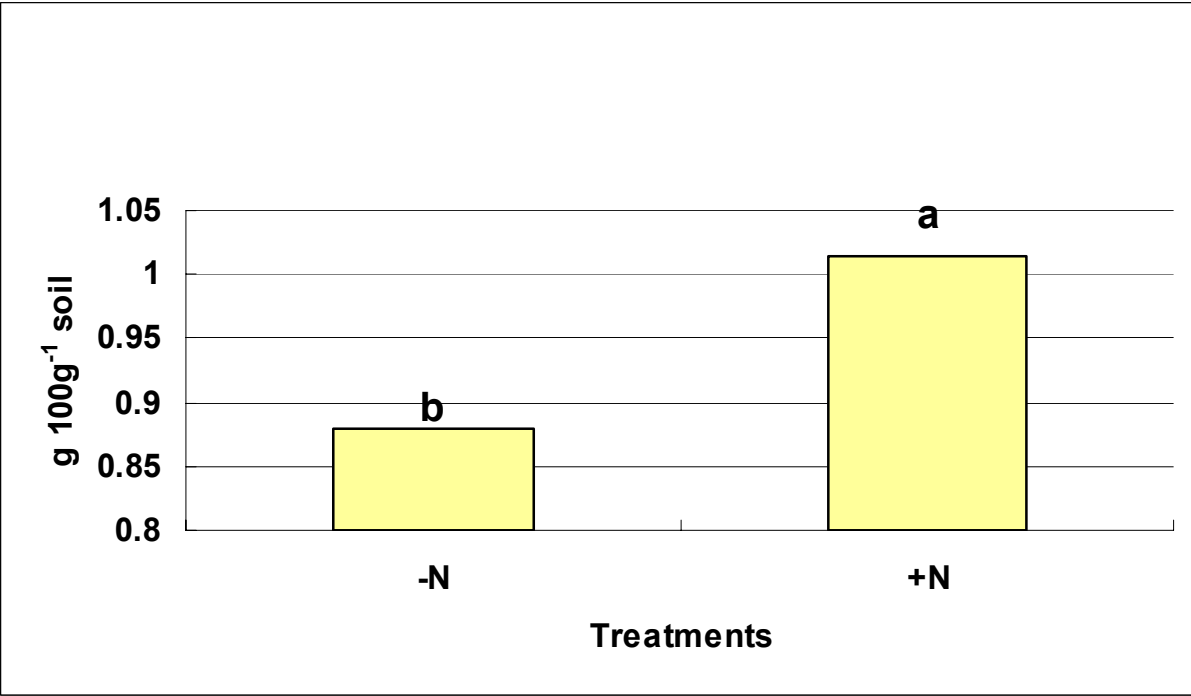
Figure 3.7. Total C in soil microaggregates (53-20 μm) at the end of the 2nd growing season. Without (-N) or with (+N) nematodes. Different letters indicate significant difference (P<0.05).
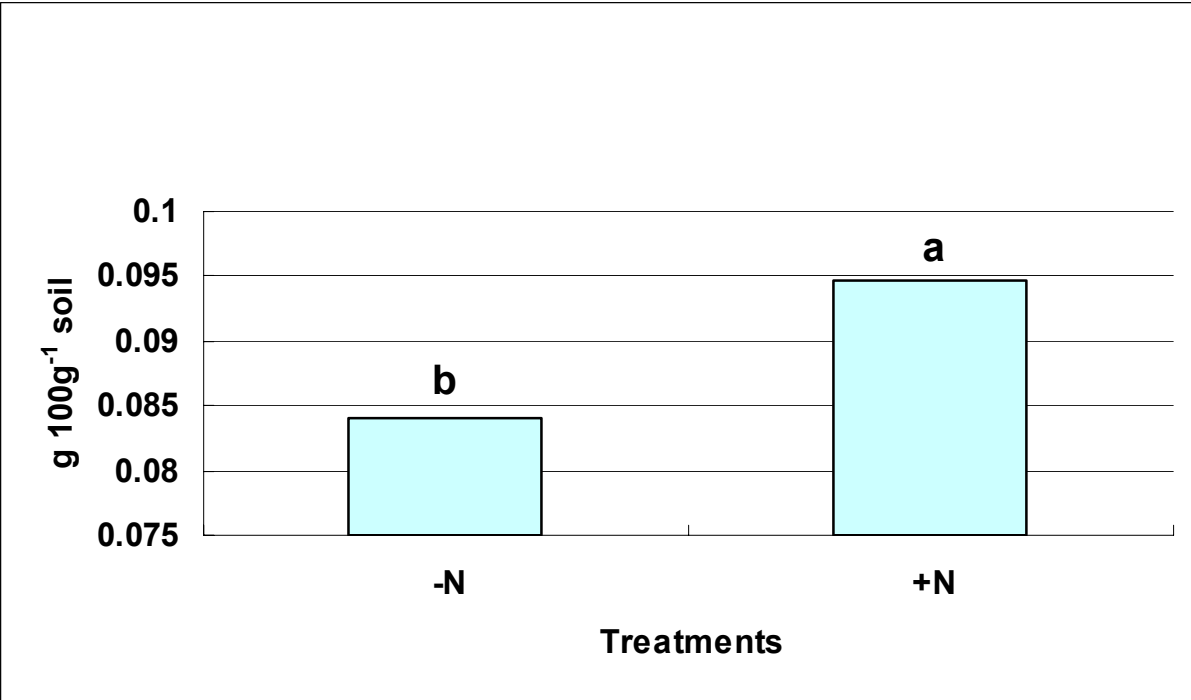
Figure 3.8. Total N in soil microaggregates (250-53 μm) at the end of the 2nd growing season. Without (-N) or with (+N) nematodes. Different letters indicate significant difference (P<0.05).
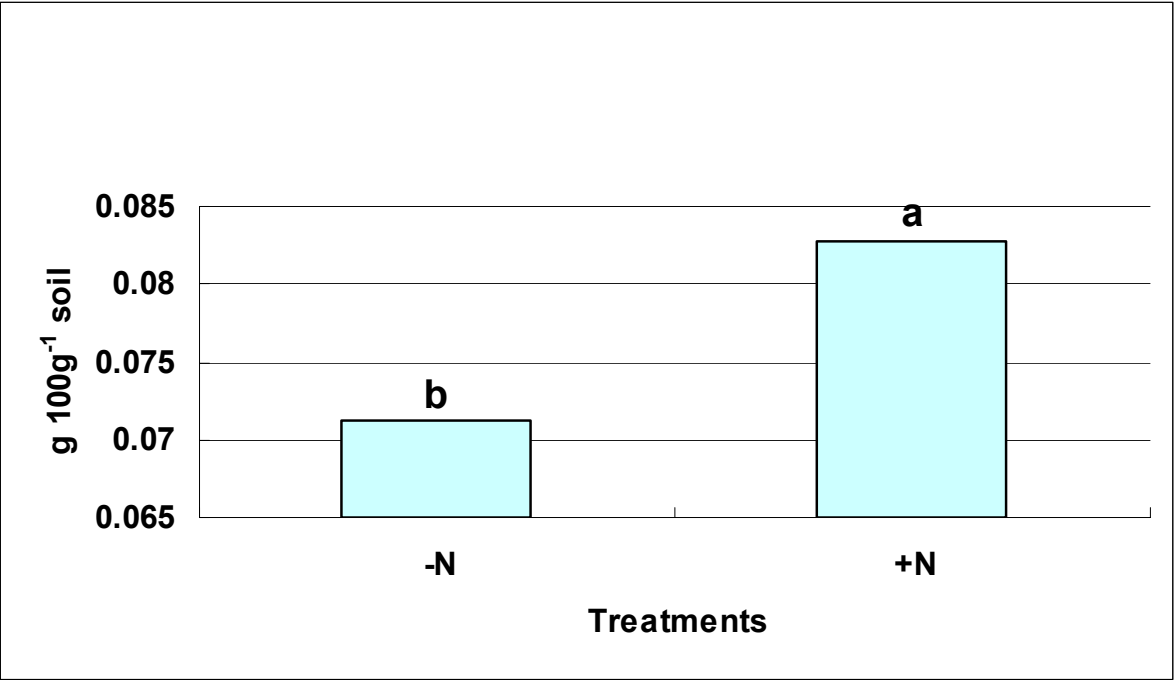
Figure 3.9. Total N in soil microaggregates (53-20 μm) at the end of the 2nd growing season. Without (-N) or with (+N) nematodes. Different letters indicate significant difference (P<0.05).
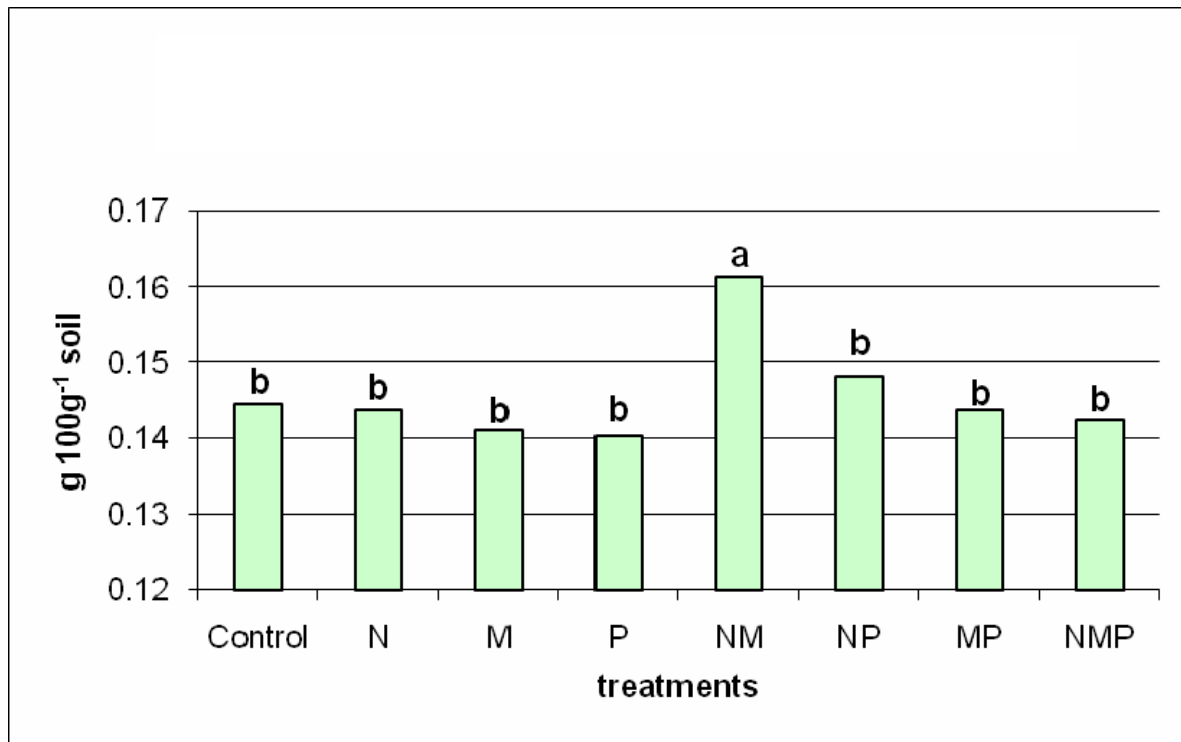
Figure 3.10. Total N in soil macroaggregates (2000-250 µm) at the end of the 3rd growing season. With (+N) or without (-N) nematodes, with (+M) or without (-M) mycorrhizae, with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).

