AN ABSTRACT OF THE THESIS OF

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The effect of serial (multiple-year) organic matter (OM) amendment on soil properties has been described in some cropping systems, although less is known about the effect of serially amended field soils on soil-borne plant diseases. The objectives of this study were to describe the effects of the third and fourth years of annual, serial amendment with dairy manure solids on 1) soil physical and biological properties and 2) severity of sweet corn root rot. Plots were amended with five rates of separated dairy manure solids annually for three years. In the fourth year, plots were split and only half of each plot was re-amended. Soil physical properties [bulk density, free and occluded particulate organic matter (POM), soil water retention, total porosity, gravimetric moisture content] and biological properties [microbial activity (as hydrolysis of fluorescein diacetate; FDA) and microbial biomass-C] were assessed each year in all treatments. Root rot severity was assessed *in situ* and in the greenhouse with multiple sweet corn (*Zea mays* L. cv Golden Jubilee) bioassays conducted in the amended field soils.

Necrosis of the radicle and nodal roots was assessed when plants reached the 6-leaf stage. Amendment rate was positively associated with increases in soil properties that serve as indicators of soil quality, such as POM content, total porosity, microbial biomass, and FDA activity. In the third year after amendment, weak root rot suppression was observed in-field and was associated with FDA activity. By the fourth year of serial amendment this trend was no longer evident, however evidence from the high-rate treatment that was not re-amended (3H-NRA) pointed to an emerging suppressive mechanism that persisted up to 13 months after the third amendment. Factors that may be interacting over time to generate observed disease suppression in these serially amended soils include: short-term post-amendment microbiostasis, soil moisture retention, inoculum potential, and a novel suppressive mechanism.

Serial Manure Amendments: Effects on Soil Properties
and Root Rot of Sweet Corn

by
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TABLE OF CONTENTS

	Page
CHAPTER 1 LITERATURE REVIEW	1
Root rot of sweet corn in the Willamette Valley	2
Organic matter management and soil quality	4
Organic-matter-mediated disease suppression	11
Effect of serial organic matter addition on plant disease	16
CHAPTER 2 IMPACTS OF SERIAL DAIRY MANURE AMENDMENT ON SOIL PHYSICAL AND BIOLOGICAL PROPERTIES IN A SWEET CORN CROPPING SYSTEM	18
Abstract	19
Introduction	21
Materials and Methods	24
Results	38
Discussion	76
Conclusions	98
CHAPTER 3 IMPACTS OF SERIAL DAIRY MANURE AMENDMENT ON THE SEVERITY OF SWEET CORN ROOT ROT	100
Abstract	101
Introduction	102
Materials and Methods	105
Results	115
Discussion	135
Conclusions	150
SUMMARY	151
LITERATURE CITED	153

LIST OF FIGURES

Figure		Page
2.1	Schematic of the experimental design for the field trial that examined effects of 5 levels of annual amendment with dairy manure solids on soil properties and severity of sweet corn root rot. In 2004, the original plots were split and half of each plot received a fresh re-amendment (RA) while half was not re-amended (NRA)..	26
2.2	Bulk density in freshly re-amended (RA) and not re-amended (NRA) treatments, 3 weeks after amendment in 2004. 4H-RA- 4 years of high-rate (H) amendment, 3H-NRA- 3 years of H amendment, 1H-RA- 1 year of H amendment. Error bars represent ±1 SE..........................	41
2.3	Free a) and occluded b) particulate organic matter (POM) throughout the four years of the field trial in the high (H), medium-low (ML), and control (C) treatments. Vertical lines represent amendment applications. Error bars, when shown, represent ±1 SE...	46
2.4	Free a) and occluded b) particulate organic matter (POM) dynamics in re-amended (RA) and not re-amended (NRA) high treatments and the control in 2004. Error bars represent ±1 SE..	51
2.5	In-field gravimetric moisture content before and 3 weeks after re-amendment (2004) in freshly re-amended (RA) and not re-amended (NRA) treatments. Error bars represent ± 1 SE	56
2.6	Gravimetric moisture content 20 h before and 120 h following an irrigation event. Soil samples were taken 11 weeks after re-amending in 2004. Irrigation occurred at 0 hrs. Error bars represent ± 1 SE (not shown for the 3H-NRA and 1H-RA treatments)..	61

LIST OF FIGURES (Continued)

Figure		Page
2.7	Soil moisture release curves for a) freshly re-amended (RA) and b) not re-amended (NRA) treatments. Soils were sampled 14 weeks after amendment in 2004. Volumetric moisture content was measured at 5 water potentials: 0 kPa, -30 kPa, -100 kPa, -300 kPa, -500 kPa. Error bars represent ±1 SE	64
2.8	Soil microbial activity (as FDA activity) throughout all sampling dates in 2003. Error bars represent ±1 SE	69
2.9	Soil microbial activity (as FDA activity) throughout all 2004 sampling dates in the a) not re-amended (NRA) treatments and b) in the freshly re-amended (RA) treatments. Error bars represent ±1 SE	70
2.10	Soil microbial activity (as FDA activity) throughout the 2003 and 2004 seasons of the field trial for the high (H), medium-low (ML) and control (C) treatments. Solid symbols represent the not re-amended (NRA) splits of the described treatments. Vertical lines represent amendment applications. Error bars, when shown, represent ±1 SE	71
2.11	Microbial biomass-C from all soil sampling dates in 2003. Error bars represent ±1 SE	73
2.12	Microbial biomass-C from all soil sampling dates in 2004 season in the a) not re-amended (NRA) treatments and b) freshly re-amended (RA) treatments. Error bars represent ±1 SE	74
2.13	Baseline (pre-amendment) values of soil microbial biomass-carbon 12 months after amendment in 2001, 2002, and 2003. Only the high (H), medium-low (ML), and control (C) treatment levels are shown. Error bars represent ±1 SE	75

LIST OF FIGURES (Continued)

Figure		Page
2.14	Correlation plots for five key soil variables: rate of hydrolysis of fluroescein diacetate (FDA), microbial biomass-carbon (MBC), free and occluded particulate organic matter (fPOM and oPOM, respectively), and gravimetric soil moisture content (MST). Each point represents a treatment mean (n=4) for one sampling date. All sampling dates in 2003 and 2004 are shown...	96
3.1	Schematic of the experimental design for the field trial examining effects of 5 levels of annual amendment with dairy manure solids on soil properties and severity of sweet corn root rot. In 2004, the original plots were split and half of each plot received a fresh re-amendment (RA) while half was not re-amended (NRA)..	108
3.2	Radicle rot severity from sweet corn grown in a) greenhouse bioassays and b) the in-field bioassay conducted in serially amended field soils in 2003. Error bars represent ±1 SE	116
3.3	Root rot severity (radicle and nodal, as specified) from sweet corn grown in serially amended field soils in 2004. Greenhouse bioassays were conducted on soils from a) freshly re-amended (RA) and b) not re-amended (NRA) treatments. Error bars represent ±1 SE..............................	118
3.4	Radicle rot severity from sweet corn grown in serially amended field soils in 2004. In-field bioassays were conducted on soils from a) freshly re-amended (RA) and b) not re-amended (NRA) treatments. Error bars represent ±1 SE...	121
3.5	Summary of results from assessments of radicle rot severity of sweet corn throughout the four years of the study. Effects of five levels of serial manure amendment on root rot severity are shown for a) 2001, b) 2002, c) and d) 2003, and e) 2004. All disease severity ratings are from greenhouse bioassays except d) which represents in-field radicle rot severity. Panel c) includes one assessment of nodal root rot severity..	126

LIST OF FIGURES (Continued)

Figure		Page
3.6	Relationships between in-field soil moisture content and radicle rot severity of sweet corn in 2003 a) before re-amendment with manure solids, and b) 8 weeks and c) 12 weeks after amendment. Graphs a) and c) represent greenhouse bioassays, b) represents an in-field assessment..	129
3.7	Relationships between soil microbial activity (as FDA activity) and radicle rot severity of sweet corn in 2003 a) before re-amendment with manure solids, and b) 8 weeks and c) 12 weeks after amendment. Graphs a) and c) represent greenhouse bioassays, b) represents an in-field assessment..	130
3.8	Pre-amendment relationships between greenhouse root rot severity of sweet corn and free particulate organic matter (fPOM) were positive across all years of the study. Samplings occurred 12 months after amendment of dairy manure solids in 2001, 2002, and 2003. Radicle rot severity is shown for 2001 and 2002. 2003 shows nodal root rot severity and excludes the high (H) treatment from the regression..	131
3.9	Eight weeks after amendment with dairy manure solids, relationships between root rot severity of sweet corn and free particulate organic matter (fPOM) changed with serial amendment. 2001 and 2002 data represent radicle rot severity from greenhouse bioassays. 2003 data represent in-field radicle rot severity. 2004 data represent radicle rot severity from greenhouse bioassays 6 weeks after amendment with the 4H-RA treatment excluded from the regression..	132

LIST OF FIGURES (Continued)

Figure | Page

3.10 Relationships between in-field soil moisture content and root rot severity of sweet corn in 2004 a) before amendment and b) 3 weeks, c) 6 weeks, d) 9 weeks, and e) 12 weeks after re-amendment. Pre-amendment (a.) greenhouse nodal root rot severity is shown. In-field radicle rot severity is shown in all other panels. In (a.) the H treatment (○) was excluded from the regression.. 133

3.11 Relationships between soil microbial activity (as FDA activity) and root rot severity of sweet corn in 2004 a) before amendment and b) 3 weeks, c) 6 weeks, d) 9 weeks, and e) 12 weeks after re-amendment. Pre-amendment, (a.) greenhouse nodal root rot severity is shown. In-field radicle rot severity is shown in all other panels. In (a.) the H treatment (○) was excluded from the regression.. 134

LIST OF TABLES

Table		Page
1.1	A summary of the literature reporting effects of mid- to long-term serial organic amendment (OA) on soil properties..........	7
2.1	Five different levels of dairy manure solids amendment were made annually over 4 years of the field trial conducted in Oregon. Amendment rates varied each year, but were consistently assigned by treatment level. Plots were split in 2004, and half of each plot was freshly re-amended (RA) while the other half was not-re-amended that year (NRA). In 2001 and 2002, amendments included composted dairy manure solids (CMS) and fresh dairy manure solids (MS)................	30
2.2	Characteristics of fresh separated dairy manure solids in 2003 and 2004..	38
2.3	Bulk density from selected sampling times before and after re-amendment in 2003 and 2004. SE in parentheses...............	39
2.4	Free and occluded particulate organic matter (POM) contents from each soil sampling in 2003....................................	43
2.5	Free and occluded particulate organic matter (POM) contents from each soil sampling in 2004....................................	49
2.6	Gravimetric moisture content from all in-field soil samplings in 2003..	54
2.7	Gravimetric moisture content from in-field (INF) and greenhouse (GH) soil samples in 2004. Standard error in parentheses..	57
2.8	Total porosity of soils sampled 14 weeks after 2004 amendment. Porosity was determined by measuring volumetric moisture content of water-saturated intact soil cores. Each value represents the treatment mean, n=4.................................	62
2.9	Soil microbial activity (as FDA activity) and soil microbial biomass-carbon from all sampling dates in 2003..................	66

LIST OF TABLES (Continued)

Table		Page
2.10	Soil microbial activity (as FDA activity) and soil microbial biomass-carbon from all sampling dates in 2004	67
2.11	Correlation coefficients (r) and associated p-values for the relationships between 5 key soil variables: microbial activity (FDA), microbial biomass-carbon (MBC), free and occluded particulate organic matter (fPOM and oPOM, respectively), and gravimetric moisture content. Relationships were determined among 64 treatment means (n=4)	97
3.1	Five different levels of dairy manure solids amendment were made annually over 4 years of the field trial conducted in Oregon. Amendment rates varied each year, but were consistently assigned by treatment level. Plots were split in 2004, and half of each plot was freshly re-amended (RA) while the other half was not-re-amended that year (NRA). In 2001 and 2002, amendments included composted dairy manure solids (CMS) and fresh dairy manure solids (MS)	107
3.2	Rating scale to assess root rot severity of sweet corn from in-field and greenhouse bioassays conducted in 2003	112
3.3	Rating scale to assess root rot severity of sweet corn from in-field and greenhouse bioassays conducted in 2004	113

CHAPTER 1

LITERATURE REVIEW

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ROOT ROT OF SWEET CORN IN THE WILLAMETTE VALLEY

The Willamette Valley in Oregon is a fertile agricultural area that produces a variety of vegetables for the processing industry. In the past three years, Oregon has ranked fourth nationally in the production of sweet corn for processing, much of which is grown in the Willamette Valley (NASS-USDA, 2004). In recent years, however, growers have observed a yield decline in corn production which has affected the profitability of corn plantings. In the 1990's root rot was identified as a significant cause of the yield reductions, and by 2004 three causal agents were identified: *Pythium arrhenomanes, Phoma terrestris, and Drechslera sp.* (Hoinacki et al., 2004).

The pathogen complex affects healthy corn roots by causing necrosis of radicle and nodal roots, leading to a reduction in overall root biomass. The primary symptoms occur as dark-brown to black necrotic lesions on the corn radicle. Symptoms may be visible as early as 3-4 weeks after plant emergence, and severely infested fields may exhibit radicles with 100% necrosis as early as 6 weeks after emergence (Hoinacki et al., 2004). Nodal root lesions occur after radicle infection, and develop slowly until the plant reaches the silking stage, after which lesions expand and whole roots become necrotic (Hoinacki et al., 2004). Secondary symptoms include leaf "firing" or chlorosis, small ears, poor tip-fill of ears, dimpled kernels, and an overall reduction in yield (Hoinacki et al., 2004).