Figure 3.11. Total C in soil macroaggregates (2000-250 μm) at the end of the 3rd growing season. With (+N) or without (-N) nematodes, with (+M) or without (-M) mycorrhizae, with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).
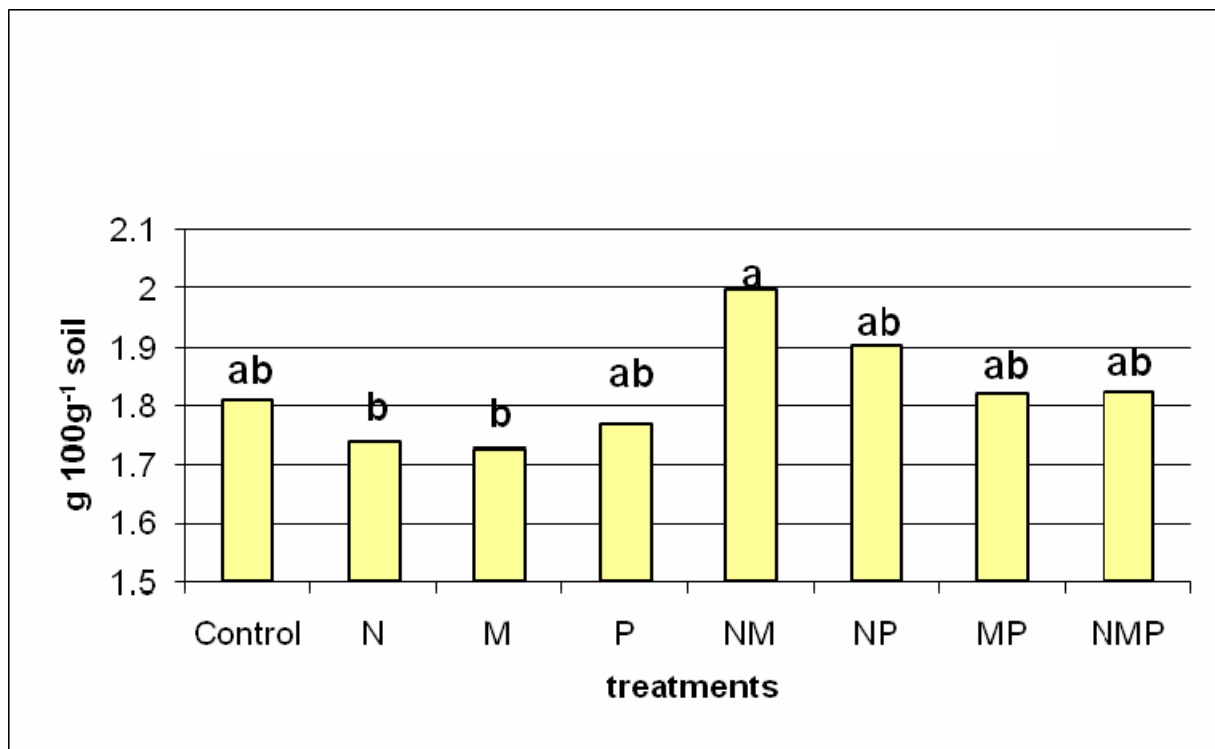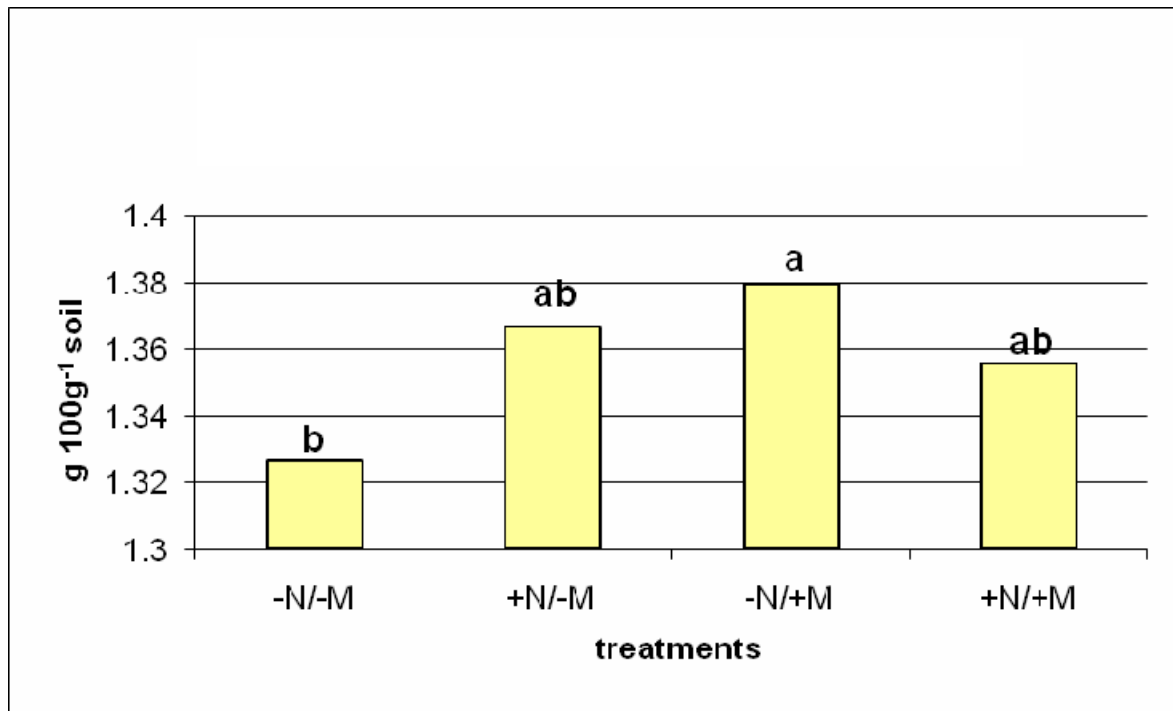
Figure 3.12. Total C in soil microaggregates (250-53 µm) at the end of the 3rd growing season. With (+N) or without (-N) nematodes, with (+M) or without (-M) mycorrhizae. Different letters indicate significant difference (P<0.05).
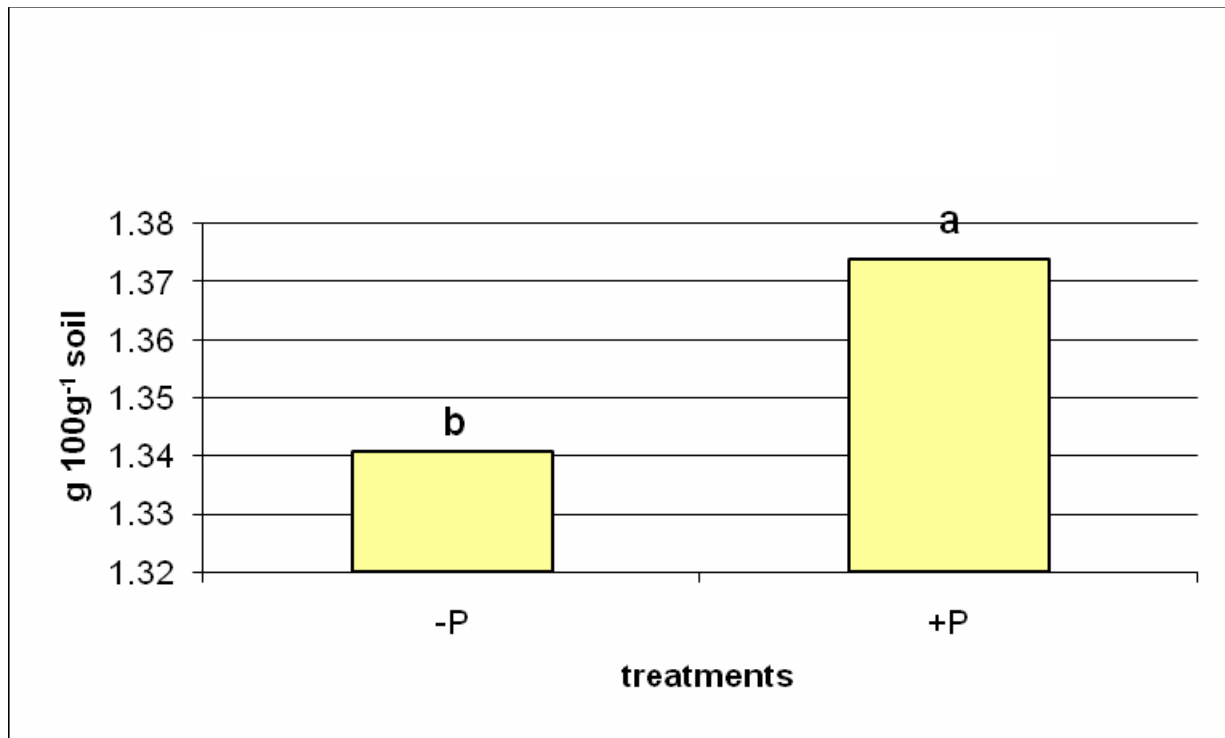
Figure 3.13. Total C in soil microaggregates (250-53 µm) at the end of the 3rd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
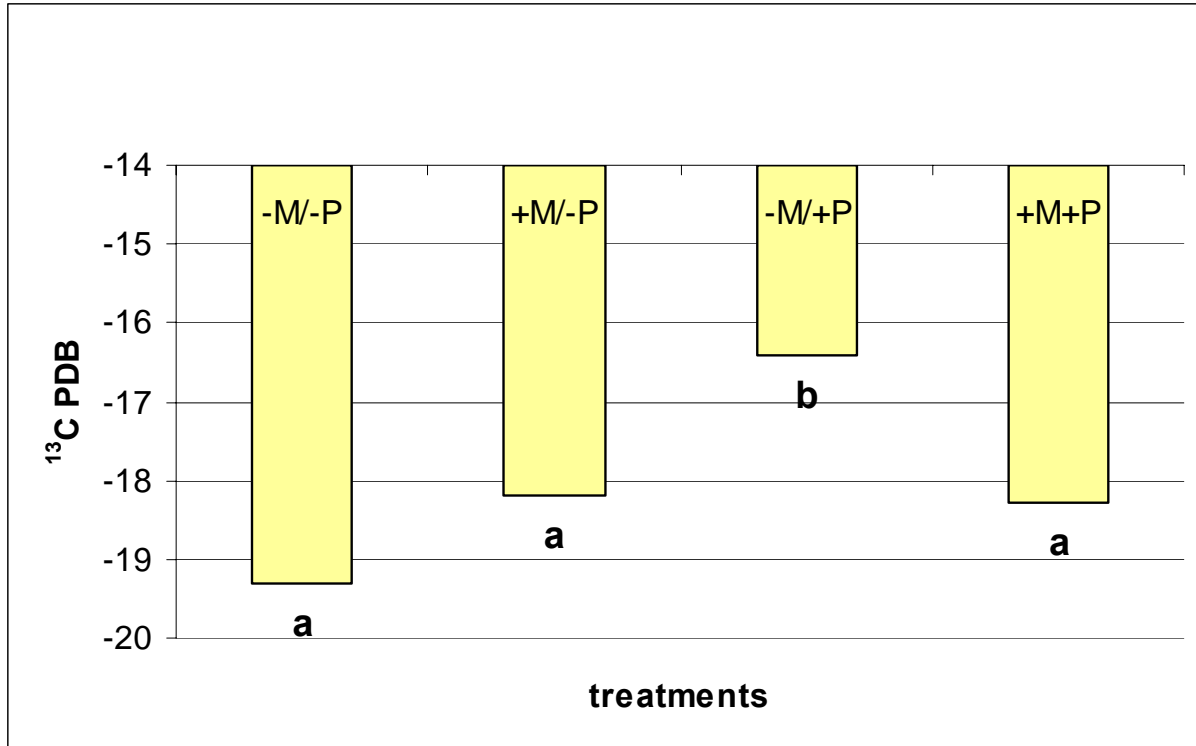
Figure 3.14. Isotopic C composition in macroaggregates (>2000 μm) at the end of the 3rd growing season. With (+M) or without (-M) and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).
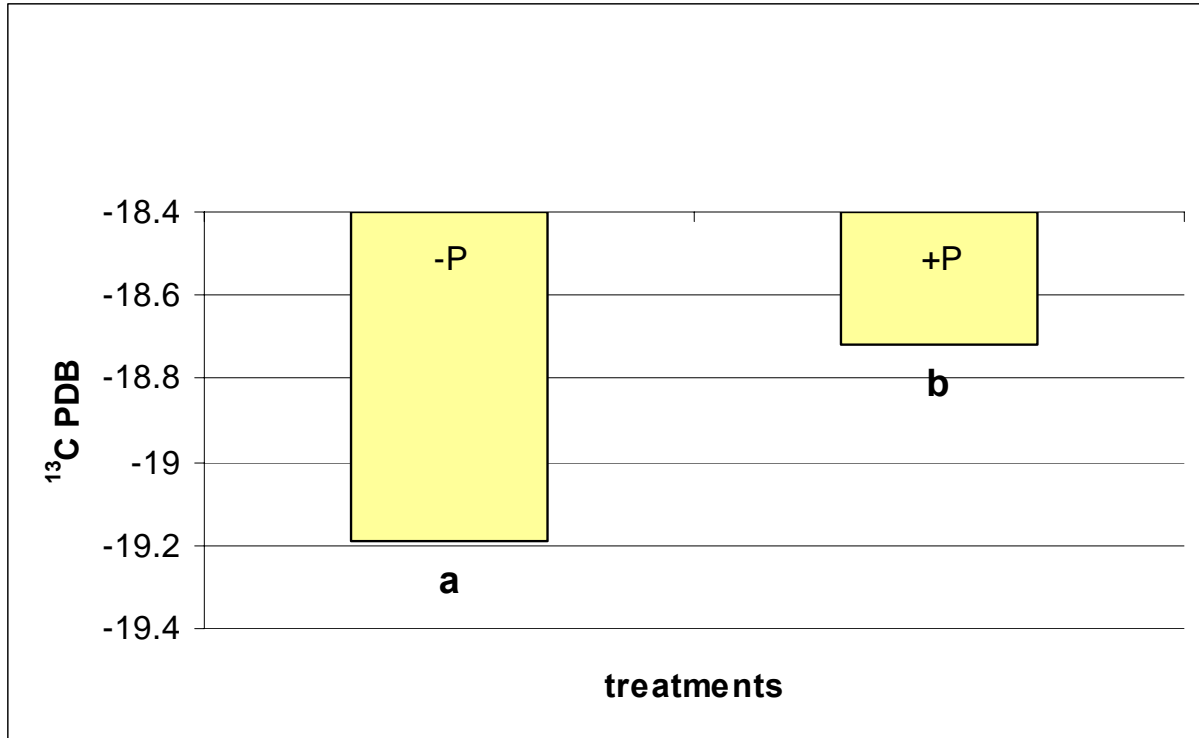
Figure 3.15. Isotopic C composition in macroaggregates (2000-250 μm) at the end of the 3rd growing season. Without (-P) or with (+P) phosphorus. Different letters indicate significant difference (P<0.05).
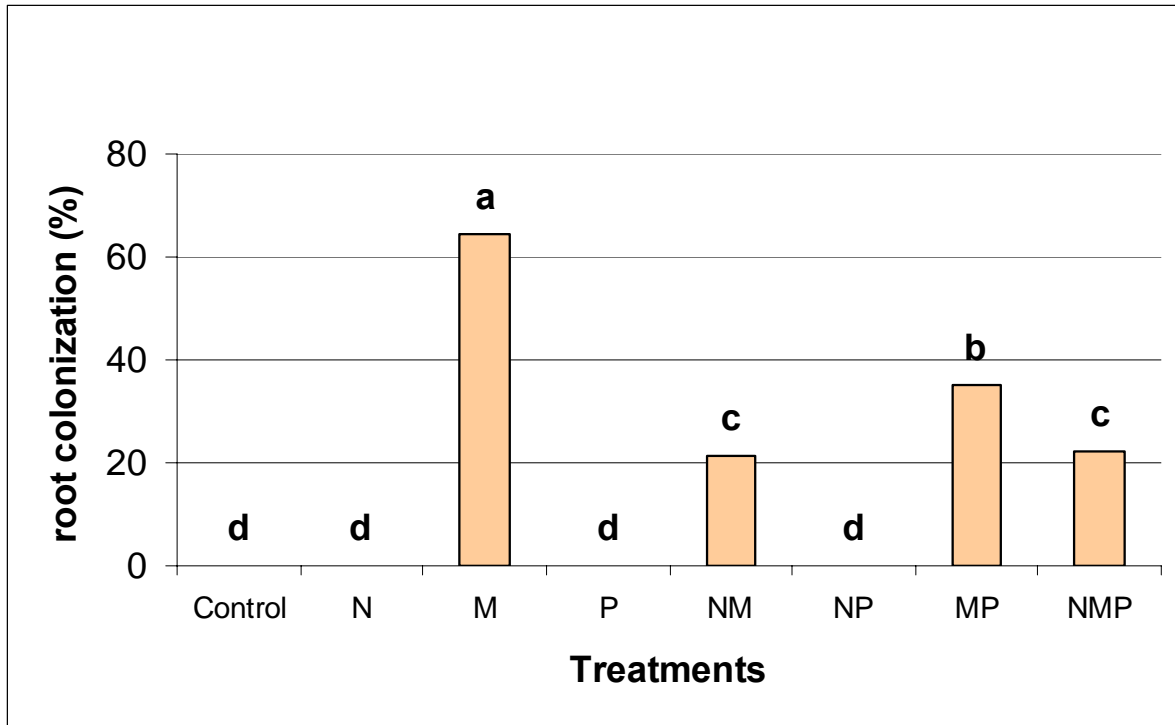
Figure 3.16. Root colonization of *Andropogon gerardii* Vit. by AM fungi at the end of the 2nd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05).
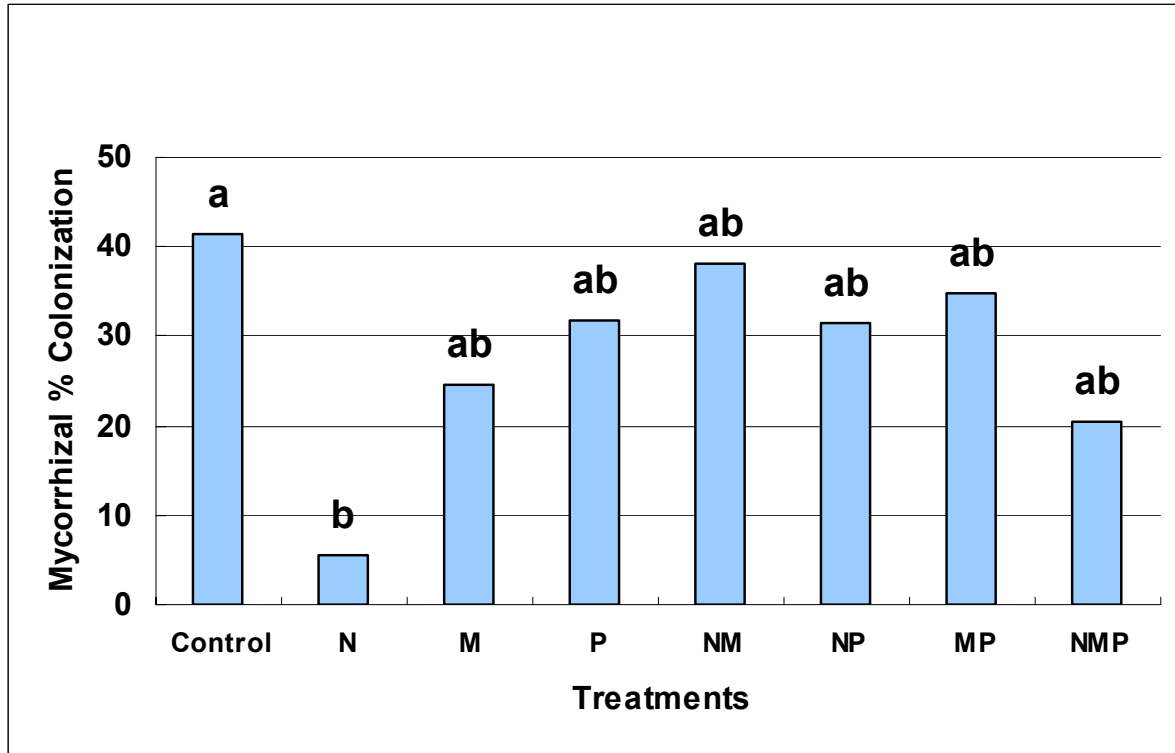
Figure 3.17. Root colonization of *Andropogon gerardii* Vit. by AM fungi at the end of the 3rd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05).

Figure 3.18. AM fungal abundance in soil indicated by 16:1 ω 5 in NLFA at the end of the 3rd growing season. With (+M) or without (-M) mycorrhizae and with (+P) or without (-P) phosphorus. Different letters indicate significant difference (P<0.05).
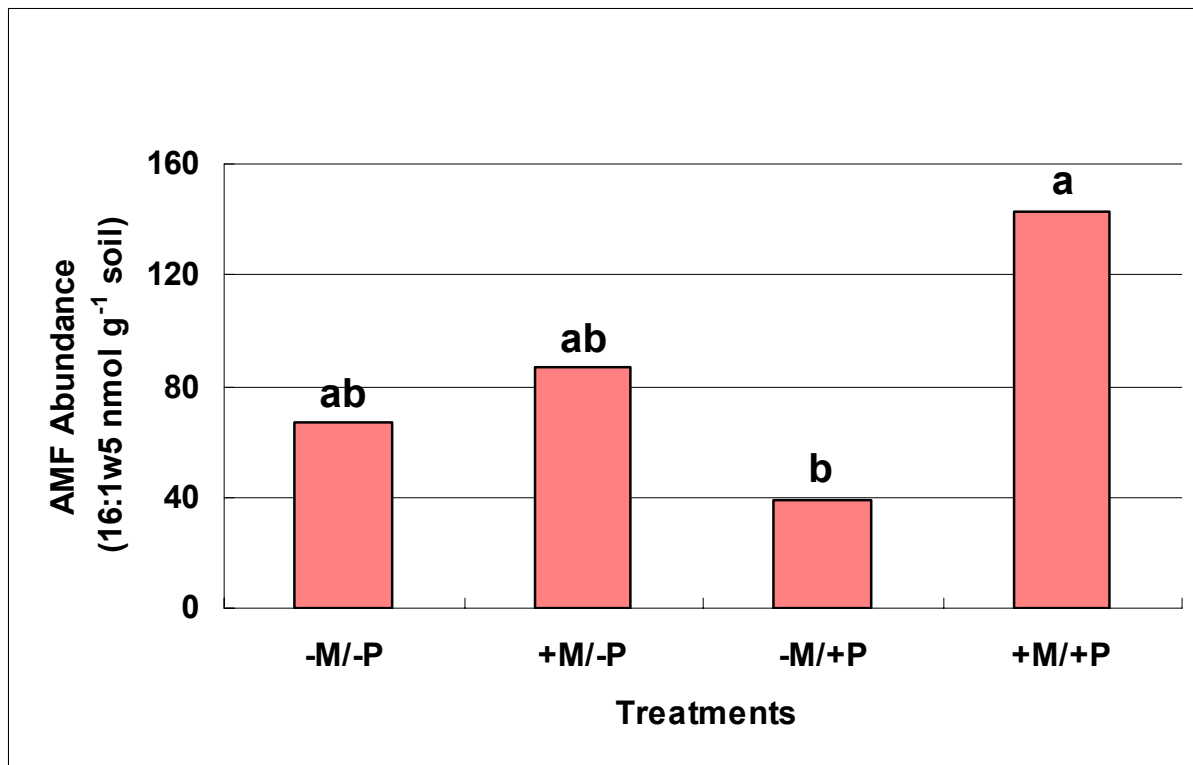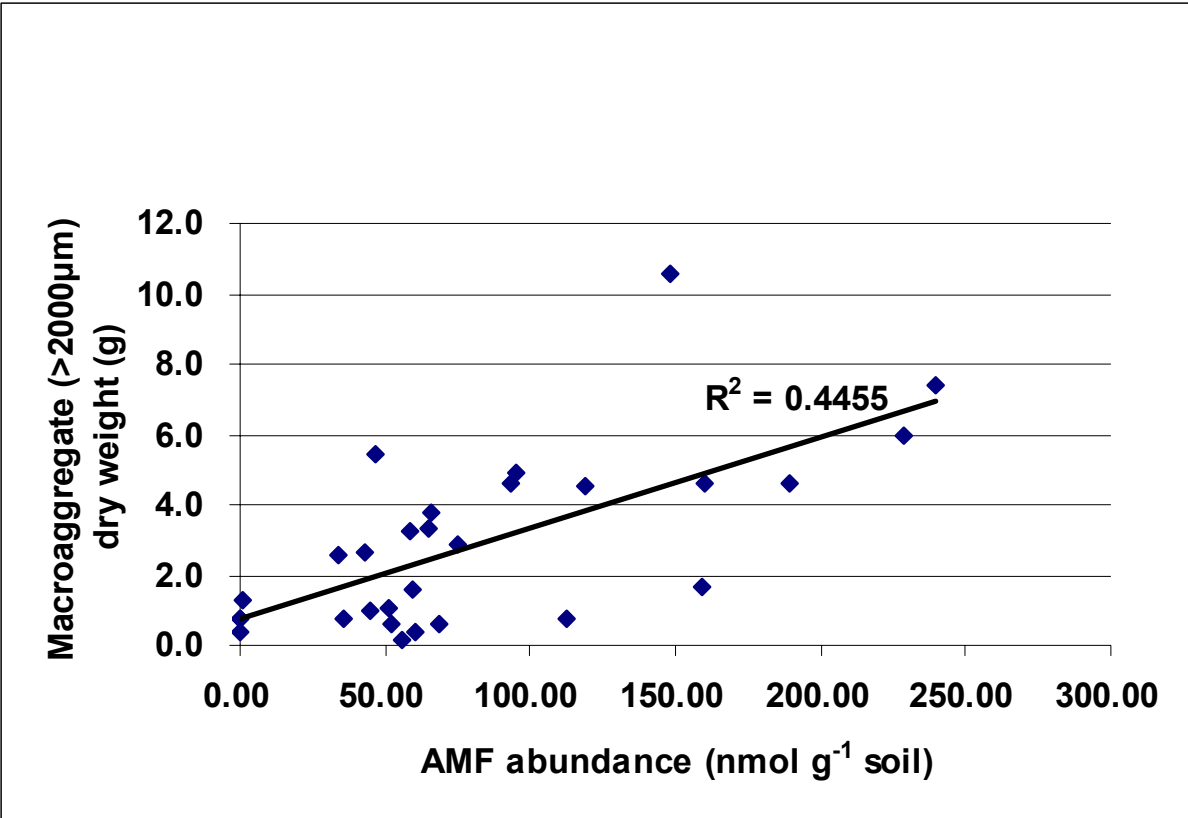
Figure 3.19. Relationship of macroaggregates (>2000μm) with abundance of AM fungi in soil indicated by 16:1 ω 5 in NLFA at the end of the 3rd growing season.