Generally root rot pathogens are classified as nonobligate parasites. They are capable of carrying out life functions as soil inhabitants, usually in association with decaying organic material (Agrios, 1997). Such organisms can overwinter as sclerotia, as spores, or as mycelium on infected plant tissue or debris (Agrios, 1997). The fungi and oomycete organisms that cause root rot of sweet corn are common soil inhabitants (Hoinacki et al., 2004). *Pythium spp.* and *Phoma terrestris* have been previously described as causal agents of root rot of corn in Ohio (Rao et al., 1978), Delaware (Mao et al., 1998) and Georgia (Sumner et al., 1990). In pathogenicity experiments with corn *Pythium arrhenomanes* was moderately virulent (Sumner et al., 1990), and the presence of the pathogen negatively affected corn growth (Yanar et al., 1997).

In seeking to manage the impact of sweet corn root rot in the Willamette Valley, early scouting of fields has been useful to assess the root rot potential of field soils. However to date, no effective management strategies other than tolerant varieties have been developed. Chemical fumigation with a combination of methyl bromide and chlorpicrin reduced root rot severity by 68%-89% and increased yields by up to 50% (Hoinacki et al., 2004), but this control strategy is not economically viable for growers.

Willamette Valley farmers are not alone in their vulnerability to the impacts of root rot pathogens. Soilborne diseases have significant impacts on yield and quality of many vegetable crops (Abawi and Widmer, 2000). Root rot diseases, by affecting below-ground plant parts, are profoundly affected by the physical, biological and chemical properties of the soil environment (van Bruggen, 1995). Frequently in root rot diseases, high soil moisture favors the growth of the pathogens and poor soil conditions can increase the severity of root rot diseases

(Abawi and Widmer, 2000; Cook and Papendick, 1972). Soil water availability and soil aeration affect the growth and mobility of fungal and bacterial plant pathogens (Griffin, 1970; Cook and Baker, 1983). In an Oregon study of the effects of irrigation level on the severity of sweet corn root rot, the most severe disease ratings (radicle and nodal roots) were observed under the highest irrigation level (Peachey et al., 2004). In a Hoytville silty-clay loam in Ohio, slow water drainage from the heavy clay soil contributed to increased susceptibility of corn to root rot caused by *Pythium arrhenomanes* (Deep and Lipps, 1996). Because corn root rot is favored by poor soil drainage and increased duration of flooding (Yanar et al., 1997), conditions of poor soil quality can contribute to disease susceptibility (Hodges and Scofield, 1983). Given the impact of soil conditions on root rot diseases, it is not surprising that indicators of soil health also serve as indicators of disease suppression (van Bruggen and Semenov, 2000).

ORGANIC MATTER MANAGEMENT AND SOIL QUALITY

Soil quality is defined as "the capacity of a soil to perform functions which sustain biological productivity and maintain environmental quality (Herrick and Wander, 1997)." Traditionally, farmers relied on regular applications of organic materials such as animal manures or crop residues to meet plant nutrient requirements and improve soil quality. More recently, increased reliance on intensive cropping systems--characterized by the use of inorganic fertilizers, chemical pesticides, short rotations, and heavy machinery--has had negative impacts on the healthy function of agricultural soils (Altieri, 1995).

Recent comparisons of conventionally managed farms and organic or biodynamic farms have illustrated management-induced changes in soil properties. In a comparison of conventionally and organically managed tomato systems in California, Drinkwater et al. (1995) reported that total organic C and N mineralization potential were higher, and inorganic N content lower, in organically managed fields compared to conventionally managed fields. In addition, incidence and severity of corky root of tomato was lower on organic farms. In New Zealand, fields managed biodynamically for at least 8 years had significantly greater soil organic matter (SOM) content, soil respiration, and mineralizable N, and lower bulk density, than conventionally farmed soils (Reganold, 1993). Other studies have found similar improvements in soil biological, physical and chemical properties under organic or alternative management practices (Jordahl and Karlen, 1993; Workneh and van Bruggen, 1994; Fraser et al., 1988). Many of the indicators of soil quality—stable aggregate formation, porosity, water-holding capacity, total organic carbon (TOC), microbial activity--are those directly associated with increases in the SOM pool (Doran and Zeiss, 2000; Fraser et al., 1988). Although organic matter comprises a small percentage of the weight of most soils, the type and amount of SOM present have far-reaching influences on most of the soil properties, and are often key determinants of soil quality (Magdoff, 1995).

SOM is comprised of living and dead organic material, and includes all of the soil carbon bound in organic forms (Herrick and Wander, 1997). Organic matter varies greatly in age, chemical composition, turnover rate, and function in the soil. Most commonly, SOM is classified into three distinct pools: the biologically active (labile), the physically protected, and the recalcitrant or stable fractions (Herrick and Wander, 1997). These pools are associated with distinct functions; labile organic matter drives biological activity in soils, while recent or slightly aged OM affects soil physical properties (Wander, 2004). The recalcitrant materials, comprising the largest SOM pool, most strongly influence the physiochemical reactivity of soils, such as through increases in sorption and cation exchange capacity (Wander, 2004).

Weil and Magdoff (2004) recently reviewed the significance of SOM to soil quality and overall soil health. High levels of SOM are generally associated with improvements in soil biological, physical, and chemical properties, and serial OM amendment has been shown to induce many of these changes. Table 1.1 presents a summary of the effects of mid- and long-term serial amendment (with various amendment types) on soil properties.

Table 1.1 A summary of the literature reporting effects of mid- to long-term serial organic amendment (OA) on soil properties.

Yrs of OA	OA type(s)	Annual rate(s)	Soil type	Key results	Citation
2	composted cattle manure	15, 30, 45 Mg ha^{-1} (wet wt)	silt loam	* Increased proportion of WSA > 4 mm, decreased WSA < 0.25 mm in compost-amended soil * Changes observed as soon as 5 months after 1st application	Whalen et al., 2003
3 apps. in 2 years	poultry manure, sewage sludge, barley straw, alfalfa	25 Mg ha^{-1}	Arlington	* Amendment increased soil respiration, aggregate stability, organic C content, and soil moisture content; decreased bulk density	Martens and Frankenberger, 1992
4	urban waste compost	10, 30, 90 t ha^{-1}	clay loam	* Compost increased total porosity (esp. elongated pores), decreased bulk density, increased water retention and plant available water (PAW), increased CEC * Soil C increase not proportional to amount of organic C added * Total porosity increased linearly with compost rate	Giusquiani et al., 1995
4	sewage sludge compost, beef manure	compost: 33, 67, 134, 268 Mg ha^{-1} manure: 67, 134 Mg ha^{-1} dry wt	loamy sand	* Compost decreased bulk density, increased water retention and PAW	Tester, 1990
4	deinking and secondary paper sludge (fresh and composted)	fresh: 8, 16, 24 t ha^{-1} compost: 24 t ha^{-1} (dry wt)	silty clay, loam, and sandy loam	* Amendment increased structural stability and decreased bulk density in the silty clay and loam soils * Air capacity and available water was increased in the silty clay only * Fresh sludge had a greater positive impact on total porosity, air capacity, and AW than compost	Nemati et al., 2000

Table 1.1 (Continued)

Yrs of OA	OA type(s)	Annual rate(s)	Soil type	Key results	Citation
5	cattle feedlot manure	60, 120, 180 Mg ha^{-1}	clay loam	* SOM in the surface 0-15 cm increased with increasing manure rate * Soil T was cooler in spring with high amendment rate * Manure amendment decreased bulk density, increased proportion of aggregates > 1 mm, decreased proportion of aggregates < 0.1 mm	Sommerfeldt and Chang, 1985
5 apps. once, alt. yrs, & annually	paper mill sludge	45, 90, 135, 180, 225 Mg ha^{-1} (dry wt)	sandy loam	* Single-year amendment did not affect physical properties five years later * High amendment rates increased soil aggregation and moisture holding properties	Zibilske et al., 2000
9	composted cattle manure (+/- biodynamic preparations)	30 Mg ha^{-1}	Fluvisol (Germany)	* Compost increased soil pH, [P] and [K], microbial biomass C (MBC), dehydrogenase activity, earthworm cast production, and cotton strip decomposition	Zaller and Kopke, 2004
11	cattle manure (4yr slurry then 7 yr solid)	7.5-27.4 Mg ha^{-1} (dry wt)	silt loam	* Manure amendment decreased bulk density by 10%, doubled K_s in surface soil, and increased soil water retention capacity	Arriaga and Lowery, 2003
18	cattle manure	20 Mg ha^{-1}	loam	* Manure amendment increased OM level of whole soil, increased formation of slake-resistant macroaggregates, did not change OM in fraction < 53 μm	Aoyama et al., 1999 a
18	cattle manure	20 Mg ha^{-1}	silt loam	* Manure increased mineralized C in most aggregate fractions * Crushing aggregates increased mineralized C * Manure amendment increased size of protected C pool (up to threefold)	Aoyama et al., 1999 b
20	farm-yard manure (FYM)	10 t ha^{-1}	loamy sand	* FYM increased soil organic C, infiltration rate, water retention, aggregation and aggregate stability	Benbi et al., 1998

Table 1.1 (Continued)

Yrs of OA	OA type(s)	Annual rate(s)	Soil type	Key results	Citation
24	beef cattle manure	60, 120, 180 Mg ha^{-1} (wet wt)	clay loam	* Bulk density was negatively related to rate, was affected by season * High amendment rates reduced air permeability * Soil T varied with amendment rate	Miller et al., 2002
70	horse manure	100 t ha^{-1}	typical Brunisol (France)	* Manure amendment increased SOM, water retention, soil stability, and porosity	Pernes-Debuyser and Tessier, 2004
90	FYM/animal slurry	variable rates based on NPK supply	sandy loam	* FYM increased SOC by 23%, CEC by 17%, increased soil water content, and decreased bulk density	Schjonning et al., 1994
23-121	FYM of various types	variable: 6-30 t ac^{-1}	various	* FYM increased porosity by increasing microporosity and decreasing macroporosity * FYM increased volumetric moisture content at all matric potentials tested, increased available water capacity	Rose, 1991

A number of long-term studies have described the effects of serial manure amendment on soil properties. The Morrow plots in Illinois have followed the effects of almost 100 years of regular manure amendment on corn yields under three different rotations: continuous corn (CC), corn-oat (CO) and corn-oat-hay (COH). Initiating manure addition led to immediate increases in corn yield, and yields in high SOM plots were greater than in lower SOM plots. In the manured CC treatment, corn yield was generally comparable to that of the non-manured long-rotation treatment (COH) indicating that either serial manure addition or long rotation alone can sustain corn productivity over the long term (Aref and Wander, 1998). A study of the effects of five years of beef feedlot manure amendment reported increases in OM content of the surface soil (0-15cm), and a decrease in bulk density at this depth in the manured treatments (Sommerfeldt and Chang, 1985). Other long-term studies have reported increased soil water-holding capacity (Rose, 1991), increased cation exchange capacity (Schjonning et al., 1994), greater enzymatic activities (Giusquiani et al., 1995), and greater total porosity (Giusquiani et al., 1995) in soils receiving multiple years of OM amendment. Although many studies have reported changes in soil properties resulting from OM additions, few have described the effects of serial amendment on soil-borne diseases affecting crops. This literature has been reviewed previously (Stone et al., 2004; van Bruggen, 1995).

Recently, traditional farming systems and organic farming systems have been studied for their benefits in maintaining soil health and improving plant health. Two ancient Mexican agricultural systems—the Chinampa system practiced by the Aztecs and the Maize Marceno (March Corn) system developed by the Mayans—have been shown to suppress plant disease caused by soil-borne pathogens (Lumsden et al., 1983). Both systems rely on the use of large amounts of OM. Resulting disease incidence was related to SOM content, Ca content, water-holding capacity, and microbial activity. Similarly, traditional Chinese farmers make large annual additions of organic materials, and have only limited occurrence and impact of root diseases (Thurston et al., 1994). In a comparative study of organic and conventional tomato cropping systems in California, the incidence and severity of both corky root and Phytophthora root rot was higher in conventional farms than in organic farms that had received at least 3 years of annual additions of legume residues, animal manure, compost, and/or earthworm castings (Workneh et al., 1993).

ORGANIC MATTER MEDIATED DISEASE SUPPRESSION

Previous work on the ability of organic amendments to induce suppression of soil-borne plant diseases has lead to an interest in studying the potential for disease suppression of sweet corn root rot in the Willamette Valley. In situations of general disease suppression, competition among the total population of soil microorganisms for nutrients and energy-sources (particularly carbon) contributes to reduced success of the pathogen (Cook and Baker, 1983; Hoitink and Boehm, 1999). In any suppressive system, often more than one mechanism contributes to disease reduction (Alabouvette, 1990). Identified mechanisms of suppression include microbiostasis, colonization or destruction of pathogen propagules, antibiosis, competition for substrate or for root-infection sites, and induced systemic resistance (Stone et al., 2004; Davis et al., 1996; Alabouvette, 1990).

The biological nature of disease suppression has been demonstrated in experiments where suppressive soil was sterilized with steam, methyl bromide, or γ-rays and suppression was lost (Scher and Baker, 1980; Alabouvette, 1990). When sterilized soil was reinoculated with a portion of suppressive soil, suppression was restored (Scher and Baker, 1980). However, the significance of soil biological factors in disease suppression does not preclude the importance of soil physical and chemical factors and the impact of soil nutrient status on plant disease (Stone et al., 2004; Alabouvette et al., 1996; Davis et al., 2001). A study of high nitrogen organic amendments (poultry and swine manure) demonstrated that ammonia, nitrous acid, and volatile fatty acid production during decomposition contributed to a reduction in viable microslerotia of *Verticillium dahliae* (Lazarovits, 2001). Pallant et al. (1997) described the beneficial impact of OM amendment on corn root growth; root networks were more dense in soil pockets rich in OM. The impact on disease of assorted abiotic factors--soil texture and structure, soil water potential, clay type, pH, and nutrient availability--will vary with the environmental and physiological requirements of a given pathogen (Alabouvette et al., 1996).