Table 3.1. Aggregate fractions at the end of the 2nd growing season from 50g soil.

Treatments	>2000μm	250-2000μm	53-250μm	20-53μm
		g		
Control	0.38	10.2	32.4	4.0
N	1.24	12.6	28.6	4.0
M	0.87	10.8	29.2	5.1
P	0.77	10.5	29.5	5.1
NM	1.80	14.7	29.3	2.9
NP	1.60	14.0	26.4	4.1
MP	1.44	11.7	29.4	4.8
NMP	0.34	9.7	32.4	3.0

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.2. Analysis of variance of aggregate fractions at the end of the 2nd growing season

Effects	>2000µm	250-2000µm	53-250µm	20-53µm
		P value		
N	0.3077	0.0197	0.3872	0.0552
M	0.7561	0.8995	0.4506	0.5530
P	0.9175	0.4703	0.6699	0.6902
N*M	0.2131	0.2147	0.0288	0.2166
N*P	0.1735	0.1427	0.4240	0.7726
M*P	0.2704	0.0778	0.0620	0.5373
N*M*P	0.1817	0.0367	0.6256	0.5517

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.3. Aggregate fractions at the end of the 3rd growing season from 50g soil.

Treatments	>2000µm	250-2000µm	53-250µm	20-53µm
		g		
Control	4.2	16.6	22.2	4.7
N	1.3	13.9	28.4	4.3
M	3.4	14.0	27.9	3.9
P	0.75	12.4	30.8	3.3
NM	5.2	9.8	27.9	4.6
NP	1.4	12.1	29.0	4.9
MP	4.2	13.1	25.3	4.7
NMP	5.3	16.8	22.4	4.2

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.4. Analysis of variance of aggregate fractions at the end of the 3rd growing season.

Effects	>2000µm	250-2000µm	53-250µm	20-53µm
		P value		
N	0.8850	0.6389	0.8643	0.4981
M	0.0221	0.8698	0.4292	0.8849
P	0.5644	0.9944	0.8967	0.8294
N*M	0.2414	0.7379	0.4061	0.6629
N*P	0.4912	0.1755	0.2187	0.7113
M*P	0.3404	0.1177	0.0546	0.5463
N*M*P	0.3251	0.4560	0.5506	0.1498

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.5. Aggregate fractions at the end of the 1st growing season (original data were divided by a factor of 2 to compare with the 2nd and the 3rd growing season). Data collected and reported by Watson (2005)

Treatments	>2000μm	250-2000μm	53-250μm	20-53μm
		g		
Control	2.3	11.0	24.8	6.6
N	1.6	12.5	25.6	6.5
M	1.9	12.5	25.2	6.1
P	2.9	15.8	21.6	5.7
NM	3.1	14.3	22.8	5.8
NP	3.8	14.4	23.7	4.3
MP	2.3	15.1	22.6	5.9
NMP	2.4	14.7	22.6	6.2

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.6. Analysis of variance of total C in agggregates at the end of the 2nd growing season

Effects	>2000µm	250-2000µm	53-250µm	20-53µm
		P value		
N	0.5624	0.5522	0.0543	0.0205
M	0.5687	0.2853	0.1030	0.4105
P	0.2938	0.9219	0.5298	0.8773
N*M	0.4004	0.8313	0.5323	0.5303
N*P	0.7283	0.5609	0.6536	0.2288
M*P	0.9719	0.6863	0.2796	0.4439
N*M*P	0.1244	0.8542	0.9264	0.8602

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.7. Analysis of variance of total N in aggregates at the end of the 2nd growing season

Effects	>2000µm	250-2000µm	53-250µm	20-53µm
	P Value			
N	0.4381	0.2439	0.0825	0.0214
M	0.2955	0.7577	0.2015	0.4371
P	0.5089	0.5276	0.6469	0.6218
N*M	0.6578	0.5573	0.7403	0.3507
N*P	0.4263	0.7818	0.3489	0.5194
M*P	0.8264	0.8683	0.7612	0.8165
N*M*P	0.2208	0.8208	0.6690	0.9059

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.8. Analysis of variance of total C in aggregates at the end of the 3rd growing season

Effects	>2000µm	250-2000µm	53-250µm	20-53µm
		P Value		
N	0.1695	0.1249	0.5718	0.6714
M	0.5420	0.4836	0.1492	0.5592
P	0.7046	0.8422	0.0267	0.3382
N*M	0.1876	0.3474	0.0334	0.9007
N*P	0.6323	0.7777	0.4175	0.1282
M*P	0.1668	0.3455	0.1998	0.8580
N*M*P	0.8752	0.0357	0.2671	0.0916

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.9. Analysis of variance of total N in aggregates at the end of the 3rd growing season

Effects	>2000μm	250-2000μm	53-250μm	20-53μm
		P value		
N	0.2894	0.0268	0.3978	0.5170
M	0.9797	0.3013	0.4095	0.9698
P	0.9272	0.1551	0.2404	0.3667
N*M	0.5860	0.2973	0.6141	0.8822
N*P	0.4621	0.2573	0.3605	0.1139
M*P	0.5816	0.1503	0.2023	0.8517
N*M*P	0.7213	0.0118	0.9859	0.1270

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.10. Analysis of variance of isotopic C in aggregates at the end of the 3rd growing season

Effects	>2000μm	250-2000μm	53-250μm	20-53μm
			P Value	
N	0.8338	0.1984	0.3968	0.7347
M	0.4516	0.1751	0.5706	0.4122
P	0.0099	0.0078	0.2224	0.4873
N*M	0.9402	0.4964	0.5869	0.8663
N*P	0.2957	0.9900	0.3137	0.3229
M*P	0.0058	0.5910	0.7561	0.7181
N*M*P	0.1483	0.2887	0.6113	0.3360

N=nematodes, M=mycorrhizae, P=Phosphorus

Table 3.11. Analysis of variance of abundance of fungi in *Andropogon gerardii* Vit. roots indicating root colonization by mycorrhizal fungi at the end of the 2nd growing season.

Effects	Colonization	AM Fungi
	P Value	
N	<0.0001	0.2521
M	<0.0001	0.6364
P	0.0010	0.1407
N*M	<0.0001	0.6066
N*P	0.0005	0.1403
M*P	0.0010	0.5711
N*M*P	0.0005	0.6736

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.12. Analysis of variance of abundance of fungi in *Andropogon gerardii* Vit. roots indicating root colonization by mycorrhizal fungi at the end of the 3rd growing season.

Effects	Colonization	AM Fungi
	P Value	
N	0.2552	0.8562
M	0.8152	0.7562
P	0.7792	0.7573
NM	0.2744	0.2635
NP	0.8031	0.4317
MP	0.4662	0.8830
NMP	0.0569	0.2385

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table 3.13. AMF and fungal abundance as estimated from NLFA derived fatty acid indicators at the end of the 3rd growing season.

Treatments	AM Fungi	General Fungi
	nmol g^{-1} soil	
Control	110.9	35.7
N	23.7	16.4
M	69.2	34.5
P	20.3	6.9
NM	92.0	35.7
NP	56.6	24.9
MP	129.4	43.8
NMP	156.0	52.4

N=nematodes, M=mycorrhizae, P=Phosphorus.

SUMMARY

Our results confirmed the positive effect of mycorrhizal symbiosis on plant aboveground biomass and rhizome biomass. Phosphorus increased plant root biomass, and decreased mycorrhizal colonization of the roots. After the 3rd growing season the non-mycorrhizal control had significant mycorrhizal colonization of the roots presumably due to contamination at the beginning of the 3rd growing season. This contamination may have confounded some of the interpretations of this study. Root colonization and AM fungal abundance in the soil and roots indicated by NLFA did not correlate, suggesting a different sensitivity of detecting AM fungal appearance or NLFA may be detecting the fatty acid from other organisms.

The nematode community changed during the 3 years of the expereiment. Microbivores increased more quickly than fungivores and herbivores and these groups dominated the first year. This was probably due to the time needed to establish root biomass because by year 3, plant-feeding nematodes were dominant, which was similar to the community composition of the native prairie. The effect of nematodes on plant growth become apparent as the herbivore population increased. Nematodes in the third year decreased both above-and belowground plant biomass.

Phosphorus increased microbial biomass due to the improvement in plant root biomass and thus increased C inputs into the soil. At the community level, the actinomycete population in the first two years was significantly higher in P treated soils. Our results also showed a consistent increase of fungi to bacterial ratio in the P treatments.

The role of AM fungi in aggregation was confirmed after three years with significantly greater distribution in the macroaggregate fraction. Aggregate distribution was not correlated with root colonization, but there was a positive relationship between AM fungal abundance in the soil and the mass of the largest macroaggregates (>2000 µm) by year 3. The effect of roots on macroaggregate (>2000 µm) fraction was less apparent. Phosphorus, which increased root biomass, did not increased macroaggregation. This strongly suggests the primary role of AMF in the formation of macroaggregates in this soil with a less important role of roots in the formation of macroaggregates. Roots had a greater role in the formation of aggregates 250-2000 µm. This

was evident where P significantly increased smaller macroaggregates (250-2000 µm), along with significantly enhanced plant root biomass.

We found mycorrhizal treatments induced more soil derived C into the macroaggregates (>2000 µm) than the nonmycorrhizal treatments when P was added to the soil. Our hypothesis was that mycorrhizae would induce more plant-derived C into the macroaggregates. The results do not support the hypothesis of direct transport of plant C into aggregates. This may due to the negative effect of P addition on mycorrhizal symbiosis and efficiency, where Watson (2005) reported a reduction on root colonization in the P-amended treatments. There was a trend for mycorrhizal-induced plant derived C into macroaggregates in non-P treatments, although it was not significant. The ^{13}C results of this macroaggregate fraction confirmed that P addition induced more plant derived C into the aggregates than the non-P amended microcosms.

To conclude, nematodes, mycorrhizal symbiosis, and phosphorus addition can significantly influence both above-and belowground plant biomass. The interaction between nematodes and mycorrhizae was significant in altering C cycle, but it was the plant roots regulated by P that played a key role in influencing soil biotic interactions, presumably due to their C inputs into soil. Mycorrhizae and associated plant roots play a key role in soil aggregate formation.

Future studies may be focused on further exploring the relationship between AM fungi and soil macroaggregates and C storage. Effects on soil aggregates became more apparent by the 3rd year; thus a longer-term study would help to decipher the role of roots and AM fungi on aggregate formation. At the same time, field studies need to be examined to compare with these greenhouse microcosm results. Nematodes showed a positive effect on soil microaggregate formation, which need to be further examined. Besides C, nitrogen may also need to be addressed and added to understand the interactions belowground. Further studies using ^{15}N to trace N allocation in plants and soil may be an important area of research to the findings reported in this thesis.
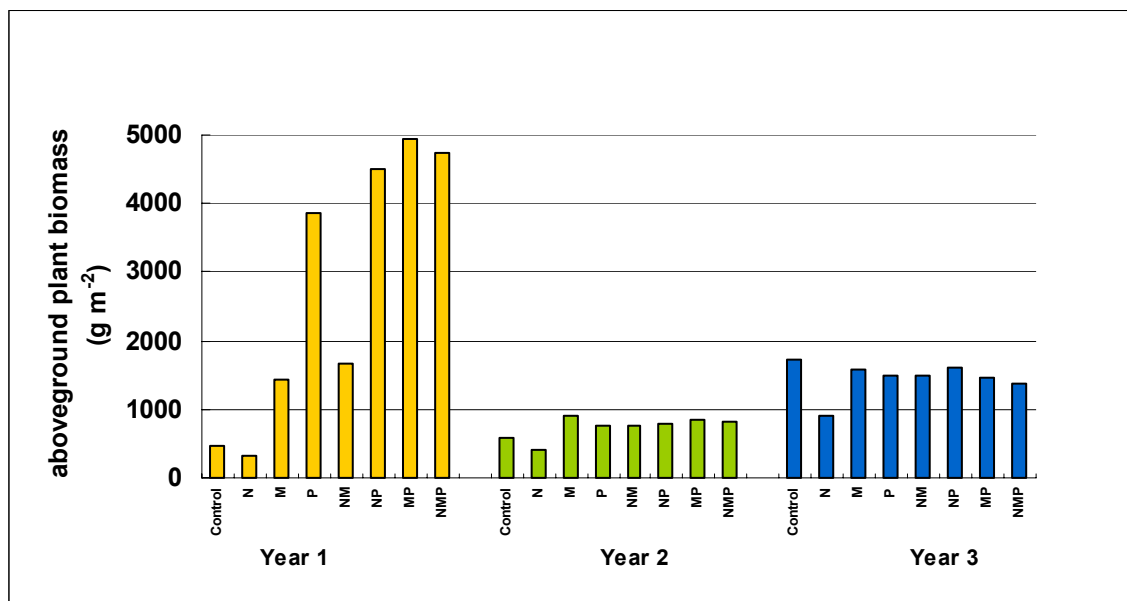
Appendix A - OTHER GRAPHS AND TABLES

Figure A.1. Aboveground plant biomass of *Andropogon gerardii* Vit. from the 1st, 2nd, and the 3rd growing season.
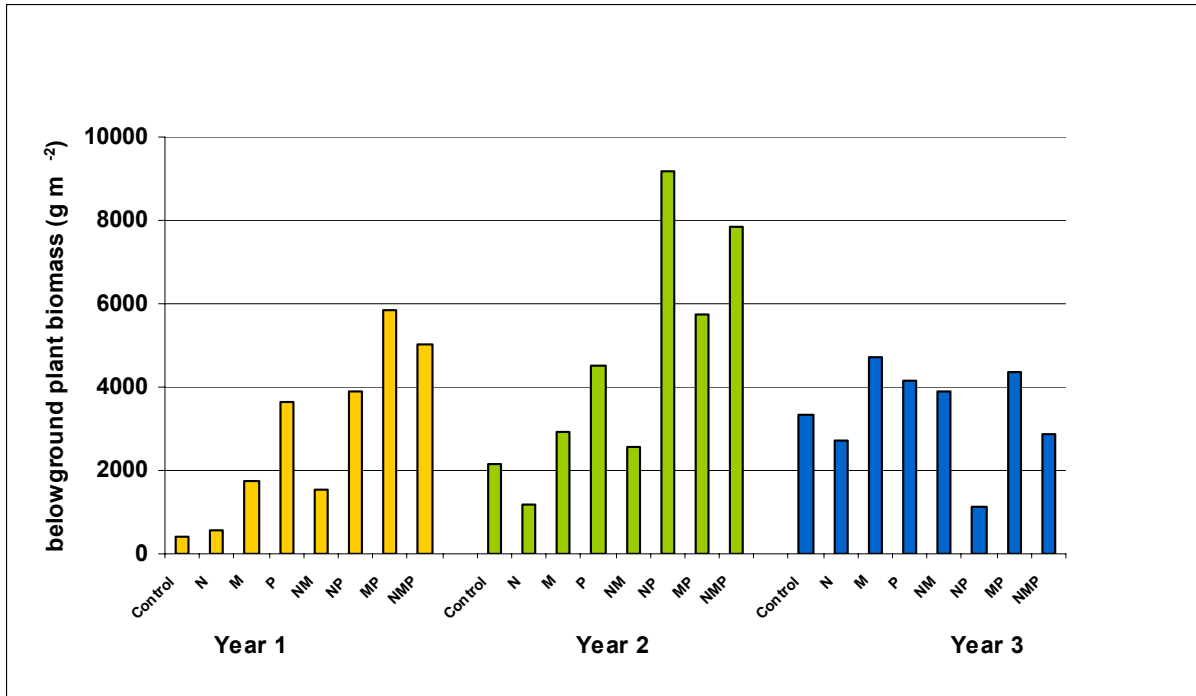
Figure A.2. Belowground plant biomass of *Andropogon gerardii* Vit. from 1st, 2nd, and the 3rd growing season.
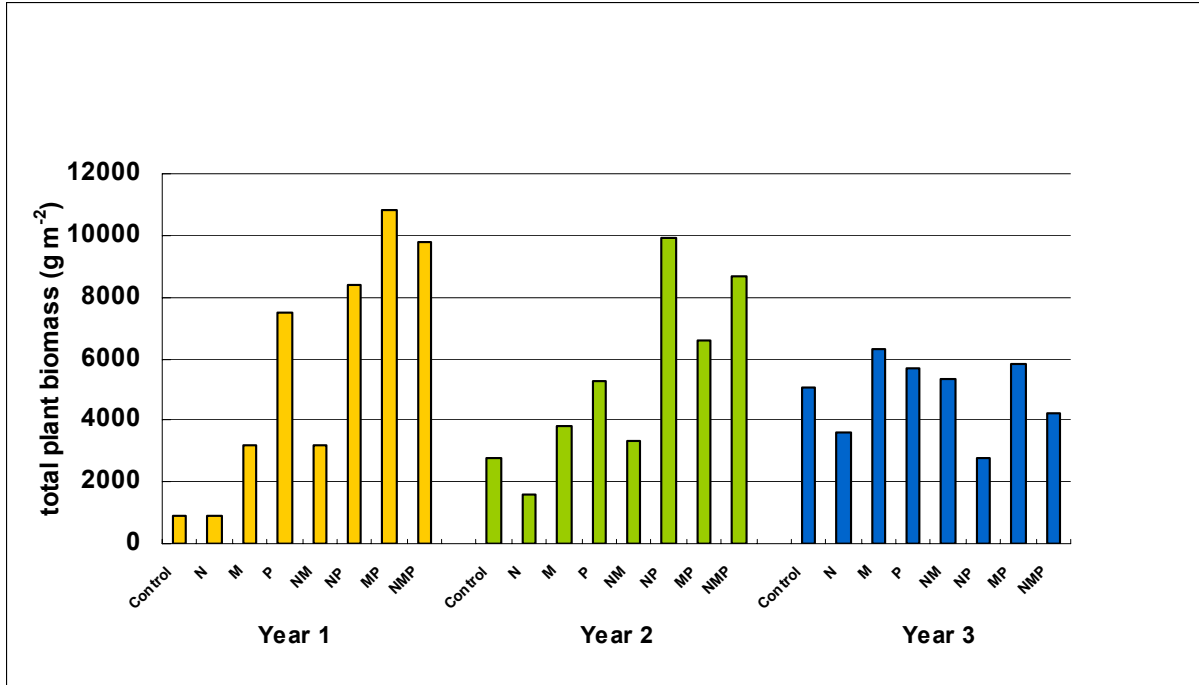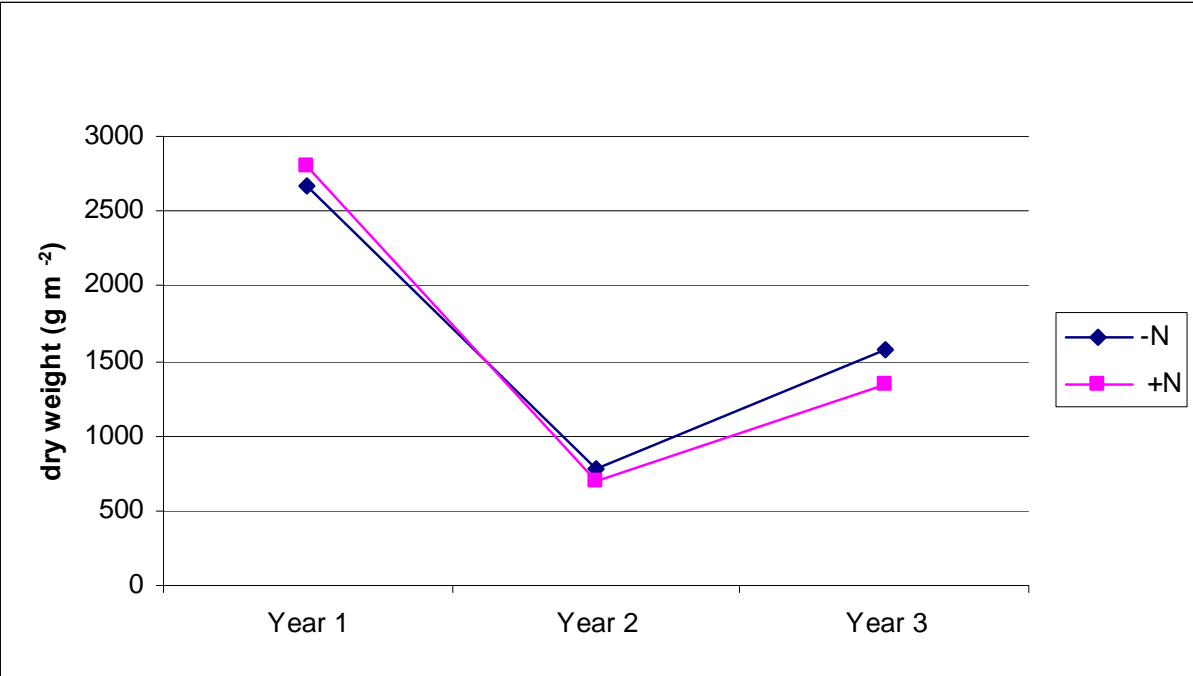
Figure A.3. Total plant biomass of *Andropogon gerardii* Vit. from the 1st, 2nd, and the 3rd growing season.
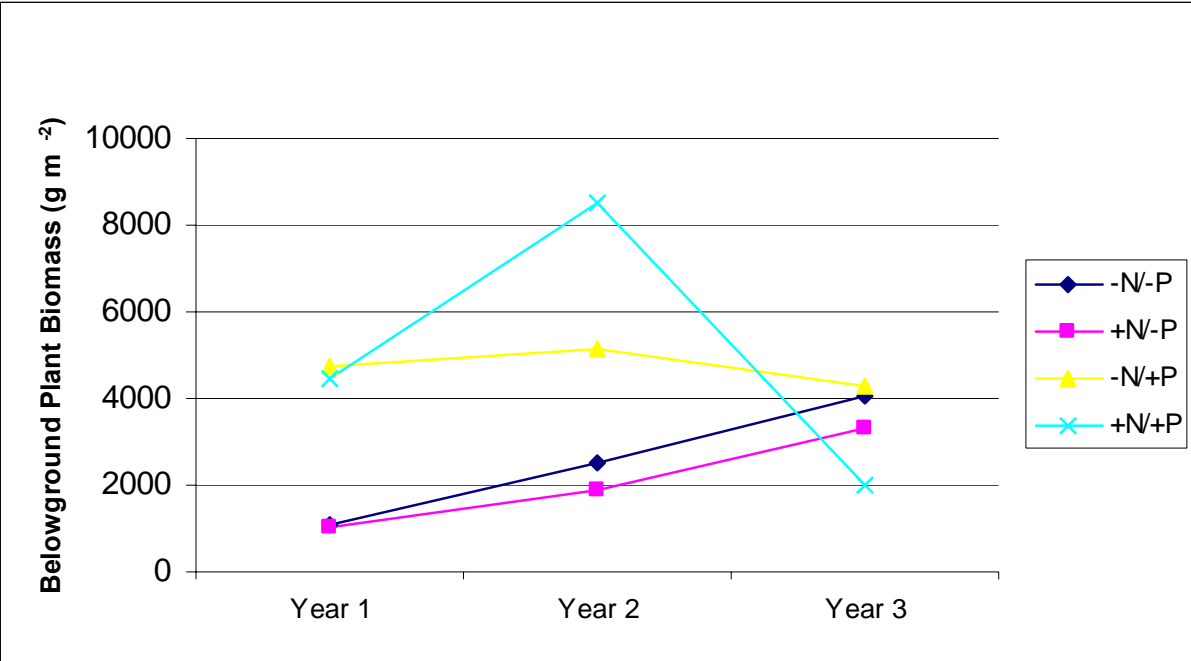
Figure A.4. Aboveground plant biomass of *Andropogon gerardii* Vit. from the 1st to the 3rd growing season. Without (-N) or with (+N) nematodes.
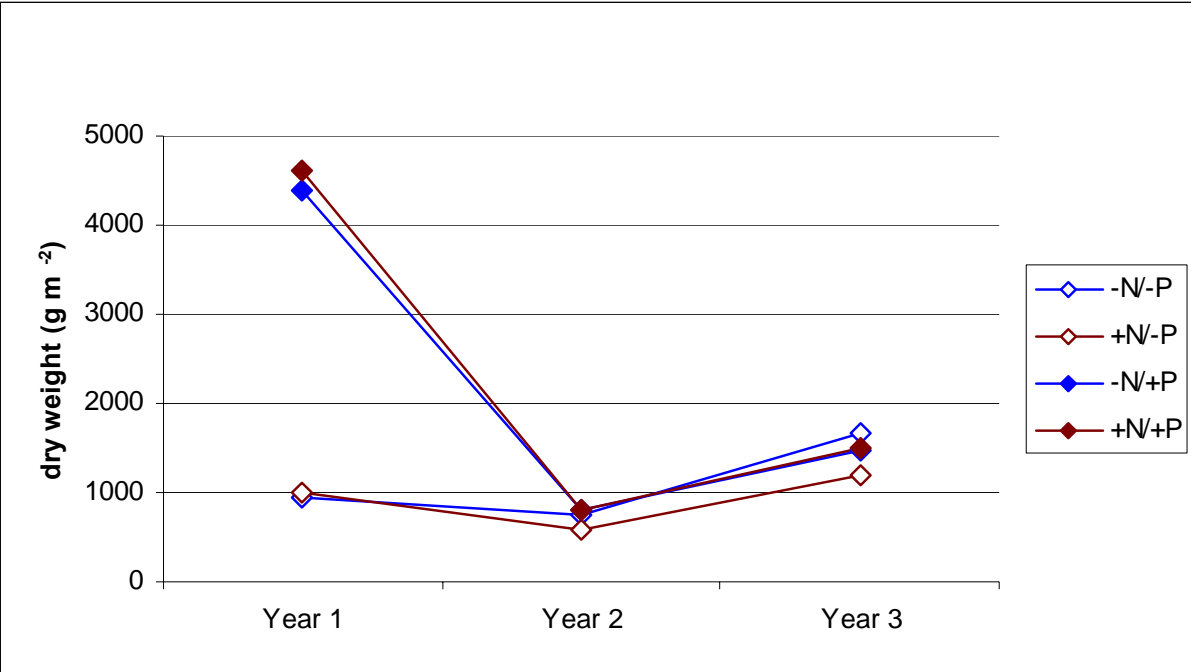
Figure A.5. Aboveground plant biomass of *Andropogon gerardii* Vit. from the 1st to the 3rd growing season. With (+N) or without (-N) nematodes and with (+P) or without (-P) phosphorus.