Recent reviews have described many examples of disease suppression in containers and field trials (Stone et al., 2004; Hoitink and Boehm, 1999). In a suppressive soil, despite the presence of the necessary conditions for disease development—pathogen, susceptible host, and conducive environment—disease incidence remains low (Alabouvette, 1990). While specific microbial antagonists have been identified in certain suppressive systems (Stone et al., 2004; Hoitink and Boehm, 1999), disease reduction in many soils is attributed to conditions of general suppression, which is related "to the total amount of microbiological activity at a time critical to the pathogen (Cook and Baker, 1983)". Fungistasis, or microbiostasis, is the best understood and documented mechanism involved in OM-mediated general suppression, although its effects are tied to OM quality which changes over time as decomposition proceeds (Stone et al., 2004).

Soil microorganisms exist in an energy-limited environment and competition for nutrients is strong (Fontaine et al., 2003; De Nobili et al., 2001). Available energy-yielding nutrients are rapidly utilized when added to a system (De Nobili et al., 2001). Under conditions of energy stress, germination and growth of microbial spores is limited, resulting in "microbiostasis" or "fungistasis" (named for the repression of fungal spores) (Lockwood, 1977). Immediately following the addition of a labile energy source, fungistasis may be lost, but resumes, often at a higher level, after slight degradation of the energy source (Lockwood, 1990).

OM-mediated fungistasis depends on both the quantity and decomposition level of the organic matter (Stone et al., 2004; Hoitink and Boehm, 1999), and is a transient response of the soil biological community to the addition of a large amount of labile organic material. Much of what is known about OM-mediated disease suppression comes from work on *Pythium* damping off (DO) in peat- and compost-amended soil-less container mixes (Hoitink and Boehm, 1999; Boehm and Hoitink, 1992). Different types of sphagnum peat representing different decomposition stages were studied for the potential to induce disease suppression in containers, clarifying the importance of OM quality in suppression (Boehm et al., 1997; Boehm and Hoitink, 1992). Light peat from the surface of a bog, representing a slightly decomposed material, was suppressive to *Pythium* DO. As the light peat decomposed, the ability to suppress DO was lost. Dark peat taken from the deeper and more highly decomposed region of the bog was conducive to DO (Boehm and Hoitink, 1992). OM decomposition level was inversely related to microbial activity, and positively related to disease severity (Boehm and Hoitink, 1992). Any peat generating a rate of FDA hydrolysis above 3.2 $\mu g\ min^{-1}\ g^{-1}$ dry weight potting mix was suppressive to *Pythium* DO and Pythium root rot (Boehm and Hoitink, 1992). OM-mediated suppression can be generated by the high-rate application of many different types of organic amendment. Such suppression is of short duration (typically lasting weeks to 1 year) and is positively related to microbial activity (Stone et al., 2004).

The effect of compost decomposition level on DO of cucumber was investigated in compost-amended sand mixes in a container study (Stone et al., 2001). Larger-size and less-decomposed POM sustained suppression of DO for up to 1 year. Suppressive POM was very similar in composition to lightly decomposed forest litter and material from soil organic horizons (Stone et al., 2001).

In the study in an Oregon field soil, raw and composted dairy manure solids (DMS) suppressed root rot of sweet corn in the first and second years of annual amendment (Darby et al., in press). As soon as 6 months after amending, suppression was lost. Interestingly, in both years there was evidence that high-rate amendments resulted in higher disease severity 12 months after amending, compared to the un-amended control (Darby et al., in press). Suppression was positively related to FDA activity and to free POM (fPOM). There is evidence that POM loses its ability to induce suppression as OM decomposes (Inbar et al., 1991). This residual POM as well as POM-induced changes to soil physical properties contribute to increased water-holding capacity of highly amended soils (Benbi et al., 1998). These OM-induced physical changes are of interest because conditions of increased soil moisture can exacerbate root diseases (Peachey et al., 2004; Kerr, 1964; Bhatti and Kraft, 1992).

A reduction in inoculum density is not necessary for disease suppression. Frequently in suppressive soils disease incidence is low even at high inoculum densities (Alabouvette, 1996). Davis et al. (1999 a) showed that large differences in Verticillium wilt incidence and severity occurred under green manure treatments with the same inoculum density. In another study, Davis et al. (1996) reported similar levels of Verticillium control both with and without a reduction in soil inoculum.

EFFECT OF SERIAL ORGANIC MATTER ADDITION ON PLANT DISEASE

To date, much of the work describing and characterizing soil suppressiveness has been conducted in soil-less container mixes, or in field soils that have received only a single year of organic amendment. While much is known about the impact of single-year OM additions on disease suppression, less is known about the effect of serial OM amendment on disease, particularly in field soils (Stone et al., 2004). Field studies assessing single-season low rate OM amendments have generally reported highly variable impacts on disease incidence and yield (Lewis et al., 1992; Lumsden et al., 1986) but high-rate amendments more consistently generate disease suppression (Stone et al., 2003; Darby et al., in press). Longer term studies have reported more predictable improvements in yield, quality, and disease suppression (Aref and Wander, 1998; Workneh et al., 1993; Darby et al., in press). However in serially amended systems, little is known about how a specific cropping system and a pathogen's life history relate to disease suppression. In addition, it is not clear if serial amendment generates mechanisms of suppression other than the commonly recognized "general suppression."

It is known that long-term OM additions alter soil properties, but the direct effect of these cumulative soil changes on plant disease is less clear. Soil water retention generally increases as OM content and aggregation increase, creating conditions of increased moisture that favors root rot of sweet corn (Peachey et al., 2004; Giusquiani et al., 1995). However, fresh OM amendment provides soil microorganisms with a pool of labile OM, elevating the level of soil microbial activity, and frequently inducing general suppression. In serially amended field soils, it is likely that an interaction between soil biological and physical properties guides disease severity.

The primary objective of this study was to 1) describe the effects of 1, 3, and 4 years of serial addition of dairy manure solids on soil biological and physical properties and 2) to describe treatment effects on severity of root rot of sweet corn. A secondary objective was to identify the soil properties most strongly associated with disease suppression in a serially amended field system. By building on the previous two years of work in this field site (Darby et al., in press), it was possible to compare the effects of first and second year OM amendment with effects following the third and fourth years of treatment.

CHAPTER 2

IMPACTS OF SERIAL DAIRY MANURE AMENDMENT ON SOIL PHYSICAL AND BIOLOGICAL PROPERTIES IN A SWEET CORN CROPPING SYSTEM

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ABSTRACT

The effect of serial (multiple-year) organic matter (OM) amendment on soil properties has been described in some cropping systems, although less is known about how OM-induced changes affect soil-borne plant diseases. The objective of this study was to describe the effects of one, three, and four years of annual, serial amendment with dairy manure solids on 1) soil physical properties and 2) soil biological properties in an agricultural field soil. Plots were amended with five rates of separated dairy manure solids annually for three years. In the fourth year, plots were split and only half of each plot was re-amended. Soil physical properties [bulk density, free and occluded particulate organic matter (fPOM and oPOM, respectively)], water-holding capacity, gravimetric moisture content) and biological properties [fluorescein diacetate (FDA) activity, microbial biomass-carbon (MBC)] were assessed each year in all treatments. After four years of serial amendment, soil water retention and total porosity increased with increasing amendment rate but neither property changed after a single high-rate amendment. Following re-amendment, soil fPOM content increased significantly but little residual increase in fPOM was observed 12 months after amendment. Bulk density was negatively related to amendment rate. Both FDA activity and microbial biomass-C (MBC) were positively related to amendment rate, and baseline (pre-amendment) levels of FDA activity and MBC increased each season. The soil properties that respond rapidly to amendment (FDA activity, fPOM content) increased markedly in re-amended (RA) treatments but not in not-re-

amended (NRA) treatments, while the properties that respond more slowly to OM amendment (MBC, oPOM, water retention, porosity) were more similar in RA and NRA treatments. Serial manure amendment enhanced the soil properties that serve as indicators of soil quality.

INTRODUCTION

The importance of organic matter (OM) amendment to agricultural soils has long been recognized (Paustian et al., 1997). Farmers have traditionally relied on the use of animal manure, cover crops, and other organic amendments to maintain soil quality and soil fertility (Paustian et al., 1997). However, recently the application of manure to agricultural fields has become less common with the increased adoption of inorganic fertilizers to meet crop nutrient needs and growing concerns over the environmental impact and ease of transport of manure for organic amendments (Sims, 1995). Early in the 20th century a renewed interest in the use of OM amendments emerged when disease suppression was identified in agricultural systems receiving OM additions, although the mechanisms driving suppression are not fully understood (reviewed in Stone et al., 2004; Hoitink and Boehm, 1999). The link between OM additions and disease suppression may be clarified by an examination of the soil property changes induced by organic amendment.

Organic matter has been identified as a key determinant in soil quality (Magdoff, 1995; Doran et al., 1996). Soil organic matter (SOM) has far-reaching influences on most of the soil properties even though it comprises a small percentage of the weight of most soils (Magdoff, 1995). SOM is classified into three distinct pools, each associated with distinct functions (Herrick and Wander, 1997). SOM in the labile pool drives biological activity in soils, the recent or slightly aged SOM affects soil physical properties, and the recalcitrant pool impacts soil physiochemical properties such as sorption and cation exchange capacity (Wander, 2004). Serial amendment with labile OM such as fresh animal manure affects each of these SOM pools over time and has the potential to impact multiple soil properties.

Manure amendments can improve soil quality by increasing microbiological activity, improving aggregation, and increasing soil organic carbon content (Benbi et al., 1998; Nemati et al., 2000; Zaller and Kopke, 2004). Many studies have described the effects of mid- and long-term organic amendment (OA) on soil physical properties. A review of the effects of various organic waste applications on soil physical properties indicated a significant negative relationship between soil organic C and bulk density, and indicated that soil water holding capacity was affected by texture and also was positively related to soil organic carbon content (Khaleel et al., 1981). Whalen et al. (2003) reported increases in the proportion of water-stable aggregates > 4mm as soon as 5 months following amendment with 15, 30, or 45 Mg ha^{-1} (wet wt basis) of composted cattle manure. Improvements in aggregation (Sommerfeldt and Chang, 1985), aggregate stability (Martens and Frankenberger, 1992; Nemati et al., 2000), total porosity (Giusquiani et al., 1995; Nemati et al., 2000), and soil water retention (Giusquiani et al., 1995; Tester, 1990; Zibilske et al., 2000) were observed in studies of the effects of 5 or fewer annual organic amendments.

Soil microorganisms exist in an energy-limited environment so the addition of a labile source of carbon can stimulate growth and activity (De Nobili et al., 2001; Fontaine et al., 2003). The size and activity of the microbial biomass is directly related to the amount and decomposition level of the organic carbon supplied by OA (Fraser et al., 1988; Powlson et al., 1987). In soils receiving 7 years of annual beef feedlot manure additions (12-14 Mg ha^{-1}, dry wt basis), soil microbial biomass contents were 10% to 26% higher compared to non-manured soils (Fraser et al., 1988). Increases in soil microbial activity (as hydrolysis of fluorescein diacetate; FDA) were observed 8 weeks after amendment with raw (16.8 or 33.6 Mg ha^{-1}, dry wt basis) or composted (28 or 56 Mg ha^{-1}, dry wt basis) dairy manure solids (Darby et al., in press).

The primary objective of this study was to describe the effects of one, three, and four years of annual, serial amendment with dairy manure solids on 1) soil physical properties and 2) soil biological properties in a sweet corn cropping system. A secondary objective was to evaluate seasonal changes in soil properties, with particular attention to sampling dates just before and just following amendment.

MATERIALS AND METHODS

Site description

In 2001, plots were established within a 30.5 m by 86.9 m parcel on the Oregon State University Vegetable Research Farm in the Willamette Valley of Oregon. The soil is classified as a Chehalis silt loam (fine-silty, mixed, mesic Cumulic Ultic Haploxeroll). The valley has a Mediterranean climate with cool, wet winters and warm, dry summers. The mean annual temperature is 11.5°C. The mean annual precipitation is 111 cm, the majority of which falls between November and March.

Cropping history

The cropping history for the field site was snap bean (*Phaseolus vulgaris* L.) in 2000 and 1999, fallow in 1998 and 1997, and sweet corn (*Zea mays* L.) in 1996 and 1995. In the first two years of this experiment, sweet corn (cv Golden Jubilee) was grown to harvest in 2001, and in 2002 snap bean (cv Oregon 91G) was grown to harvest.

Experimental design

Manure treatments were initially randomly assigned in 2001 within a complete block design (Figure 2.1). Four blocks containing ten plots each were established within the study site. The blocking factor was an observed soil texture gradient between the east and west field edges. Plots originally measured 6.1 m by

9.1 m, and each treatment was initially replicated 8 times within the field site. In the summer of 2002, a sudangrass (*Sorghum bicolor)* cover crop treatment was applied to half of the field plots. These cover cropped plots have been excluded from this study, therefore beginning in 2003 the number of replicates was reduced to four per treatment. In 2004, plots were split and a randomly assigned half of each plot received a fresh manure re-amendment (RA), including the control plots, while the other half was not amended in 2004 (NRA). Once split, the plots measured 6.1 m by 4.6 m.