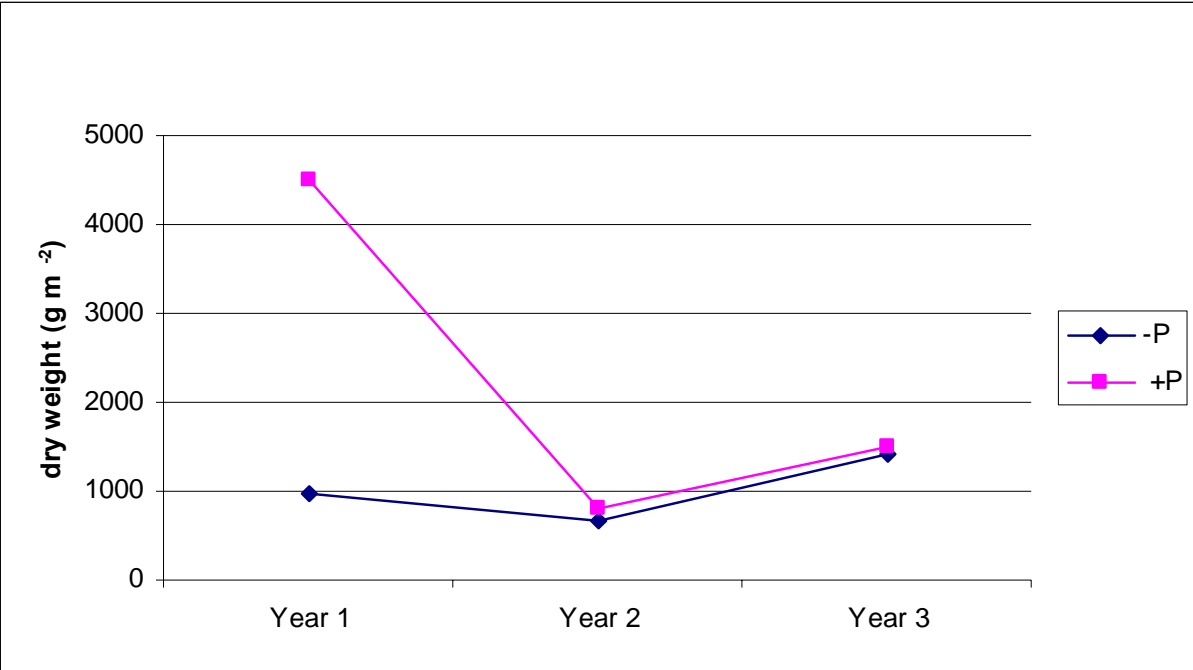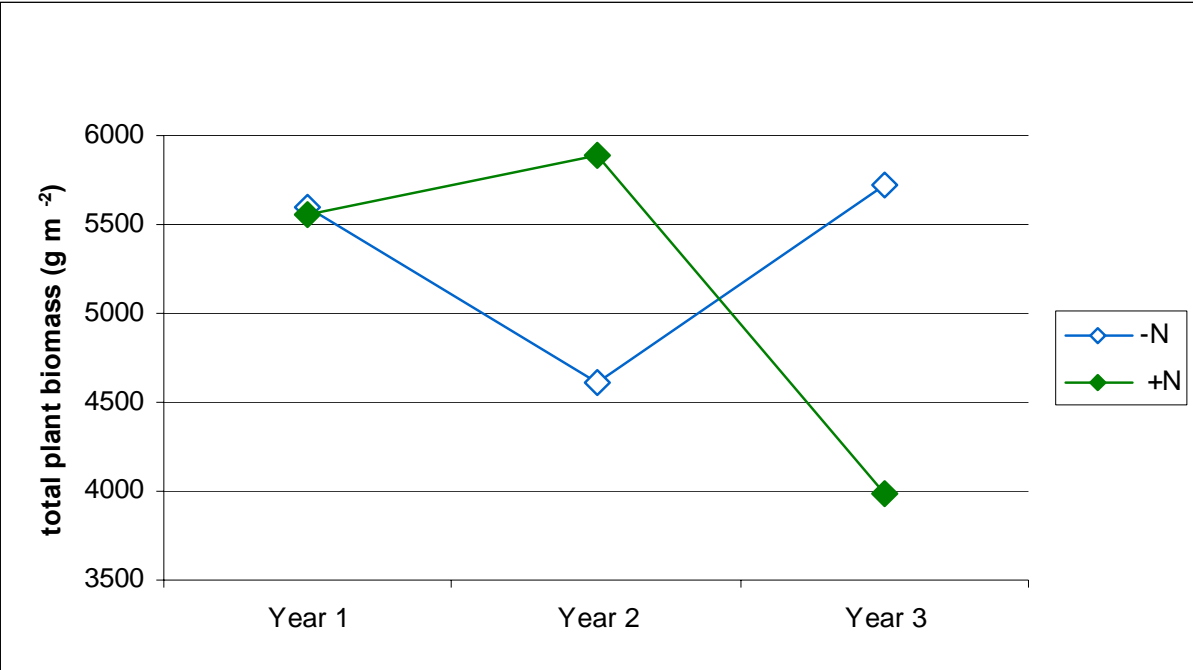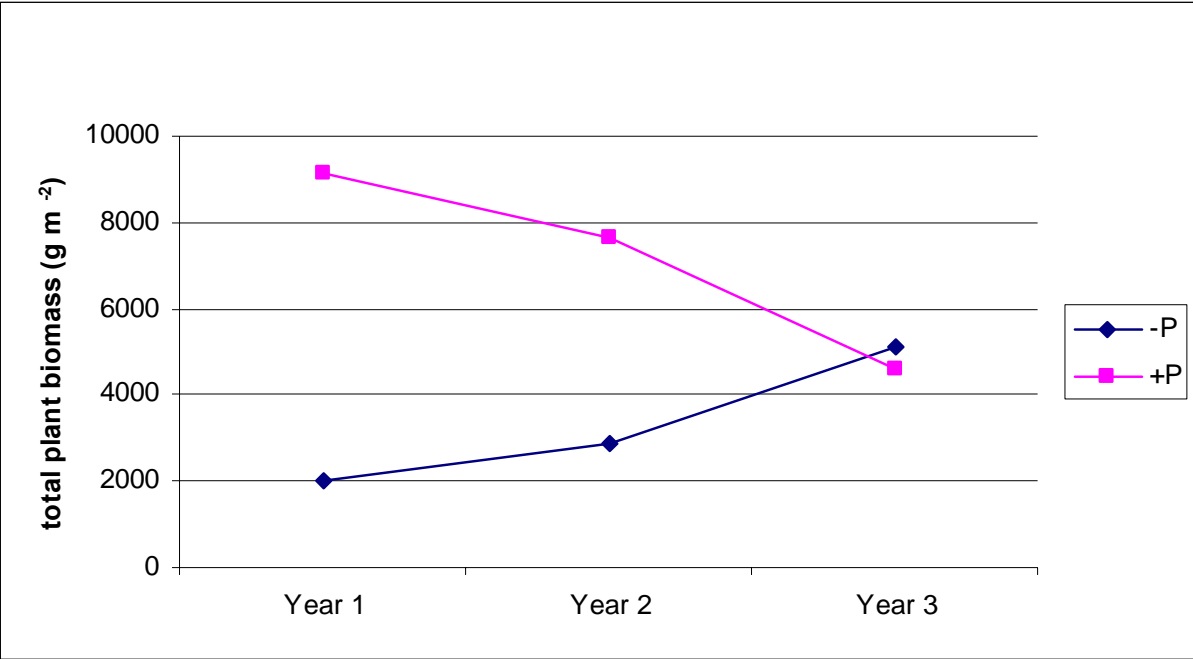
Figure A.6. Aboveground plant biomass of *Andropogon gerardii* Vit. from the 1st to the 3rd growing season. Without (-P) or with (+P) phosphorus.
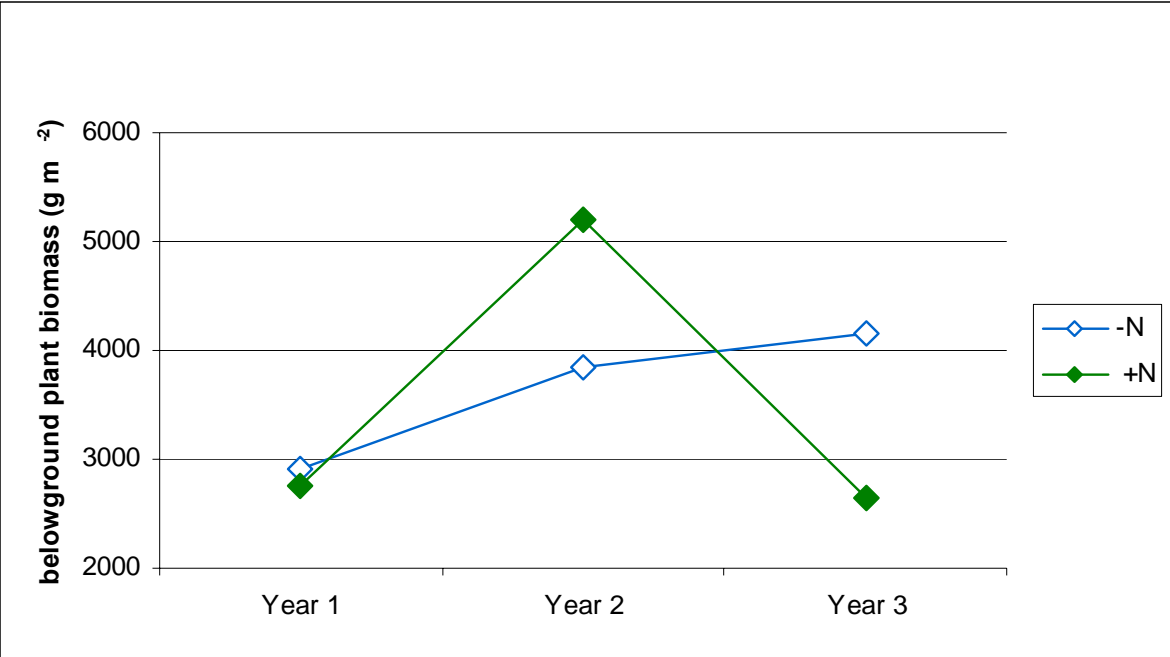
Figure A.7. Belowground plant biomass of *Andropogon gerardii* Vit. from the 1st to the 3rd growing season. Without (-N) or with (+N) nematodes.
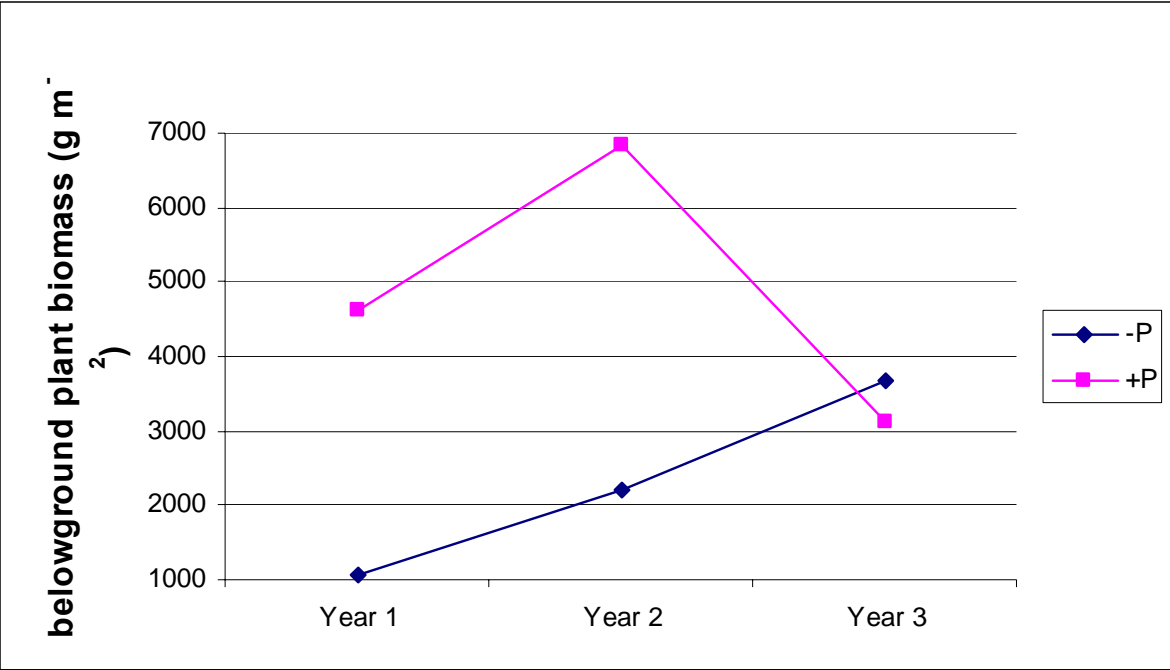
Figure A.8. Belowground plant biomass of *Andropogon gerardii* Vit. from the 1st to the 3rd growing season. Without (-P) or with (+P) phosphorus.
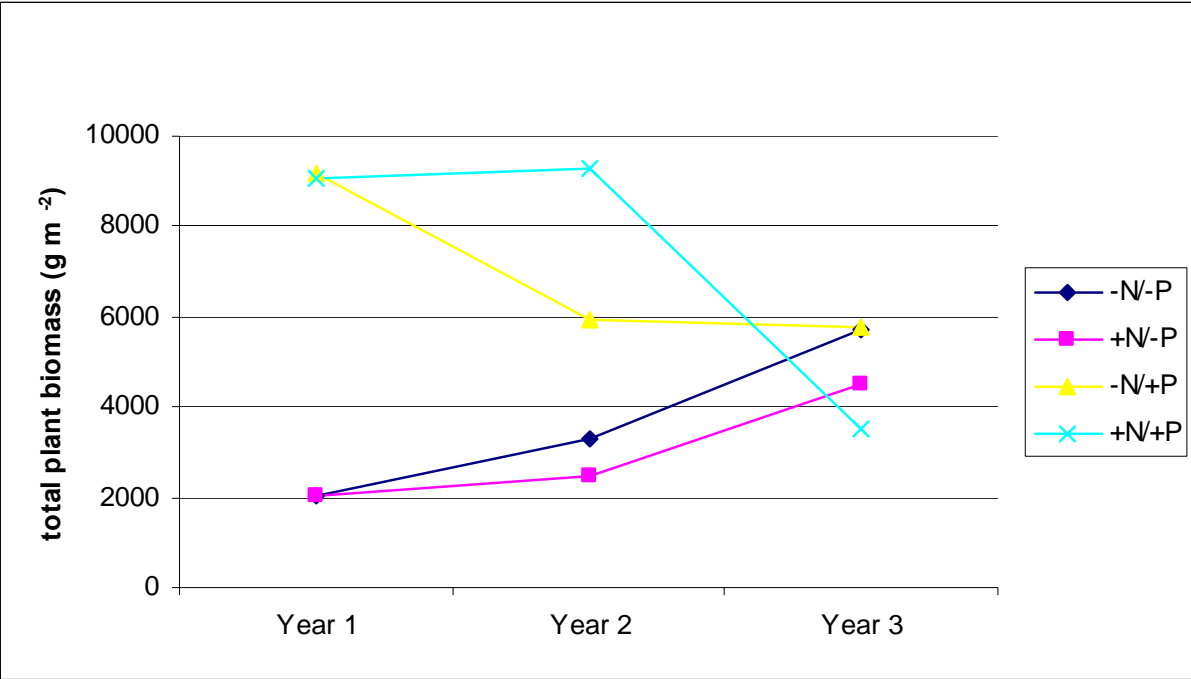
Figure A.9. Belowground plant biomass of *Andropogon gerardii* Vit. from the 1st to the 3rd growing season. With (+N) or without (-N) nematodes and with (+P) or without (-P) phosphorus.