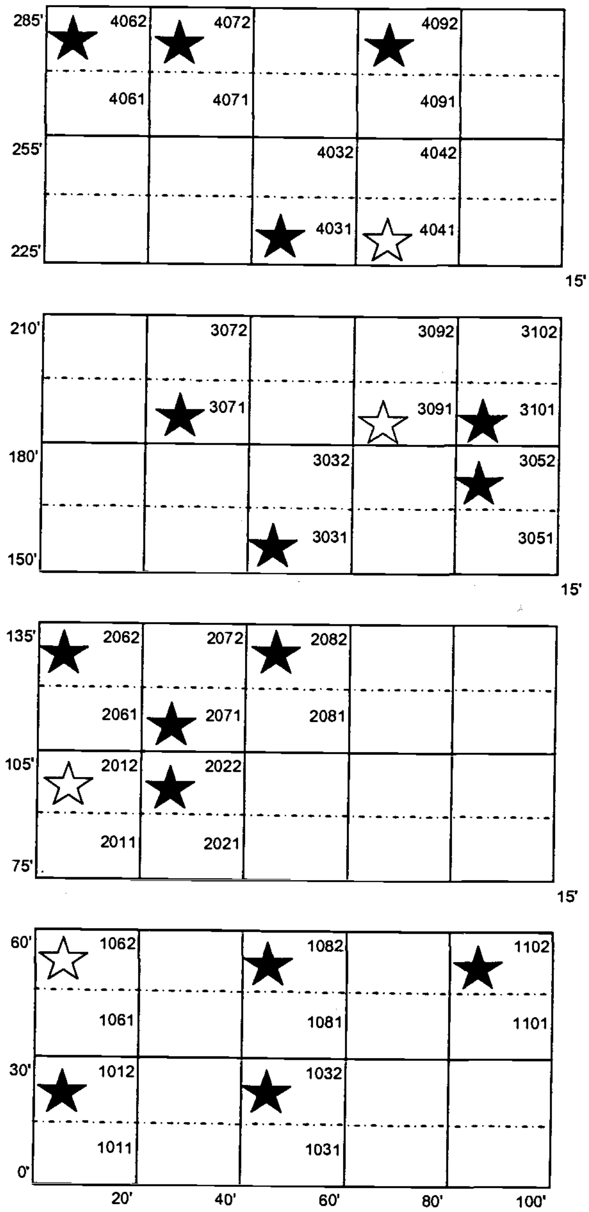
Figure 2.1 Schematic of the experimental design for the field trial that examined effects of 5 levels of annual amendment with dairy manure solids on soil properties and severity of sweet corn root rot. In 2004, the original plots were split and half of each plot received a fresh re-amendment (RA) while half was not re-amended (NRA).

← North

	Treatment assignments 2003	2004 RA	2004 NRA
Abbrev.	H	4H-RA	3H-NRA
Plot	101	1012	1011
	202	2022	2021
	307	3071	3072
	407	4072	4071
Abbrev.	MH	MH-RA	MH-NRA
Plot	103	1032	1031
	208	2082	2081
	303	3031	3032
	406	4062	4061
Abbrev.	ML	ML-RA	ML-NRA
Plot	108	1082	1081
	207	2071	2072
	305	3052	3051
	409	4092	4091
Abbrev.	L	L-RA	L-NRA
Plot	110	1102	1101
	206	2062	2061
	310	3101	3102
	403	4031	4032
Abbrev.	C	1H-RA	C
Plot	106	1062	1061
	201	2012	2011
	309	3091	3092
	404	4041	4042

★ serial RA

☆ 1st year RA



In 2003 a single planting of sweet corn (*Zea mays* L.) cv Golden Jubilee was grown to harvest, and in 2004 sweet corn was grown to the 6-leaf stage in four two-row staggered plantings three weeks apart.

The field site was irrigated weekly throughout the growing season with 2.54 cm of water delivered via overhead sprinklers.

Corn emergence was evaluated in 2003 as number of plants row-meter^{-1} to assess any treatment effects on seedling emergence. Yield data were taken at harvest in 2003, including ear weight, length, and width; kernel depth; and aboveground plant biomass. In 2004, no plants matured to harvest and no yield data were taken.

Treatments

Organic amendments were applied each spring for 4 years (Table 2.1). On May 29, 2003 and May 4, 2004, raw dairy manure solids (MS) were applied on a weight basis at different rates to give five levels of manure treatment: high (H), medium-high (MH), medium-low (ML), low (L), and no manure (control; C) (Table 2.1). The MS were purchased from a local dairy and consisted of the solid fraction of fresh dairy manure that had been separated using a screen separator. Amendments were incorporated with a small rotovator and a Roterra to a depth of 15 cm.

The MS was analyzed for nutrient content and chemical characteristics each year. On the day the MS was received, 10 shovelfuls were taken from randomly selected locations within the MS pile. The sample was composited and mixed, and a subsample was sent to Agri-Check Inc. (Umatilla, OR) for nutrient analysis. Six subsamples of approximately 30 g each were oven-dried to determine MS moisture content. This was used to calibrate actual MS application rates from treatment assignments based on dry MS. In addition, two approximately 500 g subsamples were frozen as reference material.

Actual manure application rates varied in each of the study's four years. Amendments made in 2001 and 2002 included two treatments of composted dairy manure solids (CMS) instead of MS (Table 2.1). In determining the placement of 2003 rates, the new high rate was matched to the previous high rate based on free particulate OM (fPOM) remaining 6 months after 2002 amendment (data not shown). Composted manure solids were not included in the 2003 treatments because 2001 and 2002 data indicated that composting did not enhance suppressiveness of the manure (Darby et al., in press). Rates in 2004 were increased from 2003 rates because of similarity between low rate treatments, and to widen the range of amendment rates.

In the spring of 2004, plots were split and manure amendments were applied to a randomly selected half of each plot (Figure 2.1). This permitted a comparison of disease severity throughout the season between freshly re-amended (RA) and not re-amended (NRA) split-plots. RA and NRA treatments received the same tillage management following re-amendment. When plots were split, half of the control treatment (C) was re-amended for the first time at the high rate (H); these treatments were given the designation 1H-RA, to represent the first year of high-rate amendment applied in 2004. When the H treatment was split, the re-amended half was designated 4H-RA (4 years of serial, high-rate amendment) while the not re-amended half was designated 3H-NRA (3 years of residual, serial, high-rate amendment).

Sweet corn (cv Golden Jubilee) was planted at a rate of 8 seeds m^{-1} in all treatments on June 16, 2003 and simultaneously fertilized with a 12-29-10 starter fertilizer applied at a rate of 67 kg N ha^{-1}. Plants were thinned to approximately 5 plants m^{-1} after emergence. At the 6-leaf stage, all control plots were sidedressed with an additional 112 kg N ha^{-1} (Darby, 2003). In 2004, sweet corn (cv Golden Jubilee) was planted in each plot two rows at a time on May 21, June 21, July 9, and August 3 and thinned to 5 plants m^{-1} after emergence. Fertilizer was applied at planting as described above when rows were marked at the first planting date. The August 3 planting received additional fertilizer because of the late planting date.

Table 2.1 Five different levels of dairy manure solids amendment were made annually over 4 years of the field trial conducted in Oregon. Amendment rates varied each year, but were consistently assigned by treatment level. Plots were split in 2004, and half of each plot was freshly re-amended (RA) while the other half was not-re-amended that year (NRA). In 2001 and 2002, amendments included composted dairy manure solids (CMS) and fresh dairy manure solids (MS).

Treat-ment	**Abbrev. pre-split**	**Abbrev. post-split in 2004**	**Manure amendment rate**				
			2001	**2002**	**2003**	**Plot split**	**2004**
			------------------ dry Mg ha^{-1} ------------------				dry Mg ha^{-1}
High	H	4H-RA	56 CMS	33.6 CMS	22.4 MS	RA	33.6 MS
		3H-NRA				NRA	no manure
Medium-high	MH	MH-RA	28 CMS	16.8 CMS	16.8 MS	RA	22.4 MS
		MH-NRA				NRA	no manure
Medium-low	ML	ML-RA	33.6MS	33.6 MS	11.2 MS	RA	16.8 MS
		ML-NRA				NRA	no manure
Low	L	L-RA	16.8 MS	16.8 MS	5.6 MS	RA	5.6 MS
		L-NRA				NRA	no manure
Un-amended control	C	1H-RA	no manure	no manure	no manure	RA	33.6 MS
		C				NRA	no manure

Soil sampling

Soils were sampled repeatedly throughout both growing seasons. In 2003, soils were sampled one week before amending and then 8, 12, and 18 weeks after amending. In 2004, soils were sampled earlier and more frequently in order to focus on the period just after manure amendment. Samples were taken one week before amending and then 3, 6, 9, and 12 weeks after amending. Soil samplings were conducted between 4 to 6 days after irrigation to maintain consistency in soil moisture contents across sampling dates.

From each plot, ten soil cores (15 cm depth, 2.5 cm diameter) were removed and composited. Each sample was passed through an 8mm mesh sieve, composited, and a subsample was passed through a 2mm mesh sieve and stored at 4°C for biological analysis. A 10 g subsample was oven-dried at 40°C for 48 h to determine gravimetric moisture content according to the procedure modified from Gardner (1986). Gravimetric moisture content (θ) was calculated as:

$$\theta = (\text{wet soil weight}/\text{dry soil weight}) - 1$$

The 8mm soil sample was air-dried (96 h at 25°C) and stored for light fraction analysis (Wander and Yang, 2000). Also at each sampling time, 10 wedges (approximately 15 cm by 15 cm by 5 cm) were collected from each plot with an AMS SharpShooter sampling shovel (AMS Inc., American Falls, ID) and composited. The sample was passed through a 2.54 cm mesh sieve and used for disease assessment in the greenhouse. During soil sampling, bulk density was measured by using a Model 200 impact corer (Soil Moisture Equip. Corp., Santa

Barbara, CA). One intact core measuring 5.4 cm (inner diameter) by 6 cm high was removed per plot. Bulk density was calculated as in Blake and Hartge (1986).

On August 27, 2004 a separate set of intact cores was removed from each plot using a Model 200 impact corer (Soil Moisture Equip. Corp., Santa Barbara, CA) in order to characterize soil moisture retention. One intact core measuring 5.4 cm (inner diameter) by 6 cm high was removed per plot and transferred to the laboratory for analysis.

Soil analyses

Physical properties

Free POM (fPOM) and occluded POM (oPOM) were densiometrically separated from the mineral soil using modifications of procedures developed by Golchin (1994) and Puget and Drinkwater (2001). Air-dried soil (30 g, sieved to < 8mm) was placed in a Nalgene centrifuge bottle with 70 mL of sodium polytungstate solution (SPT; Sometu-USA, Sherman Oaks, CA) adjusted to a density of 1.9 g cm^{-3}. The bottles were gently shaken for 1 h at 100 rpm on a rotary shaker, and then allowed to settle for 16 h. The fPOM on the suspension surface was aspirated into a flask, filtered through a 0.45 μ nylon filter (GE Osmonics, Inc., Minnetonka, MN) and washed with 100 mL of deionized (DI) water to remove any remaining SPT. The fPOM fraction was scraped from the filter into an aluminum tin, oven-dried (48 h, 60°C), and weighed. The remaining heavy fraction was resuspended with SPT solution and returned to the shaker for 16 h at 150 rpm to release the POM held within aggregates. After shaking, oPOM was recovered by centrifuging for 30 min at 2000_g in a swinging-bucket centrifuge (Beckman model TJ-6, Beckman Coulter, Inc., Fullerton, CA) and aspirating as described above. POM values were expressed as mg fPOM or oPOM cm^{-3} soil.

Soil moisture holding properties were characterized for each treatment in four ways: 1) by measuring in-field gravimetric moisture content on all soil sampling dates in 2003 and 2004, 2) by measuring gravimetric moisture content of greenhouse cone-tubes in 2004, 3) by measuring in-field soil moisture content repeatedly following a single irrigation event, and 4) by generating soil moisture release curves for a single sampling in 2004.

In-field gravimetric moisture content (θ) was measured as described above. Percent change in moisture contents (θ_Δ) was calculated as:

$$\theta_\Delta = [(\theta_1/\theta_2)-1]$$

Gravimetric moisture content of cone-tubes was measured in 2004 for greenhouse bioassays beginning with the 6 weeks post-amendment planting. For each field plot sampled, two additional cone-tubes were planted and then destructively sampled two and four weeks after planting to measure soil moisture. Soil from each designated moisture-assessment cone-tube was emptied, the corn plant was removed, and all soil was thoroughly mixed to homogenize the sample. A subsample was removed and gravimetric moisture content was assessed for each cone-tube as described previously.

To evaluate moisture holding properties of soil *in situ* 11 weeks after amendment in 2004, gravimetric moisture content was measured every 24 h just before and for 5 days following an irrigation event. Ten soil cores (15 cm depth, 2.5 cm diameter) were removed and composited for each plot; cores were not taken from positions directly adjacent to corn plants to avoid direct effects of corn roots on moisture content. A 10 g subsample was used to determine gravimetric moisture content for each plot, as described previously.

To generate soil moisture release curves, intact soil cores of known volume were saturated with water in the lab, and then core water retention was measured at varying pressures (Klute, 1986). Two intact soil cores measuring 5.4 cm (inner diameter) by 3 cm high were removed from each plot on August 27, 2004. One core was used to measure bulk density while the other was prepared for characterization of moisture retention. The core was fitted on the bottom with a cheesecloth square to prevent soil loss during transfer. Each core was slowly saturated from below with a 1 cm depth of water and placed on a water-saturated porous ceramic plate. The ceramic plates were placed within a pressure chamber connected to a heavy-duty pump. Five pressures were selected that represented the range of normal field conditions: 0, 30, 100, 300, and 500 kPa. The system was set to a pre-selected pressure and cores remained in the chamber until they equilibrated. At this point, cores were removed, weighed to assess moisture content, and were returned to the chamber at a sequentially greater pressure. Finally, cores were oven-dried for 48 h at 40°C to obtain soil dry weights. Volumetric soil moisture content was calculated for each core at each pressure, and used to generate moisture retention curves for each treatment (Klute, 1986). Six outliers were removed from the set of saturated (0 kPa pressure) volumetric moisture contents due to unusually large or small values. One point each was removed from the L-NRA, ML-NRA, MH-NRA, ML-RA, MH-RA, and 3H-NRA treatments. This variability was likely due to either the loss of soil during the laboratory procedure or presence of an earthworm burrow in the intact soil sample. Both of these factors were difficult to control during the procedure.

Biological properties

Soil biological properties were assessed within 48 h of soil sampling by measuring soil microbial biomass-carbon (MBC) and soil microbial activity (as the rate of hydrolysis of fluorescein diacetate; FDA). The rate of FDA hydrolysis was measured by modifying the procedure used by Zelles et. al (1991). Four replicate 1 g subsamples of field-moist soil were each mixed in an Erlenmeyer flask with 20 mL of 60mM sodium phosphate buffer (pH 7.6). To three of these flasks, 100 μL of 4.8 mM fluorescein diacetate solution (3', 6' diacetylfluorescein) was added as a substrate for the hydrolysis reaction. The remaining flask served as a control, and received 100 μL of fluorescein diacetate solution only after the addition of acetone. The hydrolysis reaction proceeded for 2 h, while shaking at 178 rpm and 25°C on an Innova 2300 platform shaker (New Brunswick Scientific Co., Inc., Edison, NJ). The addition of 20 mL acetone stopped the reaction, after which samples were centrifuged for 5 min at 4960_g (Model J2-HS, Beckman Coulter, Inc., Fullerton, CA). Samples were filtered (Whatman No. 4), and assessed colorimetrically using a spectrophotometer measuring absorbance at 490 nm (Model DU 800, Beckman Coulter, Inc., Fullerton, CA). Microbial activity was expressed as μg FDA hydrolyzed hr^{-1} g^{-1} dry wt soil by comparing sample absorbance against a standard curve. Background absorbance of the sample solutions was corrected by subtraction of the control absorbance.

The chloroform fumigation-incubation method was used to measure soil microbial biomass-C (MBC) (Jenkinson and Powlson, 1976). Briefly, from each field-moist soil sample (sieved to < 2mm), two 10 g subsamples were placed into glass scintillation vials. One vial of each pair was fumigated for 24 h in a glass vacuum desiccator containing a beaker of 50 mL ethanol-free chloroform and boiling chips. The remaining vial was left unfumigated and exposed to air for the same 24 h period. Soil samples were then transferred to 125 mL Erlenmeyer flasks, sealed with rubber septa, and incubated at 24°C for 10 d in a dark chamber. Following incubation, the headspace of each flask was sampled for CO_2 content on a Model 100 gas chromatograph (Varian Inc., Palo Alto, CA). MBC was reported as μg MBC g^{-1} dry soil, based on values obtained from a CO_2 standard curve. MBC was calculated using a k_C of 0.41 and without the subtraction of the unfumigated control (Voroney and Paul, 1984). In 2003, on three different sampling dates (pre-amendment, 12 weeks post-, 18 weeks post-) the calculated standard slope was unusually high (100% higher) compared to the slope at all other sampling times. The average slope from the other sampling dates was used to calculate MBC for these sampling dates.

Statistical Analyses

Treatment effects on soil properties were determined using analysis of variance (ANOVA) procedures, computed for individual sampling times.

Treatment means were separated using the Dunnett method to conduct multiple comparisons against the control (SAS Inst., 1999). Differences were considered significant at $p<0.05$ (unless otherwise noted). Linear regression analyses using PROC GLM were conducted in SAS to describe relationships between amendment rate and various soil properties (SAS Inst., 1999). Regression analyses were performed for individual sampling dates because relationships were not the same throughout the year. Paired two-sample t-tests were performed using S-PLUS (2001), and p-values for multiple comparisons were corrected using the Bonferonni adjustment.

RESULTS

Manure Characteristics

Nutrient content and chemical characteristics of the MS are reported for each year of amendment in Table 2.2. The nutrient characteristics for the MS and CMS applied in 2001 and 2002 are reported in Darby et al. (in press).

Table 2.2 Characteristics of fresh separated dairy manure solids in 2003 and 2004.

Chemical characteristics of separated dairy manure solids						
Dry matter	Soluble salts	Total P (P_2O_5)	Total K (K_2O)	Total N	NO_3-N	NH_4-N
----------	----------	----------	$g\ kg^{-1}$	----------	----------	----------
			2003			
201.2	3.8	5.04	7.96	15.20	0.35	2.98
			2004			
238.1	6.5	4.17	7.66	16.13	0.42	1.97

Corn Emergence, Biomass, and Yield

No treatment effects on seedling emergence, corn yield, or plant biomass were observed in 2003 (data not shown).

Soil Physical Properties

Bulk density

Pre-amendment-- 2003 and 2004 seasons

One week before amendment in both 2003 and 2004, there was no significant effect of serial amendment on bulk density (Table 2.3). However, the control consistently had the highest bulk density of all treatments, and bulk density generally decreased with increasing amendment rate.

Table 2.3 Bulk density from selected sampling times before and after re-amendment in 2003 and 2004. SE in parentheses.

	Bulk Density $g\ cm^{-3}$		
	2003		
treatment	pre-amendment	8 wks post-amendment	
C	1.21 (0.02)	1.27 (0.04)	
L	1.12 (0.01)	1.13 (0.02)	
ML	1.11 (0.05)	1.09 (0.02)	
MH	1.12 (0.02)	1.07 (0.02)	
H	1.09 (0.07)	0.96 (0.04)	
	2004		
	pre-amendment	3 wks post-amendment	
		NRA	RA
C	1.37 (0.06)	1.19 (0.03)	
L	1.37 (0.04)	1.20 (0.03)	1.15 (0.03)
ML	1.20 (0.04)	1.14 (0.03)	1.04 (0.03)
MH	1.29 (0.06)	1.11 (0.04)	1.00 (0.03)
H	1.22 (0.06)	n/a	n/a
3H-NRA	n/a	1.03 (0.02)	n/a
1H-RA	n/a	n/a	1.01 (0.05)
4H-RA	n/a	n/a	0.96 (0.02)

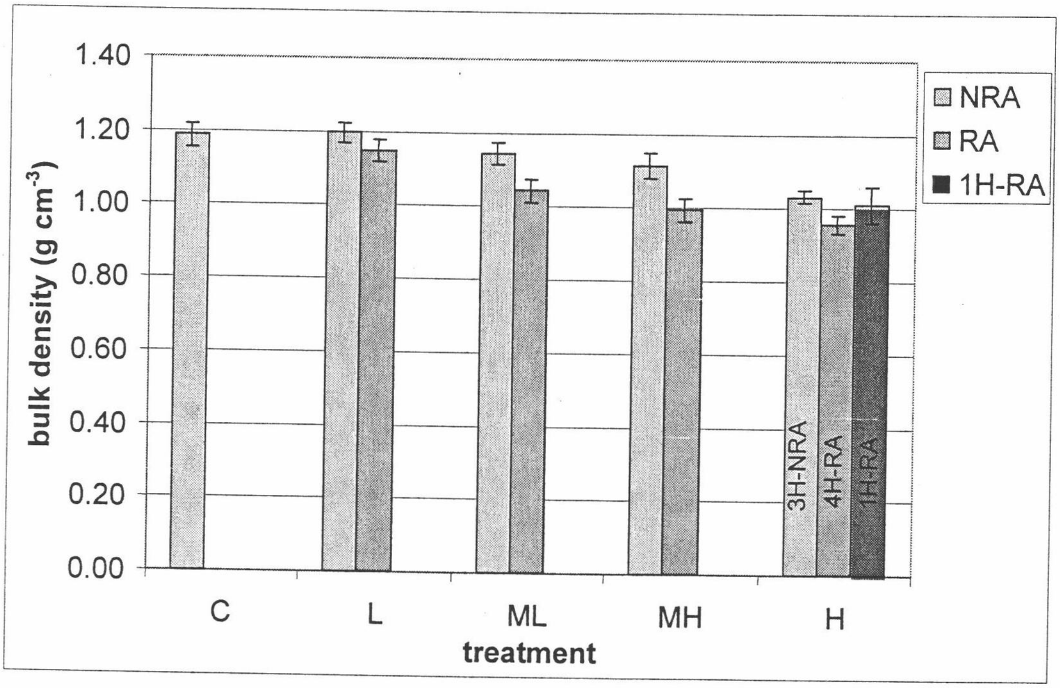
Post-amendment-- 2003 season

Eight weeks after amendment in 2003, all amended treatments had significantly lower bulk density than the control ($p<0.05$) (Table 2.3). Bulk densities in the H, MH, and ML treatments were also lower than the control 18 weeks after amendment ($p<0.05$) (data not shown). In 2003 the greatest difference in bulk densities (compared to the control) was seen eight weeks after amending. At this time H-treatment bulk density was 24% lower than the control ($p<0.05$) (Table 2.3). The treatments receiving intermediate rates of manure had bulk densities between those of the highest rate and the control (Table 2.3).

Post-amendment-- 2004 season, NRA treatments

During 2004, in the NRA split plots there was a trend of decreasing bulk density with increasing historical amendment rate (Figure 2.2). However, the only NRA treatment that was significantly different from the control was the 3H-NRA treatment 3 weeks after amending, where bulk density was 13% lower in the residually amended treatment than in the control ($p<0.05$) (Figure 2.2).

Figure 2.2 Bulk density in freshly re-amended (RA) and not re-amended (NRA) treatments, 3 weeks after amendment in 2004. 4H-RA- 4 years of high-rate (H) amendment, 3H-NRA- 3 years of H amendment, 1H-RA- 1 year of H amendment. Error bars represent ±1 SE.



Post-amendment--2004 season, RA treatments

Three weeks after re-amendment, all RA treatments except the L were of significantly lower bulk density than the control ($p<0.05$) (Figure 2.2). The bulk density of the 4H-RA treatment was 19% lower than the control, the greatest difference observed among all of the treatments at this time. Compared to the control, the bulk density of the MH-RA treatment was 16% lower, and the bulk density of the 1H-RA treatment 15% lower (Figure 2.2).

Twelve weeks after re-amendment, bulk density remained significantly lower only in the MH-RA (17%) and 4H-RA (15%) treatments compared to the control ($p<0.05$) (data not shown).

Particulate organic matter (POM)

Pre-amendment-- 2003 season

Free POM

Before amendment in 2003, all previously amended treatments contained significantly higher quantities of fPOM than the control ($p<0.05$) (Table 2.4). Compared to the control, the H treatment contained 241% more fPOM, the ML treatment contained 128% more fPOM, the MH treatment contained 104% more fPOM, and the L treatment contained 90% more fPOM.

Table 2.4 Free and occluded particulate organic matter (POM) contents from each soil sampling in 2003.

	Soil POM in 2003	
	free POM	occluded POM
	---------------mg POM cm^{-3} soil---------------	
treatment		
	52 weeks after second amendment	
C	3.95	5.38
L	7.52 *	5.96
ML	9.01 **	8.43 **
MH	8.05 *	7.60 *
H	13.45 ***	11.5 ***
	8 weeks after third amendment	
C	6.60	5.89
L	9.98	4.80
ML	16.63 **	5.94
MH	16.56 **	6.11
H	19.80 ***	7.38
	12 weeks after third amendment	
C	6.01	4.54
L	-	-
ML	12.48 *	7.75
MH	11.73 *	6.56
H	21.34 ***	9.26 *
	18 weeks after third amendment	
C	4.08	6.03
L	6.65 *	6.08
ML	8.26 **	6.83
MH	8.54 ***	8.56 **
H	12.94 ***	9.01 **

* $p<0.05$, ** $p<0.01$, *** $p<0.0001$ from multiple comparisons against the control using the Dunnett correction.

Occluded POM

Before amendment in 2003 oPOM contents followed a similar pattern to fPOM (Table 2.4). The H, MH, and ML treatments all contained significantly more oPOM than the control ($p<0.05$). The H treatment contained the most oPOM, 114% more than the control. The ML treatment contained 57% more oPOM and the MH treatment contained 41% more oPOM than the control.