**Figure A.10. Total plant biomass of *Andropogon gerardii* Vit. from the 1st, 2nd, and the 3rd growing season. Without (-N) or with (+N) nematodes.**

Figure A.11. Total plant biomass of *Andropogon gerardii* Vit. from the 1st, 2nd, and the 3rd growing season. With (+P) or without (-P) phosphorus.

Figure A.12. Total plant biomass of *Andropogon gerardii* Vit. from the 1st, 2nd, and the 3rd growing season. With (+N) or without (-N) nematodes and with (+P) or without (-P) phosphorus.
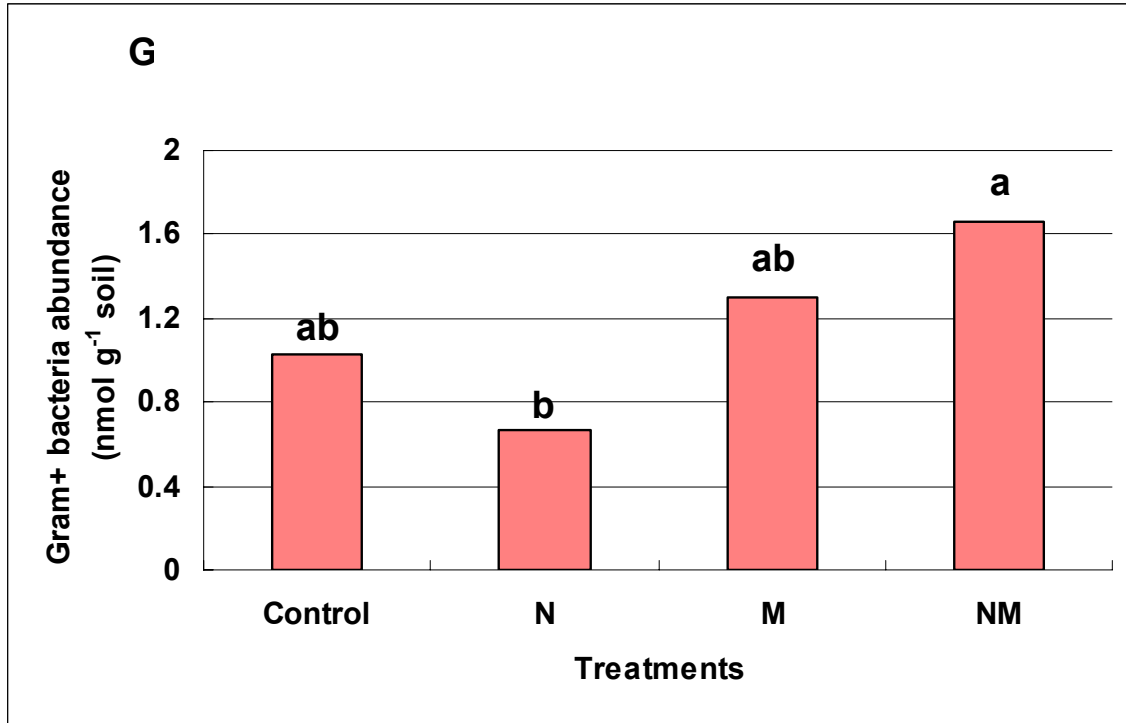
Figure A.13. Abundance of gram-positive bacteria indicated by NLFA at the end of the 3rd growing season. With (N) nematodes and with mycorrhizae (M). Different letters indicate significant difference (P<0.05).
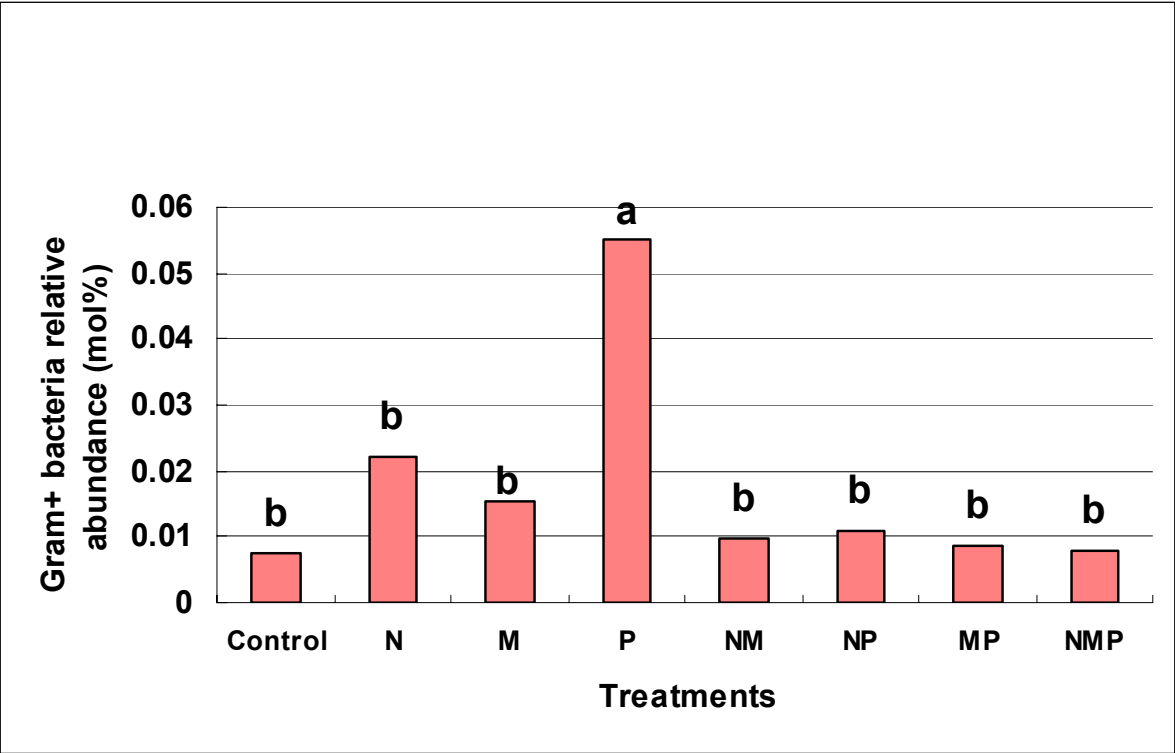
Figure A.14. Relative abundance of gram-positive bacteria indicated by NLFA at the end of the 3rd growing season. With nematodes (N), with mycorrhizae (M), and with phosphorus (P). Different letters indicate significant difference (P<0.05).
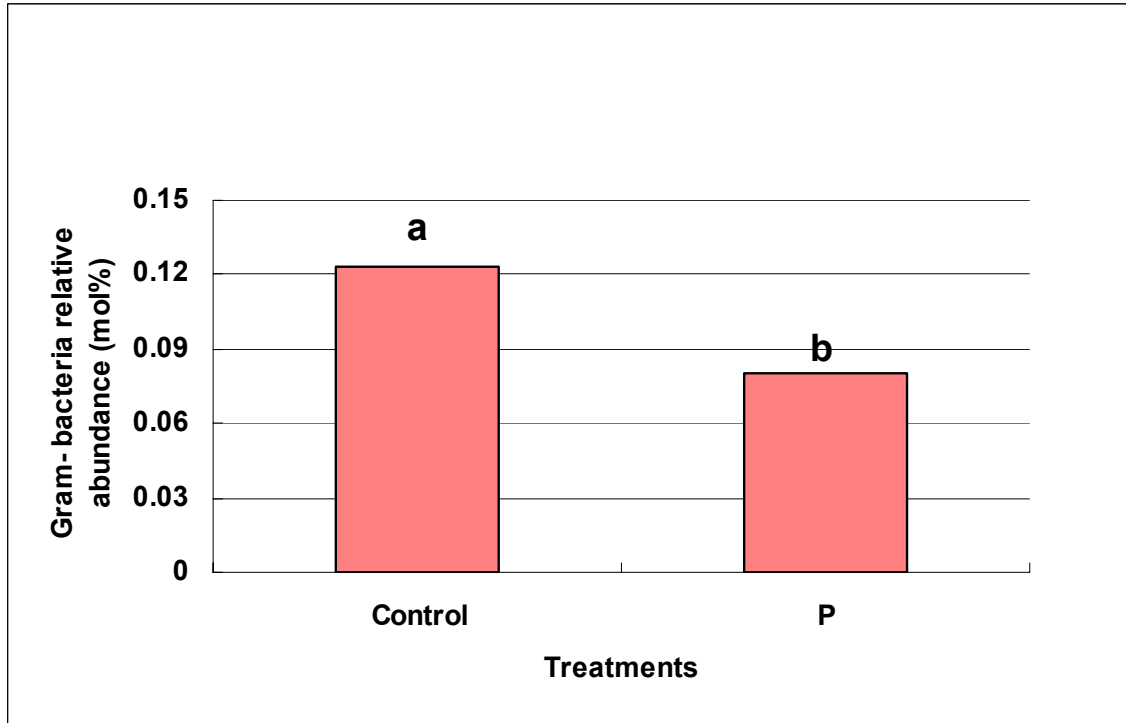
Figure A.15. Relative abundance of gram-negative bacteria indicated by NLFA at the end of the 3rd growing season. Without (Control) or with phosphorus (P). Different letters indicate significant difference (P<0.05).
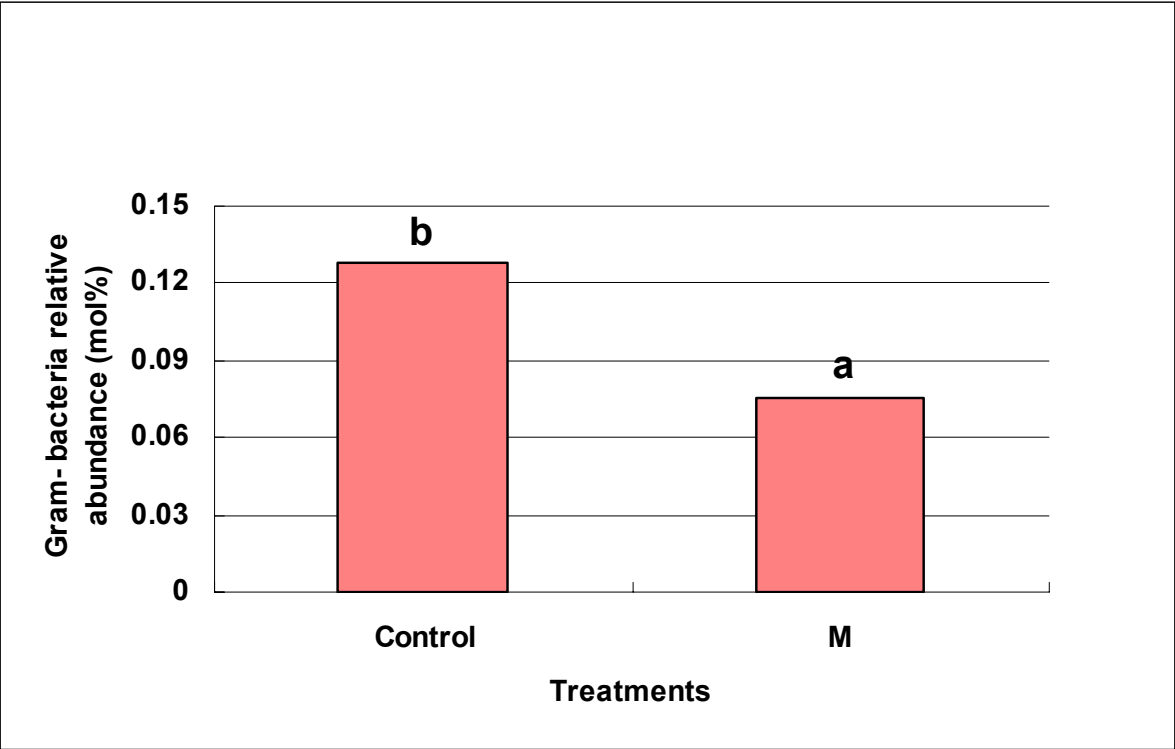
Figure A.16. Relative abundance of gram-negative bacteria indicated by NLFA at the end of the 3rd growing season. Without (Control) or with mycorrhizae (M). Different letters indicate significant difference (P<0.05).
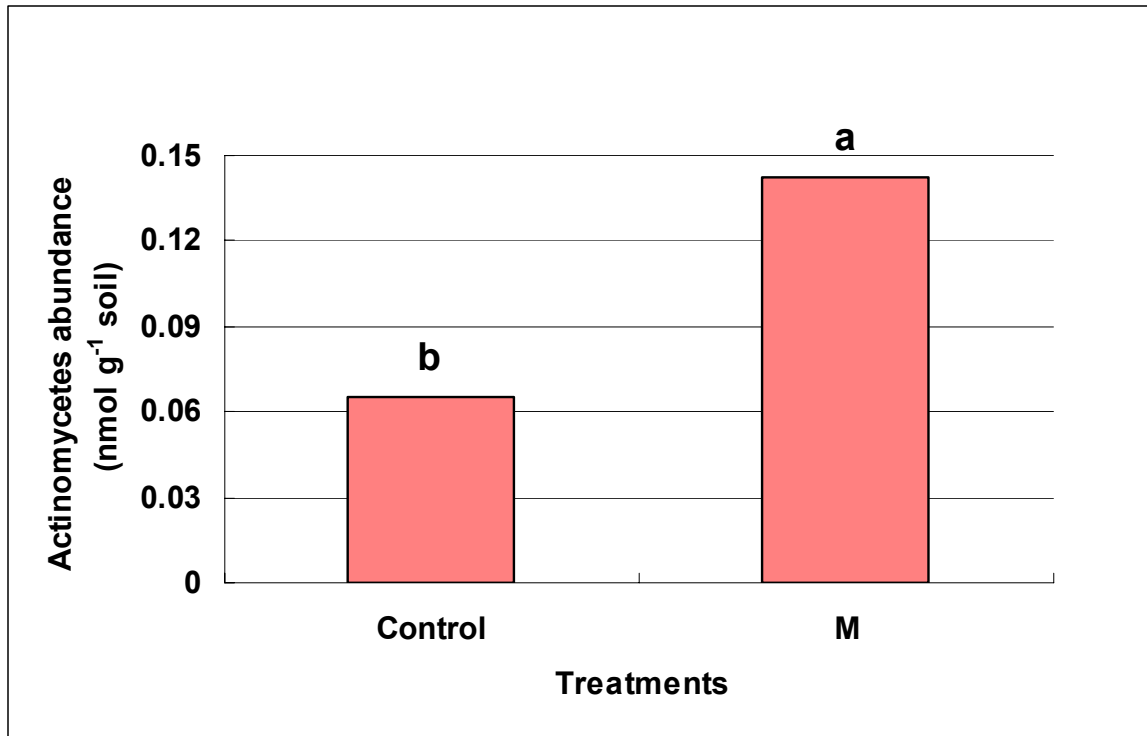
Figure A.17. Abundance of actinomycetes indicated by NLFA at the end of the 3rd growing season. Without (Control) or with mycorrhizae (M). Different letters indicate significant difference (P<0.05).
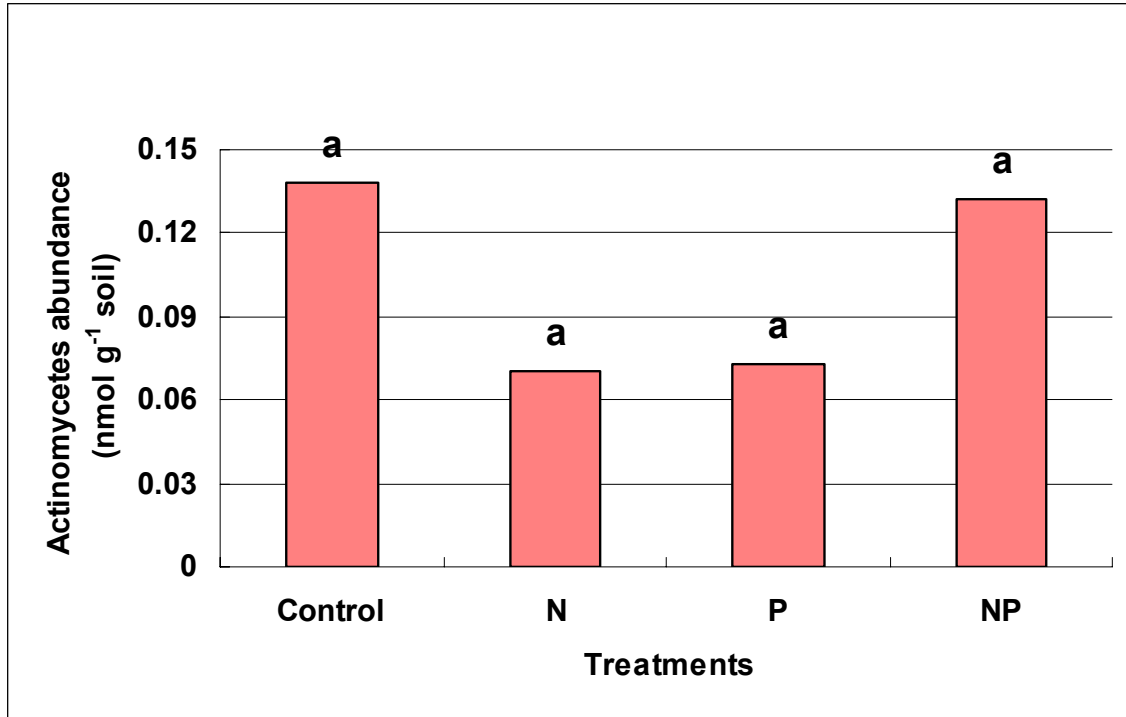
Figure A.18. Abundance of actinomycetes indicated by NLFA at the end of the 3rd growing season. With nematodes (N) and with phosphorus (P). Different letters indicate significant difference (P<0.05).
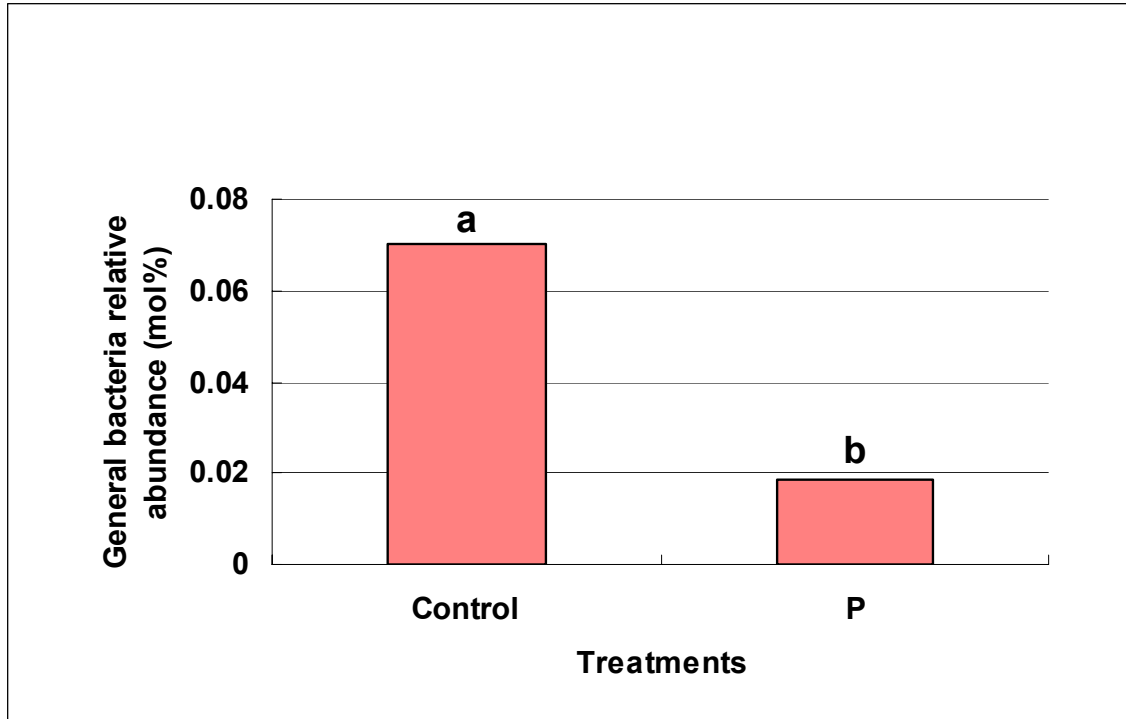
Figure A.19. Abundance ofgeneral bacteria indicated by NLFA at the end of the 3rd growing season. Without (Control) or with phosphorus (P). Different letters indicate significant difference (P<0.05).
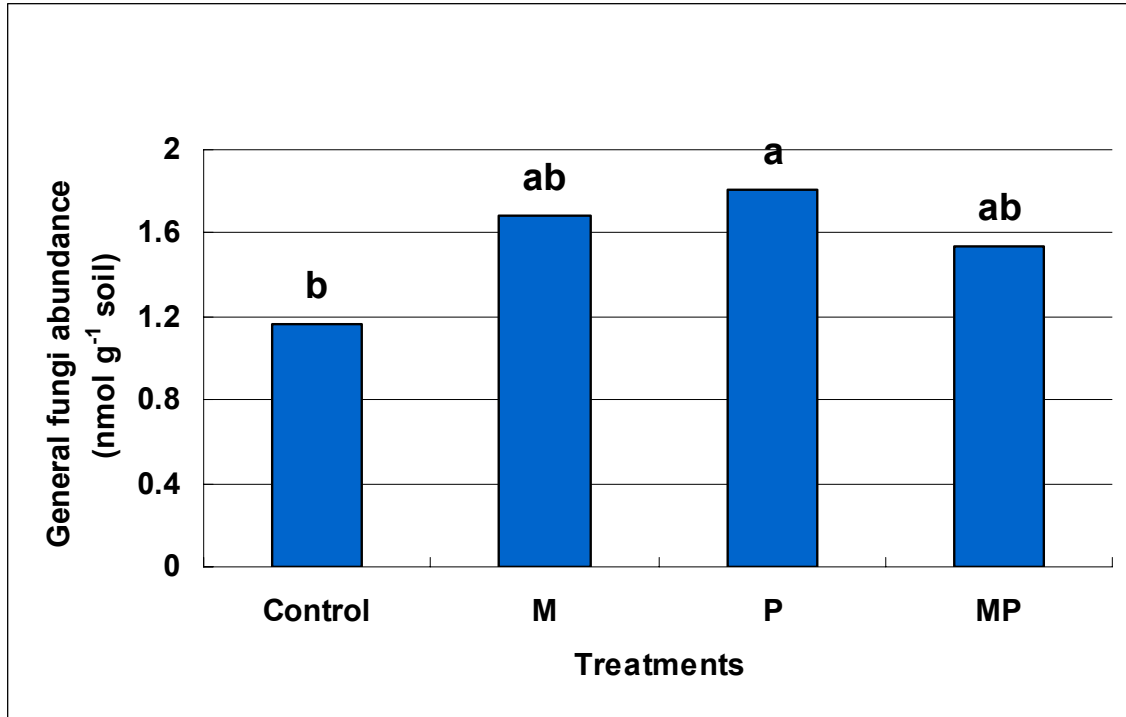
Figure A.20. Abundance general fungi indicated by PLFA at the end of the 3rd growing season. With phosphorus (P) and with mycorrhizae (M) . Different letters indicate significant difference (P<0.05).
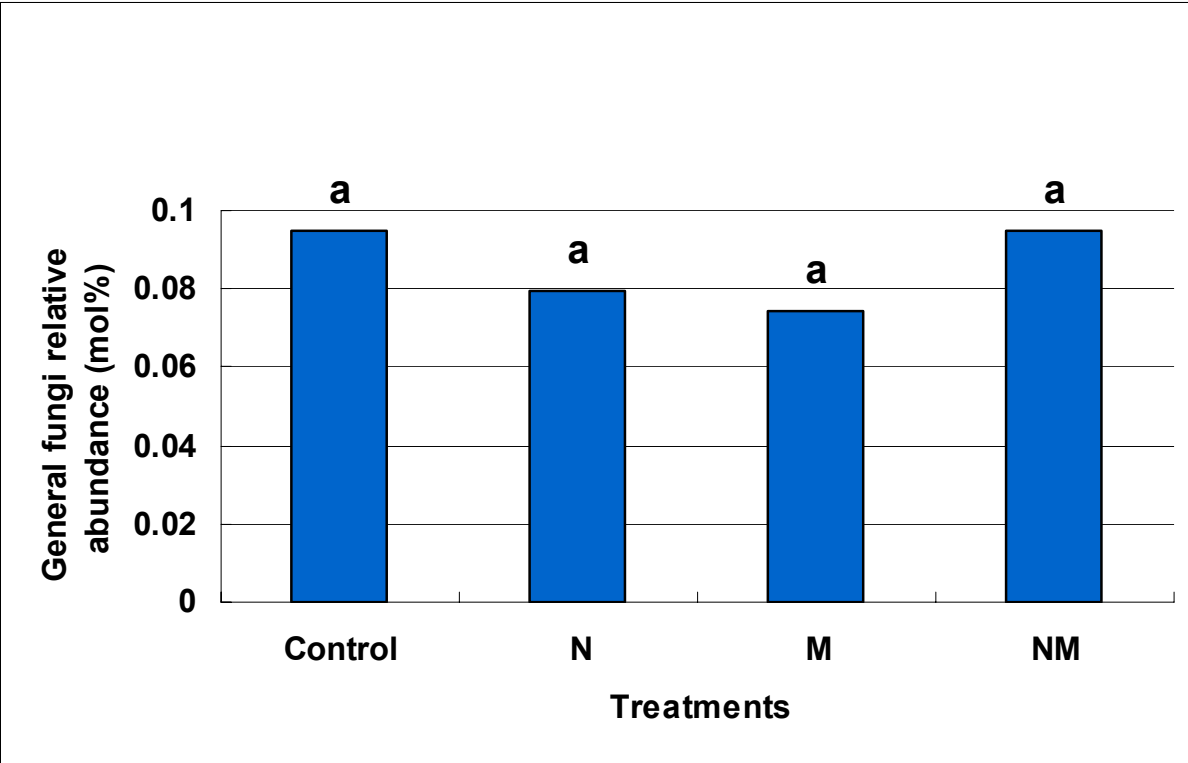
Figure A.21. Relative abundance general fungi indicated by PLFA at the end of the 3rd growing season. With nematodes (N) and with mycorrhizae (M). Different letters indicate significant difference (P<0.05)
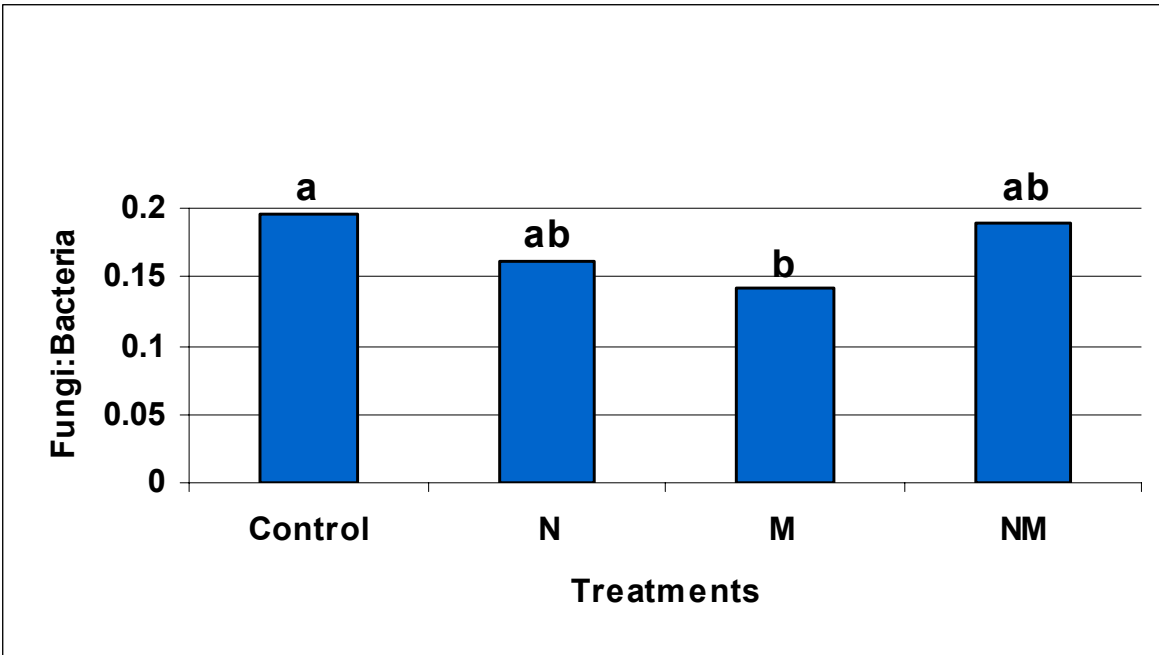
Figure A.22. Fungi to bacteria ratio indicated by PLFA at the end of the 3rd growing season. With nematodes (N) and with mycorrhizae (M). Different letters indicate significant difference (P<0.05)

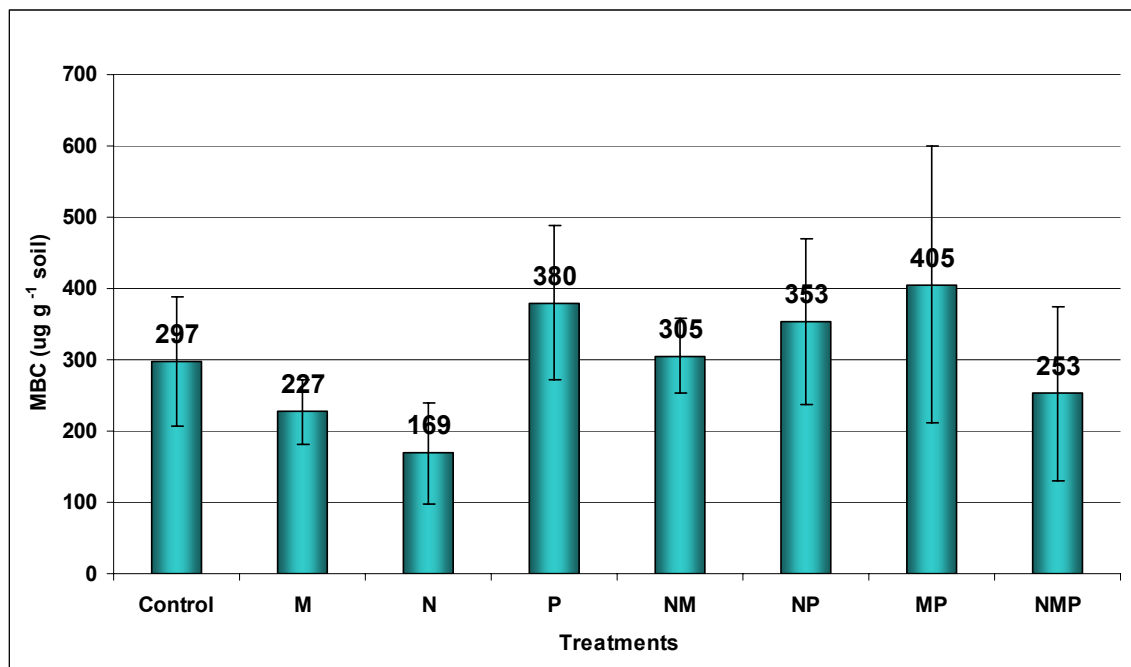**Figure A.23. Miocrobial biomass C measured by fumigation method at the end of the 3rd growing season. With nematodes (N) and with mycorrhizae (M). Different letters indicate significant difference (P<0.05).**
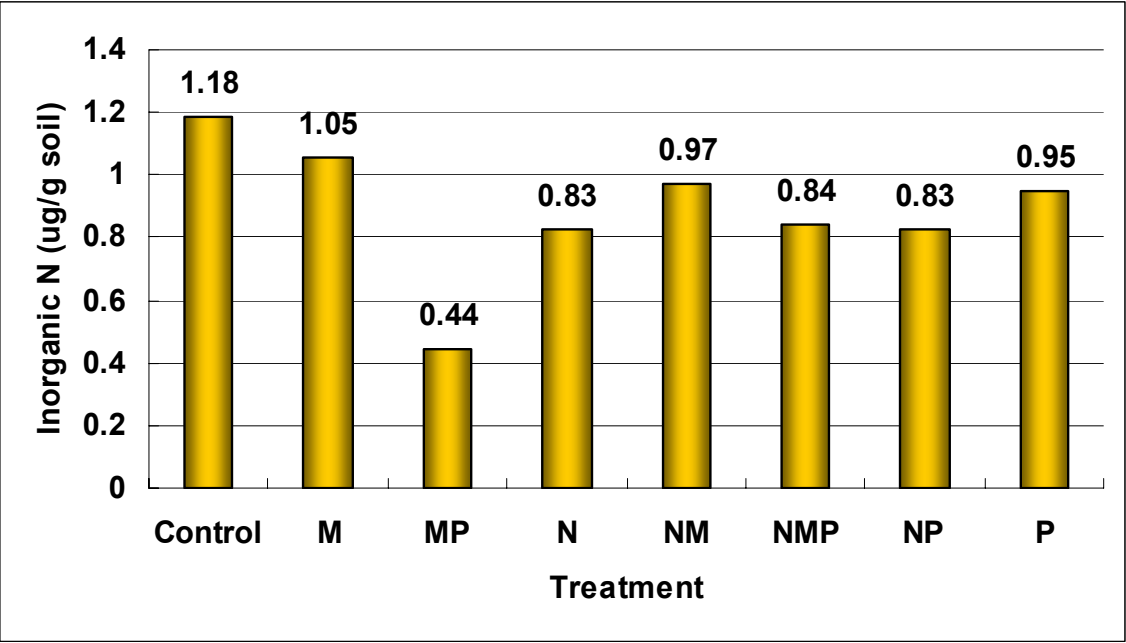
Figure A.24. Inorganic N in soil at the end of the 3rd growing season. With nematodes (N) and with mycorrhizae (M). Different letters indicate significant difference (P<0.05).
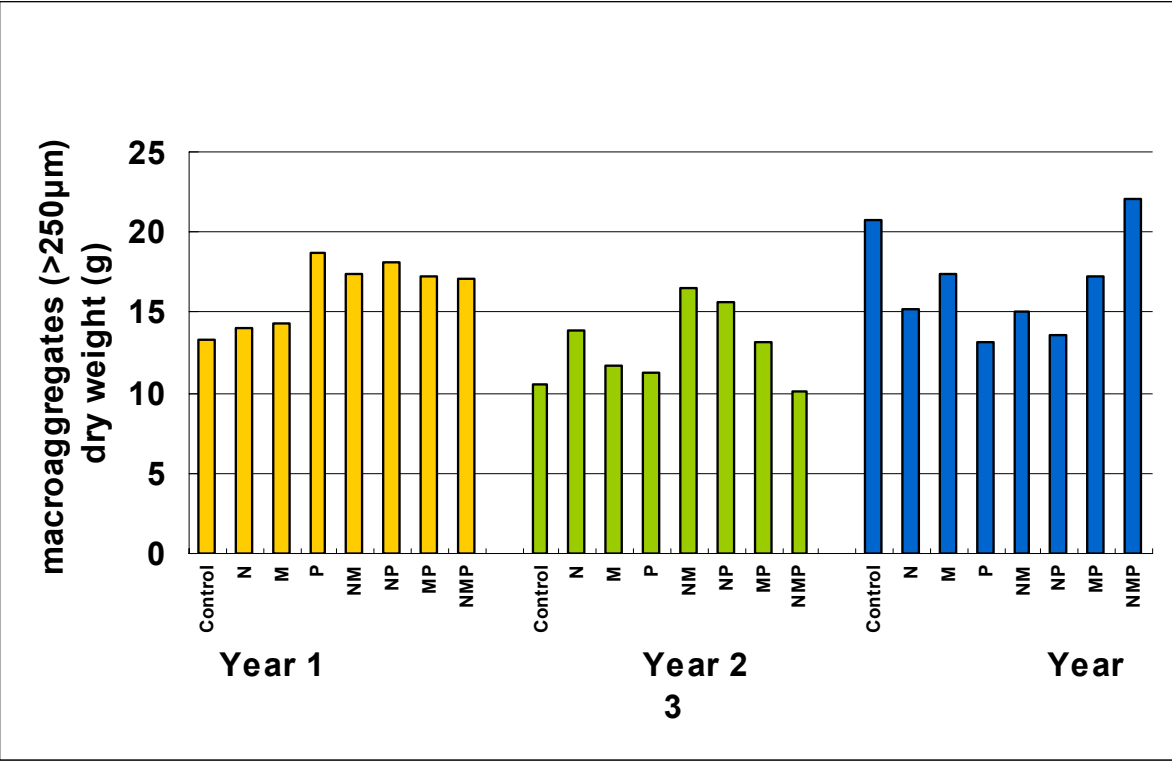
Figure A.25. Macroaggregates (>250 μm) from the 1st, 2nd, and the 3rd growing season.
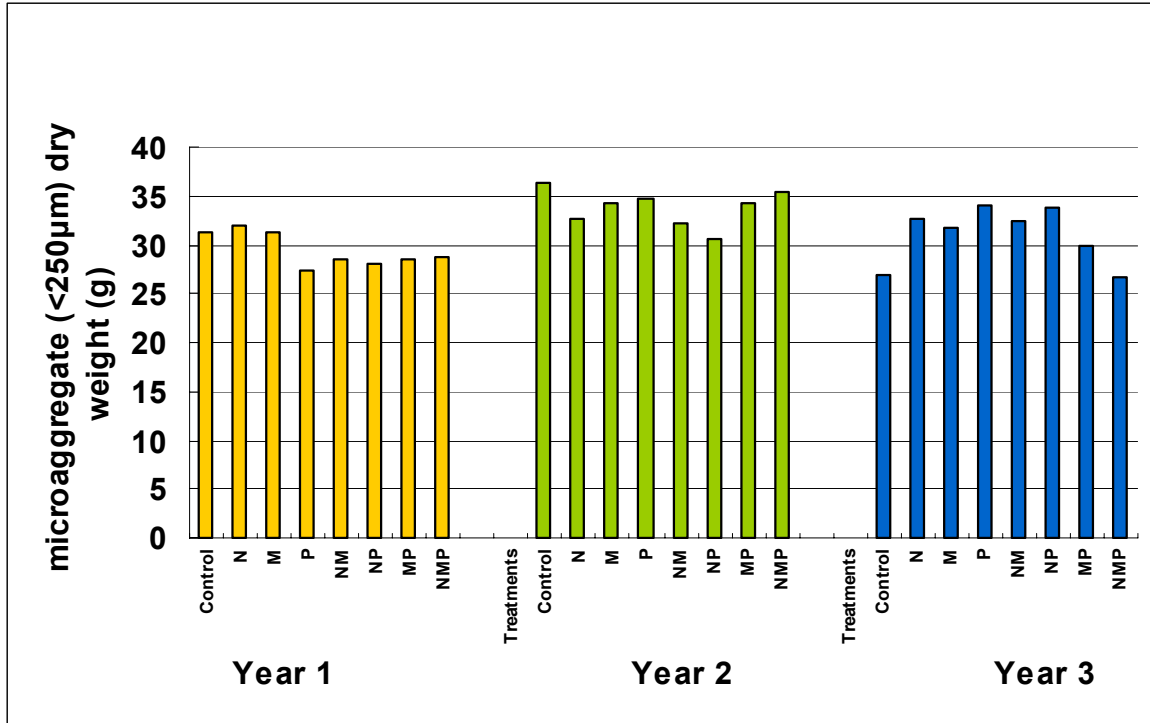
Figure A.26. Microaggregates (<250 μm) from the 1st, 2nd, and the 3rd growing season.

Figure A.27. Total N in macroaggregates (>2000 μm) from the 1st, 2nd, and the 3rd growing season.