Post-amendment-- 2003 season

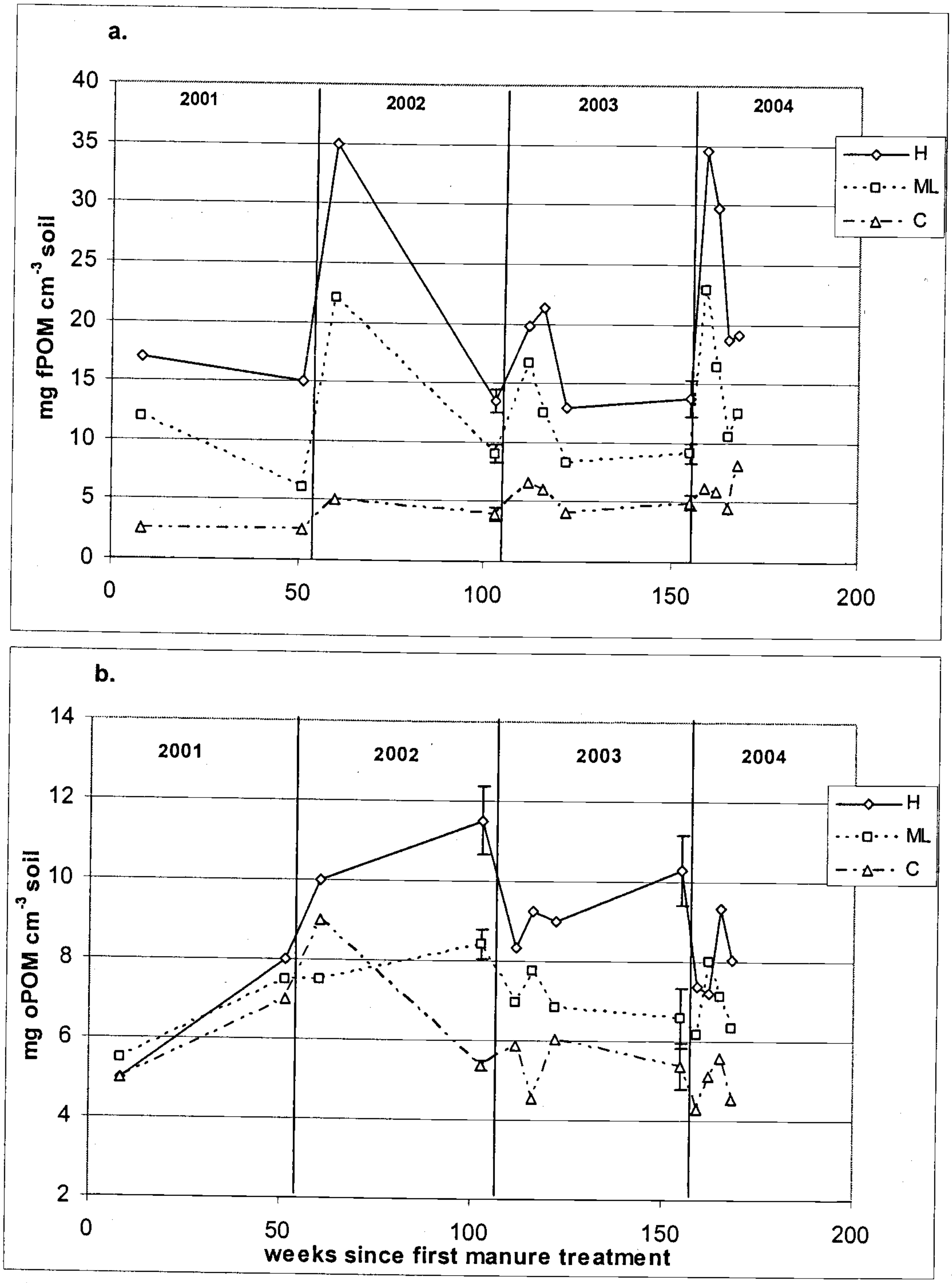
Free POM

Eight weeks after amendment in 2003, fPOM values were numerically higher than pre-amendment values in the H, MH, and ML treatments, although the differences were not significant at $p=0.05$ with the Bonferonni adjustment for multiple comparisons (Figure 2.3). Within the 8 week post-amendment sampling, compared to the control most amended treatments had higher fPOM contents (Table 2.4). Free POM contents were 200% higher in the H treatment, 189% higher in the MH treatment, and 152% higher in the ML treatment.

Twelve weeks after amendment, the H treatment had 256% more fPOM than the control, and fPOM contents in the MH and ML treatments were still significantly higher than the control ($p<0.05$) (Figure 2.3, Table 2.4). The quantity of fPOM in the MH and ML treatments decreased from the eight week sampling to the twelve week sampling. The L treatment was not sampled at this time.

By the sampling 18 weeks after amendment, fPOM contents in all treatments had declined to pre-amendment values ($p>0.55$, from multiple paired two-sample t-tests). However, all amended treatments still had significantly more fPOM than the control ($p<0.05$) (Figure 2.3, Table 2.4). Compared to the control, the H treatment contained 217% more fPOM, the MH contained 109% more fPOM, the ML treatment contained 102% more fPOM, and the L treatment contained 63% more fPOM ($p<0.05$) (Table 2.4).

Figure 2.3 Free a) and occluded b) particulate organic matter (POM) throughout the four years of the field trial in the high (H), medium-low (ML), and control (C) treatments. Vertical lines represent amendment applications. Error bars, when shown, represent ±1 SE.



Occluded POM

The quantity of oPOM present changed very little throughout the sampling season, which ran from 1 week before re-amendment to 18 weeks after amendment (Figure 2.3, Table 2.4). There were, however, consistent trends in 2003 and 2004; in general oPOM contents decreased immediately after amendment and then increased later in the season (Figure 2.3, Table 2.4).

Eight weeks after amendment, there were no treatment effects on oPOM (Table 2.4) and there was suggestive evidence that oPOM decreased just after application of high-rate amendments. At this time oPOM content in the H treatment was 27% lower than at the pre-amendment sampling ($p=0.09$). There was no difference in oPOM contents in the control between these sampling times.

Twelve weeks after amendment, only the H treatment contained significantly more oPOM (104% more) than the control ($p<0.05$) (Table 2.4).

Eighteen weeks after amendment, both the H and MH treatments contained more oPOM than the control ($p<0.05$) (Table 2.4). Compared to the control, oPOM contents in the H and MH treatments was 50% and 42% higher, respectively.

Pre-amendment-- 2004 season

Free POM

One week before amendment in 2004, fPOM content was 176% higher in the H treatment, 96% higher in the MH treatment, and 83% higher in the ML treatment compared to the control ($p<0.05$). Baseline (pre-amendment) fPOM values did not increase between 2003 and 2004 in any of the treatments (Figure 2.3, Table 2.5).

Occluded POM

Before re-amendment, oPOM content was 91% higher in the H treatment ($p<0.05$) and 54% higher in the MH treatment compared to the control ($p=0.07$) (Figure 2.3, Table 2.5).

Table 2.5 Free and occluded particulate organic matter (POM) contents from each soil sampling in 2004.

	Soil POM in 2004			
	free POM		occluded POM	
	---------------------------- mg POM cm^{-3} soil ----------------------------			
treatment				
	52 weeks after third amendment			
C	4.98		5.37	
L	7.55		7.01	
ML	9.12 *		6.59	
MH	9.77 *		8.29	
H	13.75 **		10.28 **	
	3 weeks after fourth amendment			
	RA	NRA	RA	NRA
C	6.13	6.13	4.31	4.31
L	13.13	10.80	5.87	5.56
ML	22.84***	11.95	6.16	5.92
MH	26.77 ***	12.43	6.16	6.85 **
1H-RA	25.92 ***	n/a	4.45	n/a
3H-NRA	n/a	15.07 *	n/a	6.70 *
4H-RA	34.58 ***	n/a	6.51 *	n/a
	6 weeks after fourth amendment			
	RA	NRA	RA	NRA
C	5.84	5.84	5.12	5.12
L	12.61	8.77	5.66	5.29
ML	16.45 *	9.45	7.99 *	7.29
MH	15.05 *	9.29	6.53	7.44
1H-RA	15.33 *	n/a	4.08	n/a
3H-NRA	n/a	12.06	n/a	9.22 **
4H-RA	29.78 ***	n/a	7.22	n/a
	9 weeks after fourth amendment			
	RA	NRA	RA	NRA
C	6.31	6.31	5.58	5.58
L	9.06	6.12	5.96	6.22
ML	10.47	7.13	7.14	7.31
MH	14.03 **	7.92	6.71	7.06
1H-RA	15.80 **	n/a	4.92	n/a
3H-NRA	n/a	9.72	n/a	8.11
4H-RA	18.60 ***	n/a	7.39 *	n/a

Table 2.5 (Continued)

		12 weeks after fourth amendment		
	RA	NRA	RA	NRA
C	8.08	8.08	4.55	4.55
L	8.86	6.62	4.02	5.14
ML	9.72	7.97	6.32	5.50
MH	11.77	7.38	5.28	5.79
1H-RA	15.32 **	n/a	4.34	n/a
3H-NRA	n/a	14.91 **	n/a	9.98 **
4H-RA	19.09 ***	n/a	8.05 *	n/a

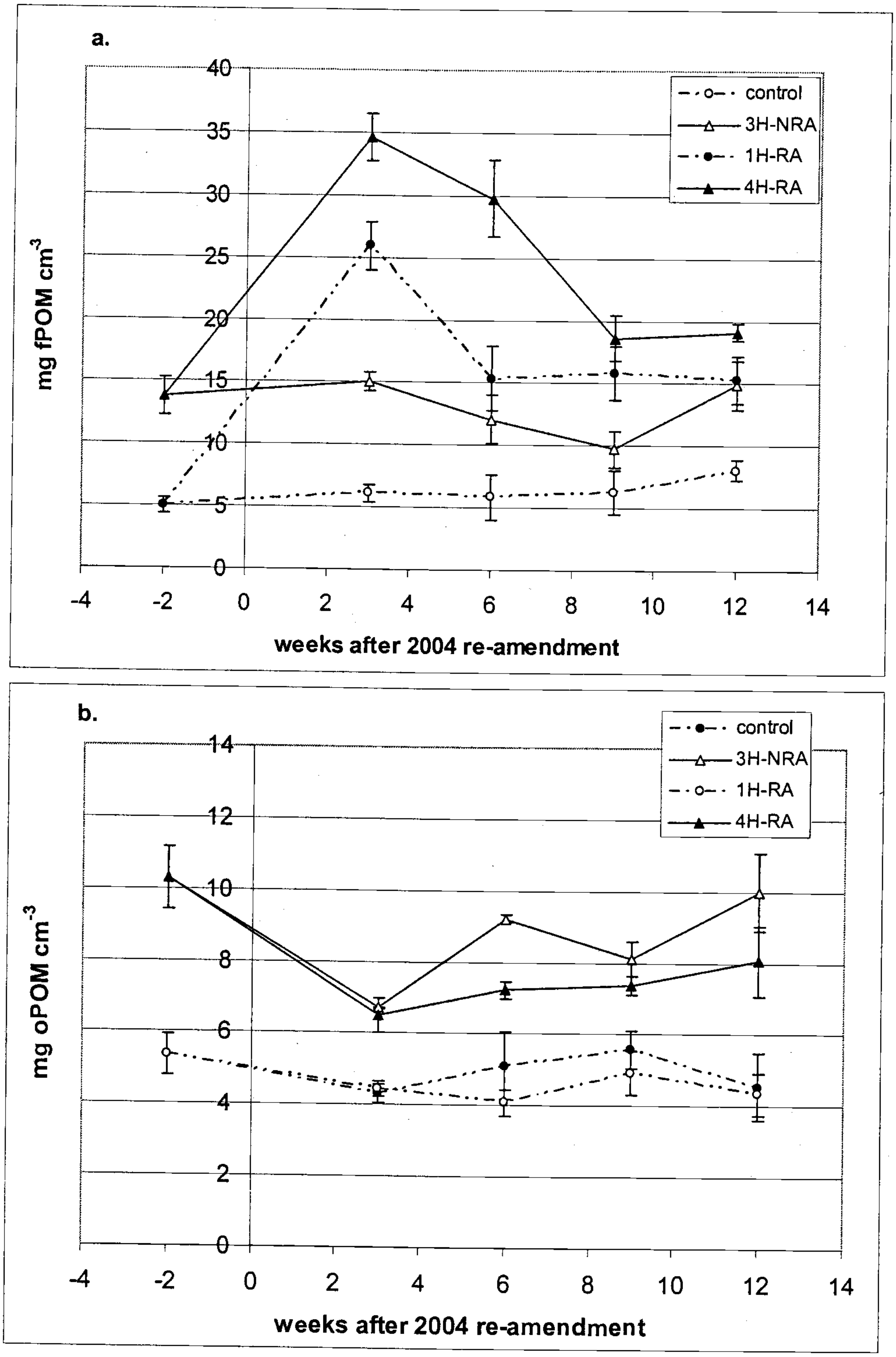
* $p<0.05$, ** $p<0.01$, *** $p<0.0001$ from multiple comparisons against the control using the Dunnett correction.

Post-amendment-- 2004 season

Free POM

The highest fPOM contents throughout 2003 and 2004 were recovered in the soils from the 3 week post-amendment sampling in 2004 (Figure 2.3, Table 2.5). Three weeks after amendment in 2004, fPOM contents in all RA treatments were significantly higher than pre-amendment values ($p<0.05$). Within the 3 week post-amendment sampling, the 4H-RA treatment contained 262% more fPOM than the control ($p<0.0001$) and the 1H-RA treatment contained 172% more fPOM than the control ($p<0.0001$), indicating a positive residual effect of serial amendment on fPOM (Figure 2.4). Free POM content in the 4H-RA treatment was 33% higher than in the 1H-RA treatment ($p=0.018$). The quantity of free POM in the L-RA treatment was not significantly different from the control ($p>0.05$).

Figure 2.4 Free a) and occluded b) particulate organic matter (POM) dynamics in re-amended (RA) and not re-amended (NRA) high treatments and the control in 2004. Error bars represent ±1 SE.



With three exceptions, all amended treatments had significantly more fPOM than their NRA counterparts at 3, 6, and 9 weeks after amendment ($p<0.03$) (Table 2.5). Three weeks after amendment, there was no significant difference in fPOM content between the L-RA and L-NRA treatments ($p=0.33$). Six weeks after amendment the 1H-RA contained more fPOM than the control ($p=0.08$). Nine weeks after amendment, the increase in fPOM in the ML-RA treatment compared to the ML-NRA was significant at $p=0.07$. Twelve weeks after amendment, the 4H-RA ($p=0.06$), 1H-RA ($p=0.03$), and MH-RA ($p=0.02$) treatments contained more fPOM than their NRA counterparts (multiple two-sample t-tests) but no differences were significant with the Bonferonni adjustment for multiple comparisons.

Overall, fPOM values declined in each subsequent sampling following the 3 week post-amendment measure, except in the control and L-RA treatments (Table 2.5).

<u>Occluded POM</u>

Occluded POM contents varied only slightly across the different sampling times in 2004, although there was generally less oPOM in RA treatments than the associated NRA treatments soon after re-amendment (Figure 2.3, Table 2.5). There was 22% less oPOM in the 4H-RA treatment than the 3H-NRA treatment six weeks after amending ($p=0.006$) (Figure 2.4).

Occluded POM content in the 4H-RA and 3H-NRA treatments remained higher than the control 3 weeks after amendment. Compared to the control, there was also significantly more oPOM in the MH-NRA treatment 3 weeks after amendment ($p<0.05$) (Table 2.5). Even 12 weeks after amendment oPOM content in the 1H-RA treatment was not higher than the control.

Occluded POM values from NRA treatments did not differ significantly from pre-amendment values in any of the post-amendment sampling times ($p>0.05$) (Table 2.5). Among RA treatments, the 4H-RA and MH-RA treatments consistently contained less oPOM in post-amendment samplings compared to the pre-amendment sampling. However, these differences were statistically significant only in the MH-RA treatment, 12 weeks after amendment ($p<0.001$) (Table 2.5).

Soil moisture characteristics

In-field gravimetric moisture

2003 season

Before amendment, treatment means for in-field soil moisture increased with increasing amendment rate, although there were no significant differences from the control (Table 2.6). Eight weeks after amendment again soil moisture increased with amendment rate; soil moisture in the H treatment was 15% greater than the control ($p=0.007$) (Table 2.6). Twelve weeks after amendment soil moisture in the ML treatment was 11% higher than the control ($p=0.027$) (Table 2.6). Eighteen weeks after amendment soil moisture in the H treatment was 8% higher than the control ($p=0.05$) (Table 2.6).

Table 2.6 Gravimetric moisture content from all in-field soil samplings in 2003.

treatment	Gravimetric moisture content ---------- g water g^{-1} dry soil ----------
	pre-amendment 2003
C	0.17
L	0.18
ML	0.19
MH	0.19
H	0.19
	8 weeks after third amendment
C	0.19
L	0.20
ML	0.20
MH	0.20
H	0.22 **
	12 weeks after third amendment
C	0.22
L	-
ML	0.25 *
MH	0.24
H	0.24
	18 weeks after third amendment
C	0.24
L	0.24
ML	0.25
MH	0.26
H	0.26 *

* $p<0.05$, ** $p<0.01$ from multiple comparisons against the control using the Dunnett correction.

2004 season

Before re-amendment in 2004, soil moisture content in the H treatment was 27% higher ($p=0.006$), and in the ML treatment was 17% higher ($p=0.09$) compared to the control (Figure 2.5, Table 2.7).

Three weeks after amendment soil moisture content in the 3H-NRA treatment was 19% higher than the control ($p=0.027$). Soil moisture in the 3H-NRA treatment was 14% higher than the control 9 weeks after amendment ($p<0.001$), and 17% higher than the control 12 weeks after amendment ($p=0.002$). Soil moisture in the ML-NRA treatment was 9% higher than the control 9 weeks after amendment ($p=0.019$).

Three weeks after amendment in 2004 the 4H-RA and MH-RA treatments had 34% and 25% higher moisture contents, respectively, compared to the control ($p<0.05$) (Figure 2.5, Table 2.7). Six, 9, and 12 weeks after amendment all RA treatments except L had significantly higher moisture content than the control ($p<0.05$) (Table 2.7). Across the four post-amendment samplings, the highest soil moisture contents were consistently observed in the 4H-RA treatment, and ranged between 25% and 34% greater than the control. Soil moisture contents in the 1H-RA treatment ranged between 13% and 18% higher than the control in the post-amendment samplings ($p<0.05$). Soil moisture content was lower in the 1H-RA treatment than in the 4H-RA treatment at 3, 6, 9, and 12 weeks after amendment ($p=0.09$, $p=0.03$, $p=0.002$, and $p=0.10$, respectively).

Figure 2.5 In-field gravimetric moisture content before and 3 weeks after re-amendment (2004) in freshly re-amended (RA) and not re-amended (NRA) treatments. Error bars represent ± 1 SE.

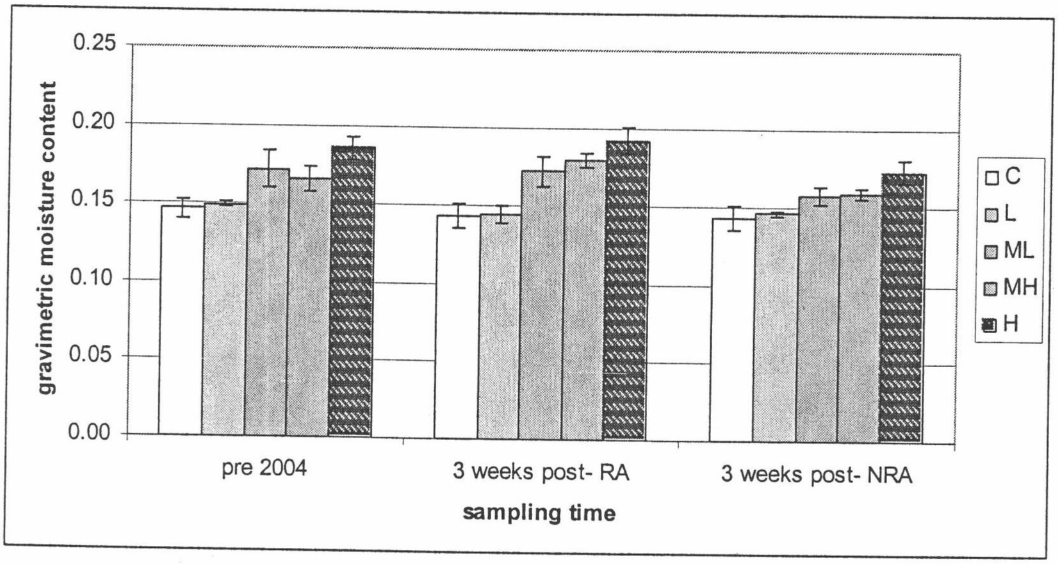


Table 2.7 Gravimetric moisture content from in-field (INF) and greenhouse (GH) soil samples in 2004. Standard error in parentheses.

Gravimetric moisture content

----------------------g water g^{-1} dry soil----------------------

pre-amendment 2004

treatment	INF
C	0.15 (0.01)
L	0.15 (0.00)
ML	0.17 (0.01)
MH	0.17 (0.01)
H	0.19 (0.01)

3 weeks after fourth amendment

	RA		NRA	
treatment	INF	GH	INF	GH
C	0.14 (0.01)	-	0.14 (0.01)	-
L	0.14 (0.01)	-	0.15 (0.00)	-
ML	0.17 (0.01)	-	0.16 (0.01)	-
MH	0.18 (0.00)	-	0.16 (0.00)	-
1H-RA	0.17 (0.01)	-	n/a	-
3H-NRA	n/a	-	0.17 (0.01)	-
4H-RA	0.19 (0.01)	-	n/a	-

6 weeks after fourth amendment

	RA		NRA	
treatment	INF	GH	INF	GH
C	0.19 (0.00)	0.23 (0.00)	0.19 (0.00)	0.23 (0.00)
L	0.20 (0.00)	0.23 (0.01)	0.20 (0.01)	0.21 (0.00)
ML	0.22 (0.00)	0.25 (0.01)	0.20 (0.00)	0.20 (0.01)
MH	0.21 (0.00)	0.23 (0.00)	0.19 (0.01)	0.22 (0.01)
1H-RA	0.22 (0.00)	0.28 (0.01)	n/a	n/a
3H-NRA	n/a	n/a	0.20 (0.01)	0.20 (0.01)
4H-RA	0.24 (0.01)	0.27 (0.04)	n/a	n/a

Table 2.7 (Continued)

9 weeks after fourth amendment

treatment	RA INF	RA GH	NRA INF	NRA GH
C	0.18 (0.01)	0.21 (0.00)	0.18 (0.01)	0.21 (0.00)
L	0.18 (0.00)	0.20 (0.00)	0.18 (0.00)	0.20 (0.00)
ML	0.20 (0.01)	0.22 (0.01)	0.19 (0.00)	0.22 (0.01)
MH	0.21 (0.00)	0.23 (0.01)	0.19 (0.00)	0.21 (0.01)
1H-RA	0.20 (0.00)	0.22 (0.03)	n/a	n/a
3H-NRA	n/a	n/a	0.20 (0.01)	0.22 (0.01)
4H-RA	0.22 (0.00)	0.22 (0.01)	n/a	n/a

12 weeks after fourth amendment

treatment	RA INF	RA GH	NRA INF	NRA GH
C	0.18 (0.00)	0.27 (0.01)	0.18 (0.00)	0.27 (0.01)
L	0.19 (0.00)	0.25 (0.00)	0.18 (0.00)	0.26 (0.01)
ML	0.20 (0.00)	0.27 (0.01)	0.19 (0.01)	0.28 (0.00)
MH	0.21 (0.00)	0.28 (0.01)	0.19 (0.00)	0.26 (0.02)
1H-RA	0.20 (0.00)	0.29 (0.01)	n/a	n/a
3H-NRA	n/a	n/a	0.21 (0.01)	0.29 (0.00)
4H-RA	0.22 (0.01)	0.29 (0.02)	n/a	n/a

Greenhouse gravimetric moisture

Greenhouse moisture data was not taken in 2003 or in 2004 at pre-amendment or 3 weeks post-amendment sampling times.

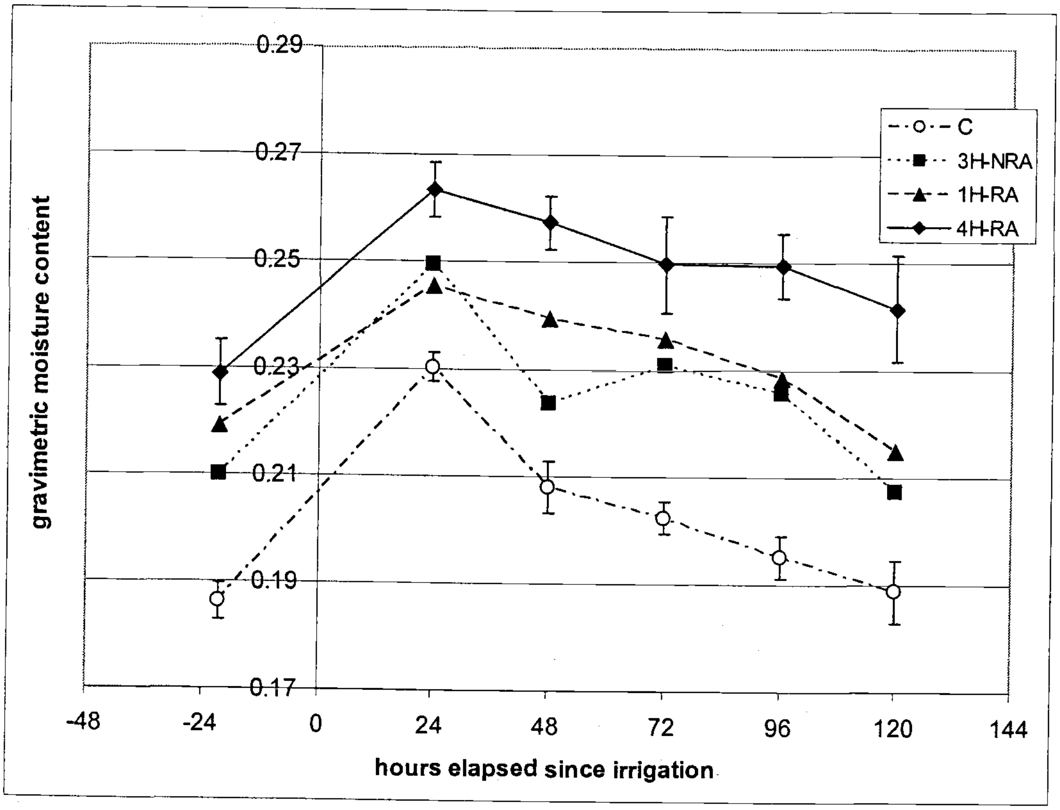
2004 season

Moisture content treatment means from the greenhouse were always numerically equal to or higher than those recorded in the field for a given sampling time (Table 2.7). Greenhouse moisture contents of NRA plots did not vary significantly between treatments at any of the six sampling times ($p > 0.12$). In the RA plots, six weeks after amendment the 4H-RA treatment had 35% higher moisture content and the 1H-RA treatment had 29% higher moisture content than the control ($p < 0.05$). In general, greenhouse soil moisture data was highly variable (Table 2.7).

Soil moisture following an irrigation event

Gravimetric soil moisture content in the 4H-RA treatment was consistently the highest across all sampling times, ranging between 14.3% and 28.0% greater than the control (Figure 2.6). The moisture contents of the 3H-NRA and 1H-NRA treatments were never significantly different from each other at any sampling date (Figure 2.6). Soil moisture content in the 1H-RA treatment ranged from 6.5% to 17.7% greater than the control. Soil moisture content in the 3H-NRA treatment ranged from 7.5% to 15.7% greater than the control. At all sampling times, soil moisture content in the 4H-RA treatment was higher than the control ($p < 0.05$, from multiple ANOVA comparisons against the control). Soil moisture content in the 3H-NRA treatment was significantly higher than the control 20 hours before irrigating, and also 72 and 96 hours after irrigation ($p<0.05$). Soil moisture content in the 1H-RA treatment was significantly higher than the control 48, 72, 96, and 120 hours after irrigation ($p<0.05$). At all six sampling dates, the control treatment consistently had the lowest soil moisture content.

Figure 2.6 Gravimetric moisture content 20 h before and 120 h following an irrigation event. Soil samples were taken 11 weeks after re-amending in 2004. Irrigation occurred at 0 hrs. Error bars represent ± 1 SE (not shown for the 3H-NRA and 1H-RA treatments).



Soil moisture release curves

<u>NRA treatments</u>

In the NRA treatments, the total porosities of the 3H-NRA, MH-NRA and ML-NRA treatments were not significantly different from each other (0.56, 0.54 and 0.50, respectively, $p>0.05$), and were significantly higher than the control ($p<0.05$) (Table 2.8). The total porosities of the L and C treatments were not significantly different from each other (Table 2.8).