Figure A.28. Total N in macroaggregates (250-2000 μm) from the 1st, 2nd, and the 3rd growing season.
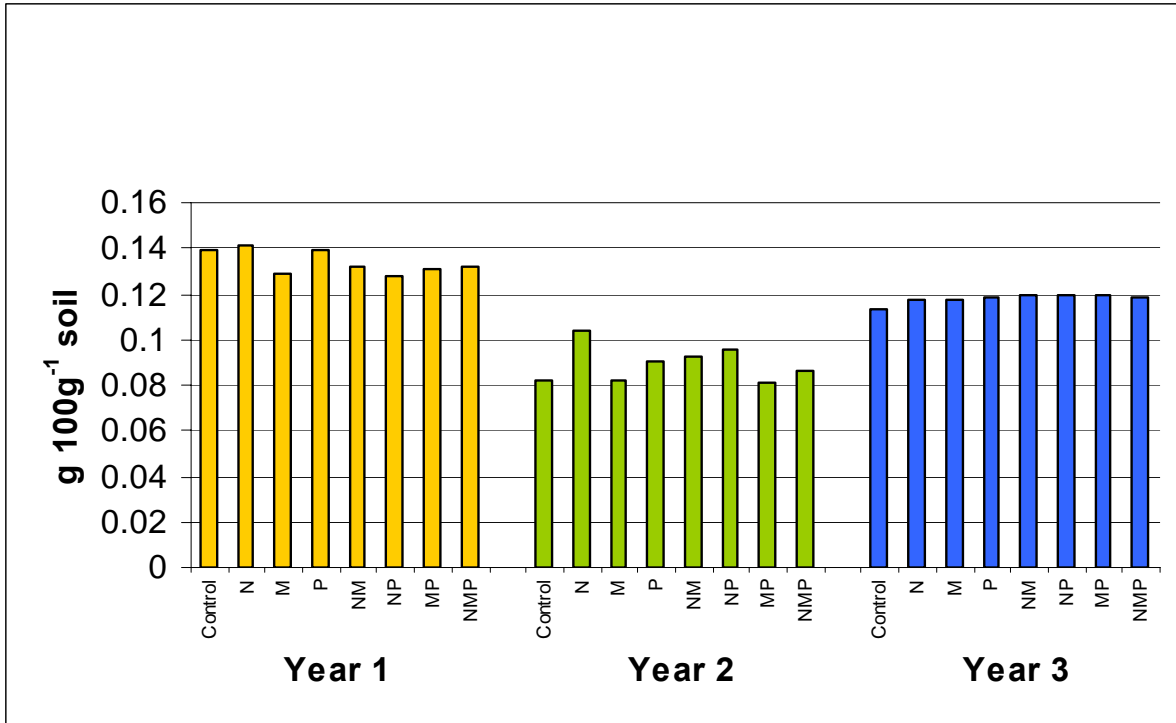
Figure A.29. Total N in microaggregates (53-250 μm) from the 1st, 2nd, and the 3rd growing season.
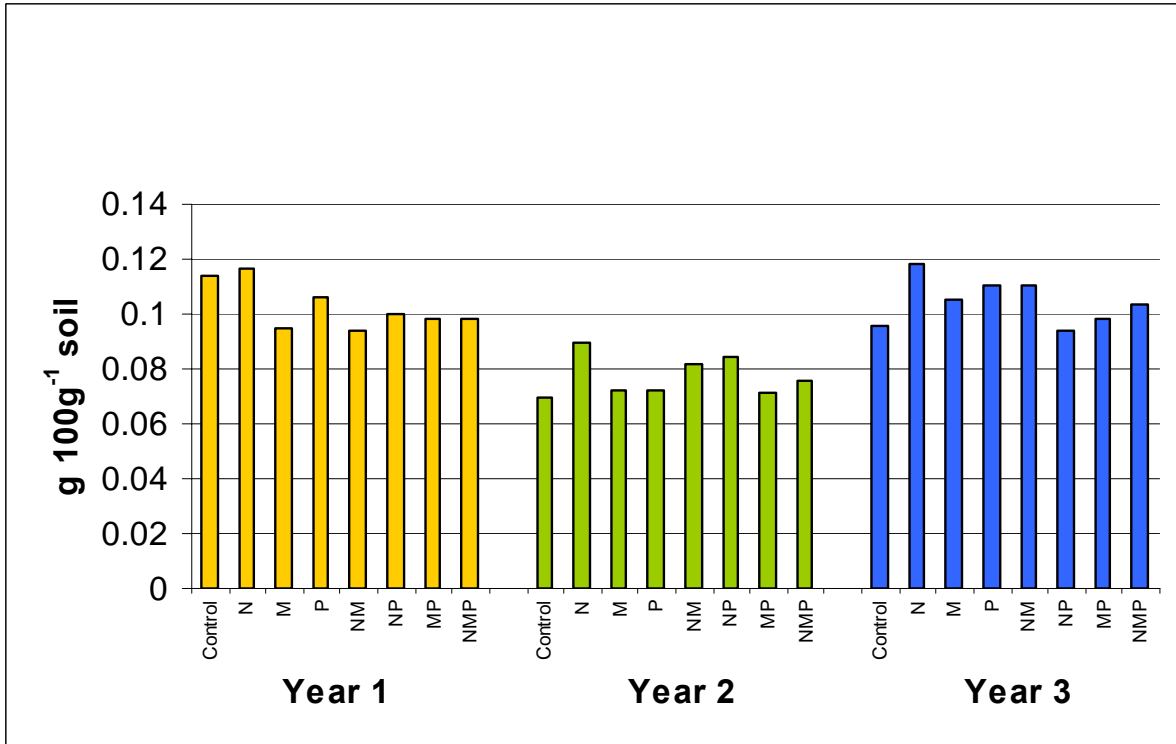
Figure A.30. Total N in microaggregates (53-20 μm) from the 1st, 2nd, and the 3rd growing season.
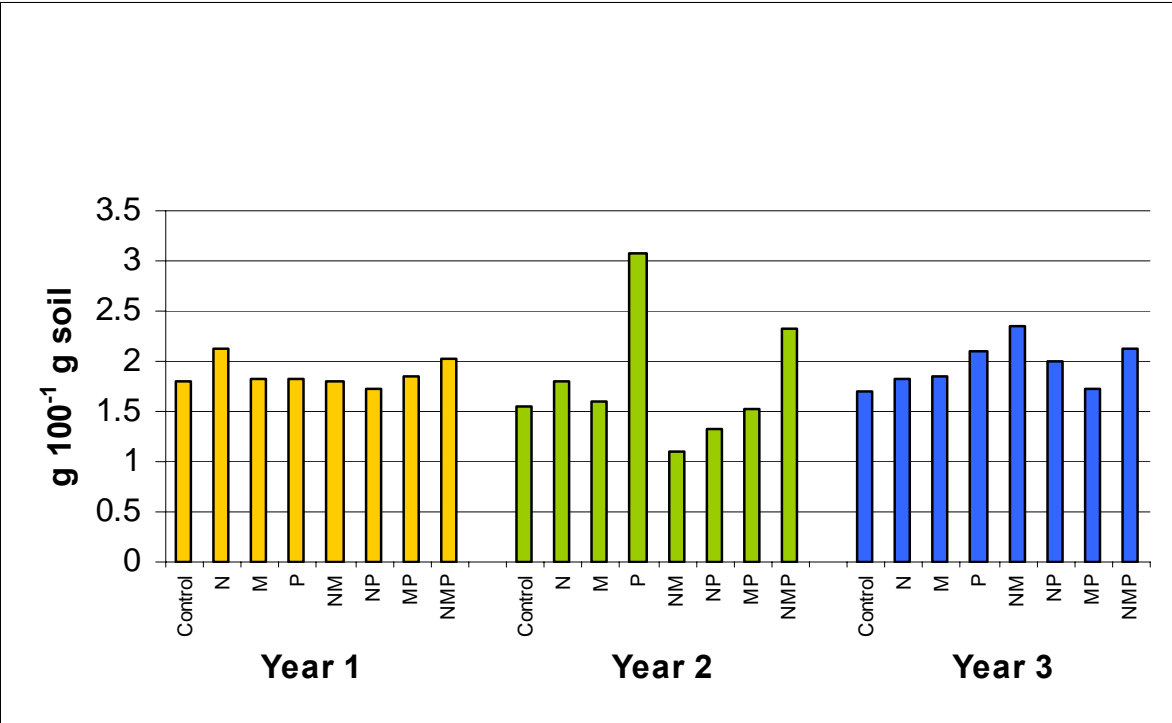
Figure A.31. Total C in macroaggregates (>2000 μm) from the 1st, 2nd, and the 3rd growing season.
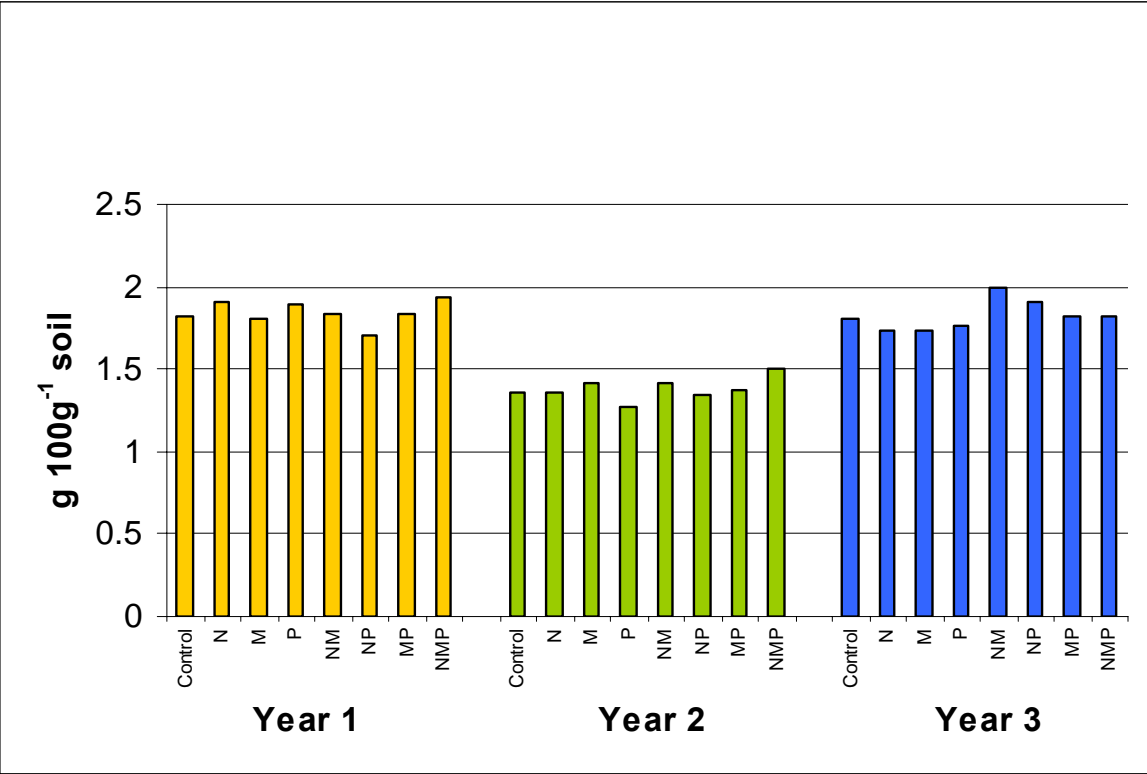
Figure A.32. Total C in macroaggregates (250-2000 µm) from the 1st, 2nd, and the 3rd growing season.
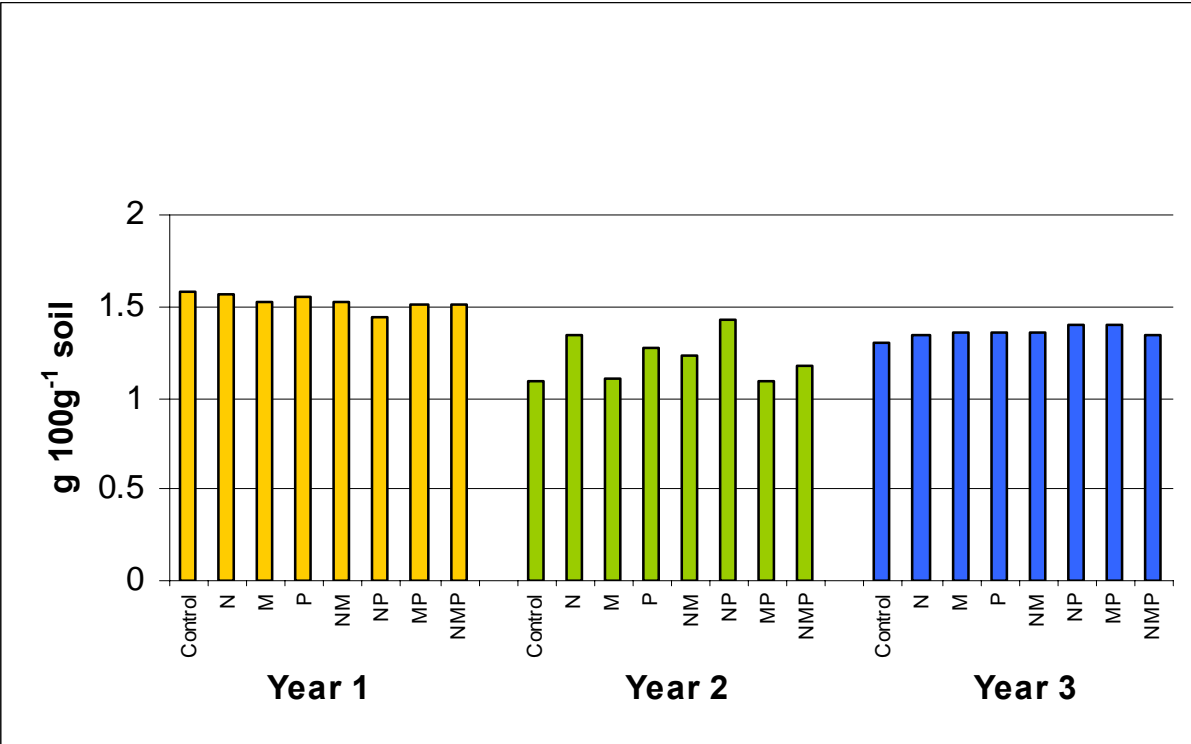
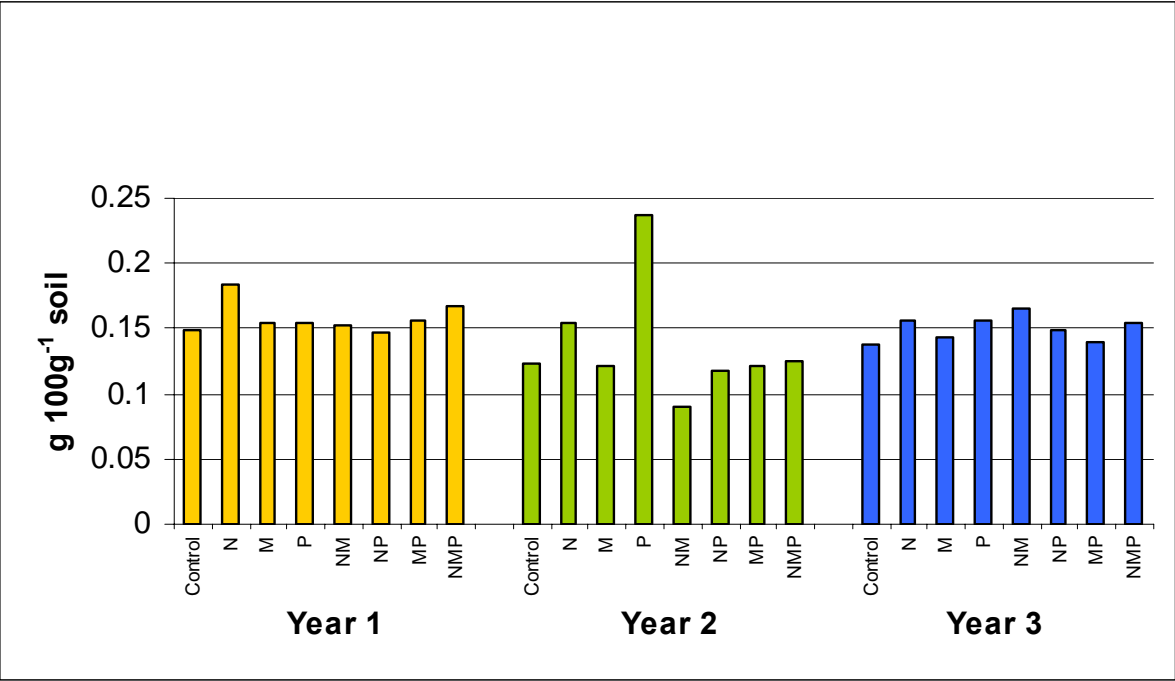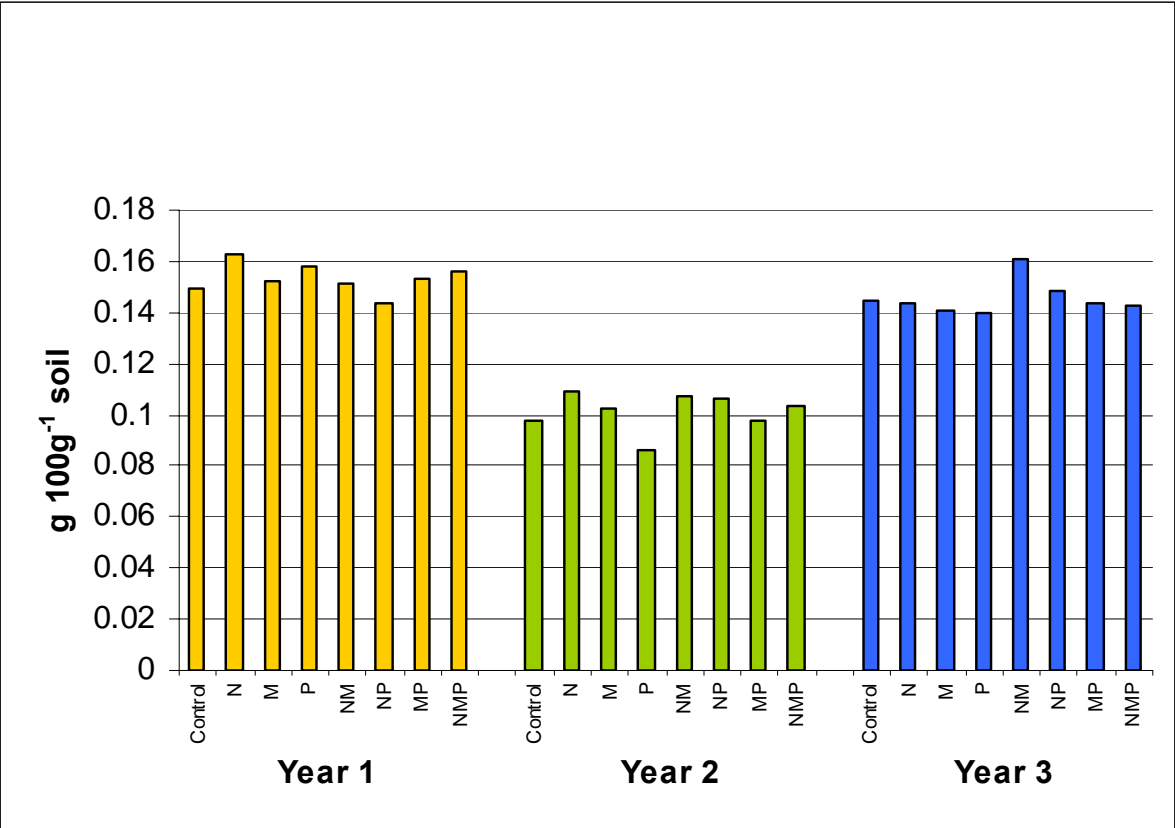**Figure A.33. Total C in microaggregates (53-250 µm) from the 1st, 2nd, and the 3rd growing season.**
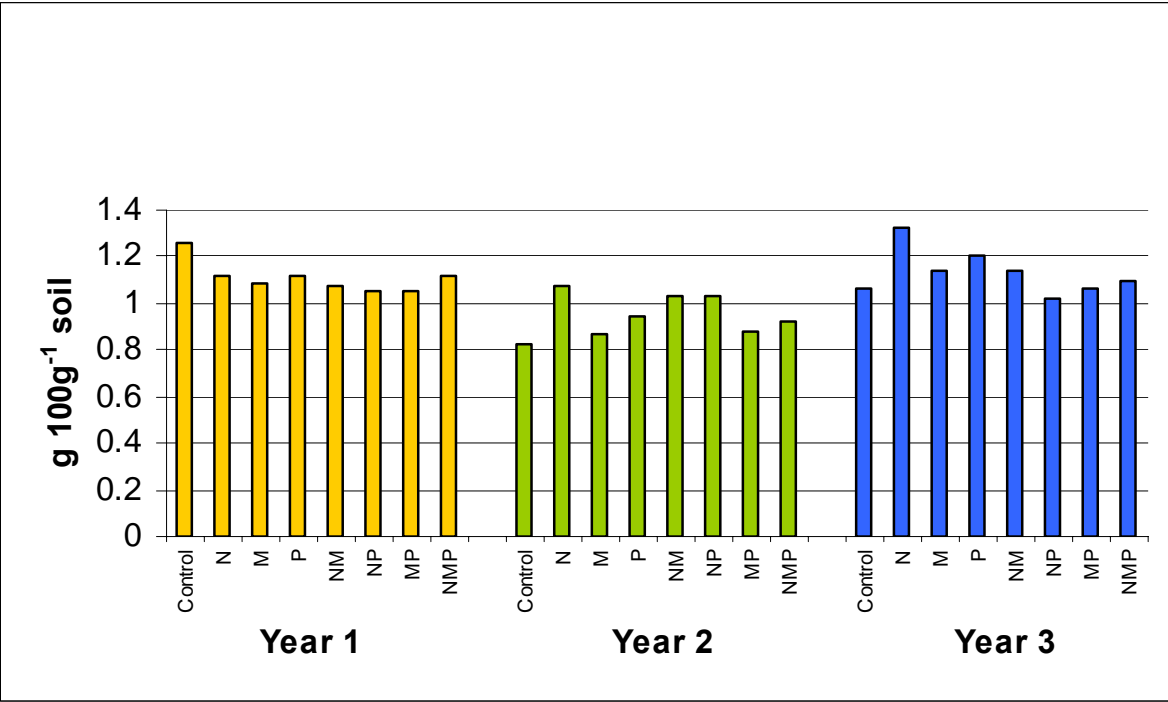
Figure A.34. Total C in microaggregates (20-53 µm) from the 1st, 2nd, and the 3rd growing season.

Table A.1. Above and belowground plant biomass of *Andropogon gerardii* Vit. at the end of the 2nd growing season.

Treatments	Aboveground Mean (g m^{-2})	SD	Belowground Mean (g m^{-2})	SD
Control	573.6	190.1	2175.4	3439.4
N	411.2	175.8	1200.1	536.5
M	903.1	54.0	2908.0	2715.9
P	752.0	79.2	4523.5	1219.1
NM	765.0	86.0	2551.9	772.5
NP	775.5	75.3	9173.1	4010.7
MP	858.4	174.5	5737.6	2246.4
NMP	816.9	406.6	7856.4	4457.6

N=nematodes, M=mycorrhizae, P=Phosphorus.

SD=standard deviation.

Table A.2. Analysis of variance of estimated soil microbial biomass C at the end of the 2nd growing season.

Effects	Microbial Biomass C
	P Value
N	0.3006
M	0.1040
P	0.0146
N*M	0.9654
N*P	0.9086
M*P	0.6990
N*M*P	0.0031

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table A.3. Fungi in *Andropogon gerardii* Vit. roots at the end of the 2nd growing season indicating root colonization by AM fungi.

Treatments	Colonization	AM Fungi
	%	µmol g^{-1} root
Control	0	0.015
N	0	0.10
M	64.25	0.020
P	0	0.022
NM	21.25	0.20
NP	0	0
MP	35.25	0.013
NMP	22.25	0

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table A.4. Abundance of fungi in *Andropogon gerardii* Vit. roots indicating root colonization by AM fungi at the end of the 3rd growing season.

Treatments	Colonization	AM Fungi
	%	μmol g^{-1} root
Control	41.5	0.25
N	5.5	0.11
M	24.5	0.22
P	31.8	0.05
NM	38.0	0.10
NP	31.5	0.43
MP	34.8	0.29
NMP	20.5	0.07

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table A.5. Analysis of variance of AMF and fungi to total bacterial abundance ratio as estimated from NLFA derived fatty acid indicators for fungi and PLFA derived fatty acid indicators for bacteria at the end of the 3rd growing season.

Effects	AMF:Bacteria	Fungi:Bacteria
	P Value	
N	0.9658	0.3724
M	0.0092	0.0009
P	0.2051	0.3565
N*M	0.1032	0.1768
N*P	0.0886	0.0325
M*P	0.0080	0.0051
N*M*P	0.2380	0.3646

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table A.6. AMF and all fungi to total bacterial relative abundance ratios as estimated from NLFA derived fatty acid indicators for fungi and PLFA derived fatty acid indicators for bacteria at the end of the 3rd growing season.

Treatments	AMF:Bacteria	Fungi:Bacteria
Control	1.16	0.38
N	0.51	0.64
M	1.05	0.71
P	0.60	0.94
NM	1.29	0.71
NP	1.16	0.41
MP	1.28	0.72
NMP	1.35	0.55

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table A.7. Analysis of variance of AMF and general fungal biomass as estimated from NLFA derived fatty acid indicators at the end of the 3rd growing season.

Effects	AM Fungi	General Fungi
	P Value	
N	0.8866	0.7222
M	0.0044	0.0019
P	0.4996	0.8107
N M	0.1626	0.6386
N*P	0.1569	0.0693
M*P	0.0417	0.0612
N*M*P	0.1046	0.2177

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table A.8. Aggregate Total C at the end of the 2nd growing season.

Treatments	>2000µm	250-2000µm	53-250µm	20-53µm
	g 100g^{-1} soil			
Control	1.5	1.4	1.1	0.83
N	1.8	1.4	1.3	1.1
M	1.6	1.4	1.1	0.87
P	3.1	1.3	1.3	0.94
NM	1.1	1.4	1.2	1.0
NP	1.3	1.3	1.4	1.0
MP	1.5	1.4	1.1	0.88
NMP	2.3	1.5	1.2	0.92

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table A.9. Aggregate isotopic C at the end of the 2^(nd) growing season.

Treatments	>2000µm	250-2000µm	53-250µm	20-53µm
		^{13}C PDB		
Control	-19.6	-17.7	-17.2	-16.7
N	-18.9	-18.0	-17.2	-17.1
M	-18.0	-18.1	-17.5	-17.1
P	-16.2	-17.5	-17.0	-16.7
NM	-16.9	-17.3	-16.5	-16.5
NP	-18.9	-18.0	-17.5	-17.3
MP	-18.0	-17.8	-17.5	-16.8
NMP	-18.4	-18.4	-18.0	-17.5

N=nematodes, M=mycorrhizae, P=Phosphorus.

Table A.10. Analysis of variance of aggregate isotopic C at the end of the 2nd growing season.

Effects	>2000µm	250-2000µm	53-250µm	20-53µm
		P Value		
N	0.9505	0.5612	0.9393	0.4595
M	0.7785	0.7426	0.6771	0.9155
P	0.8447	0.5234	0.2682	0.6025
N*M	0.3700	0.3820	0.5477	0.5659
N*P	0.5405	0.1518	0.1921	0.3239
M*P	0.2602	0.3714	0.3478	0.7432
N*M*P	0.4847	0.2726	0.5307	0.4495

N=nematodes, M=mycorrhizae, P=Phosphorus

Table A.11. Aggregate isotopic C at the end of the 3rd growing season.

Treatments	>2000µm	250-2000µm	53-250µm	20-53µm
	^{13}C PDB			
Control	-18.6	-19.2	-18.6	-18.2
N	-20.0	-19.3	-18.6	-18.2
M	-18.3	-19.0	-18.7	-18.3
P	-17.0	-18.6	-18.5	-18.2
NM	-18.1	-19.3	-18.7	-18.1
NP	-15.9	-19.1	-18.5	-18.2
MP	-18.2	-18.6	-18.4	-18.0
NMP	-18.4	-18.5	-18.7	-18.2

N=nematodes, M=mycorrhizae, P=Phosphorus.