Table 2.8 Total porosity of soils sampled 14 weeks after 2004 amendment. Porosity was determined by measuring volumetric moisture content of water-saturated intact soil cores. Each value represents the treatment mean, n=4.

treatment	Total porosity -- cm^3 H_2O cm^{-3} dry soil --
C	0.45
	NRA
L-NRA	0.46
ML-NRA	0.50*
MH-NRA	0.54**
3H-NRA	0.56**
	RA
L-RA	0.46
ML-RA	0.53*
MH-RA	0.45
1H-RA	0.45
4H-RA	0.52*

* $p<0.05$, ** $p<0.01$ from multiple comparisons against the control using the Dunnett correction

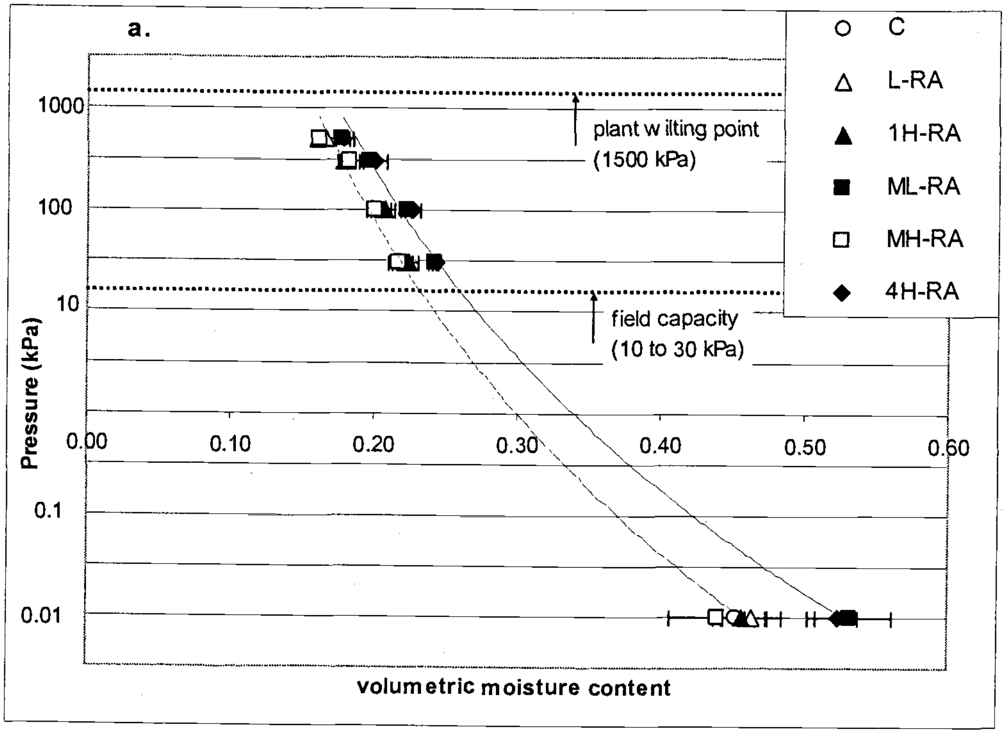
The moisture release curves for the NRA treatments followed this same pattern, where the 3H-NRA, MH-NRA, and ML-NRA had greater volumetric moisture contents at all pressures than either the control or the L-NRA treatments. This resulted in two distinct curves, a high-moisture-retention (HMR) and a low-moisture-retention (LMR) curve (Figure 2.7).

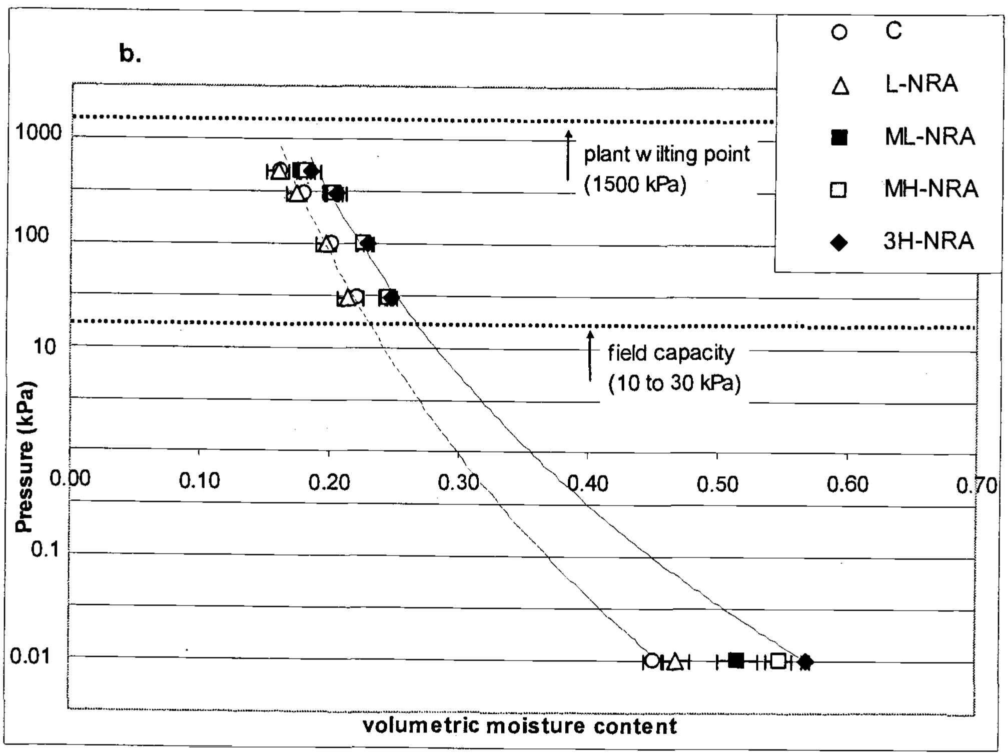
RA treatments

Total porosities among the RA plots also differed significantly by treatment (Table 2.8). Similar to the NRA results, the 4H-RA and ML-RA treatments had significantly greater total porosities than the control (p=0.01). Interestingly, following re-amendment total porosity in the MH-RA treatment was not different than the control (p=0.99). In addition, total porosity in the 1H-RA treatment, which received its first high-rate amendment in 2004, was not different than the control (Table 2.8).

The moisture release curves for the RA treatments again formed two distinct groups, a high-moisture-retention (HMR) group (4H-RA and ML-RA) and a low-moisture-retention (LMR) group (1H-RA, MH-RA, L-RA, and control) (Figure 2.7).

Figure 2.7 Soil moisture release curves for a) freshly re-amended (RA) and b) not re-amended (NRA) treatments. Soils were sampled 14 weeks after amendment in 2004. Volumetric moisture content was measured at 5 water potentials: 0 kPa, -30 kPa, -100 kPa, -300 kPa, -500 kPa. Error bars represent ±1 SE.





Soil Biological Properties

Hydrolysis of fluorescein diacetate (FDA)

Pre-amendment—2003 and 2004 seasons

Twelve months after amendment in both 2003 and 2004, FDA activity was significantly higher in all previously amended treatments (except the L treatment), compared to the control ($p<0.05$) (Figures 2.8, 2.9, 2.10). In 2003 and 2004, FDA activity in the H treatment was 80% and 118% higher than the control ($p<0.01$), respectively, indicating a trend towards increasing residual FDA activity (Figures 2.8, 2.9).

Table 2.9 Soil microbial activity (as FDA activity) and soil microbial biomass-carbon from all sampling dates in 2003.

	Soil Biological Properties in 2003	
	FDA activity	Microbial biomass-C
	μg FDA hydrolyzed hr^{-1} g^{-1} dry soil	μg C g^{-1} dry soil
treatment		
	52 weeks after second amendment	
C	26.07	84.13
L	38.43	122.88 *
ML	44.51 *	141.32 **
MH	41.73 *	122.39 *
H	46.90 **	180.13 ***
	8 weeks after third amendment	
C	25.94	128.37
L	42.09 **	219.49
ML	62.04 ***	347.94 **
MH	58.33 ***	327.51 **
H	74.45 ***	477.73 ***
	12 weeks after third amendment	
C	31.04	230.35
L	-	-
ML	61.59 ***	395.69 *
MH	56.77 **	438.15 **
H	68.15 ***	449.16 **
	18 weeks after third amendment	
C	35.70	199.97
L	52.95 *	314.51 *
ML	63.85 **	392.29 **
MH	64.31 **	386.53 **
H	75.97 ***	547.31 ***

* p<0.05, ** p<0.01, *** p<0.0001 from multiple comparisons against the control using the Dunnett correction.

Table 2.10 Soil microbial activity (as FDA activity) and soil microbial biomass-carbon from all sampling dates in 2004.

	Soil Biological Properties in 2004			
	FDA activity		Microbial biomass-C	
	μg FDA hydrolyzed hr^{-1} g^{-1} dry soil		----------- μg C g^{-1} dry soil ------	
treatment				
	52 weeks after third amendment			
C	28.24		135.78	
L	37.58		205.31 *	
ML	49.86 **		219.87 *	
MH	47.61 **		230.21 **	
H	61.43 ***		318.63 ***	
	3 weeks after fourth amendment			
	RA	NRA	RA	NRA
C	40.44	40.44	183.27	183.27
L	60.66 ***	47.27	208.79	169.86
ML	86.39 ***	59.44 **	359.90 **	221.48
MH	84.00 ***	60.75 ***	421.94 ***	243.49
1H-RA	76.90 ***	n/a	356.81 **	n/a
3H-NRA	n/a	70.55 ***	n/a	398.48 **
4H-RA	107.44 ***	n/a	566.24 ***	n/a
	6 weeks after fourth amendment			
	RA	NRA	RA	NRA
C	38.04	38.04	178.43	178.43
L	57.59 **	45.64	305.59	206.29
ML	75.88 ***	53.32 *	298.54	243.02
MH	74.49 ***	55.19 **	417.66 **	209.49
1H-RA	68.18 ***	n/a	341.95 *	n/a
3H-NRA	n/a	59.89 **	n/a	269.36
4H-RA	100.29 ***	n/a	484.70 ***	n/a
	9 weeks after fourth amendment			
	RA	NRA	RA	NRA
C	39.22	39.22	159.83	159.83
L	52.82 *	45.32	191.81	150.94
ML	65.51 ***	48.30	249.13	173.48
MH	70.57 ***	50.88	317.24 **	198.32
1H-RA	71.73 ***	n/a	350.85 **	n/a
3H-NRA	n/a	57.76 **	n/a	270.15
4H-RA	86.83 ***	n/a	343.16 **	n/a

Table 2.10 (Continued)

	12 weeks after fourth amendment			
	RA	NRA	RA	NRA
C	39.47	39.47	233.52	233.52
L	47.56 *	42.46	300.69	249.47
ML	66.97 ***	46.76	386.22	263.33
MH	64.86 ***	46.95	312.97	255.97
1H-RA	61.76 ***	n/a	403.16	n/a
3H-NRA	n/a	56.18 ***	n/a	270.86
4H-RA	79.45 ***	n/a	374.74	n/a

* $p<0.05$, ** $p<0.01$, *** $p<0.0001$ from multiple comparisons against the control using the Dunnett correction.

Figure 2.8 Soil microbial activity (as FDA activity) throughout all sampling dates in 2003. Error bars represent ±1 SE.

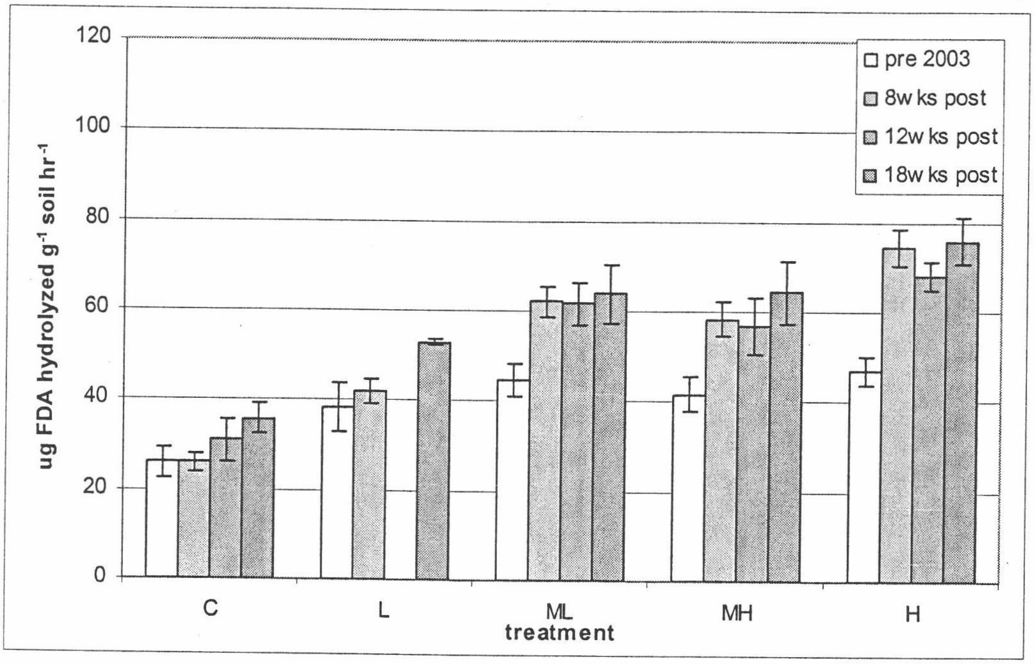


Figure 2.9 Soil microbial activity (as FDA activity) throughout all 2004 sampling dates in the a) not re-amended (NRA) treatments and b) in the freshly re-amended (RA) treatments. Error bars represent ±1 SE.

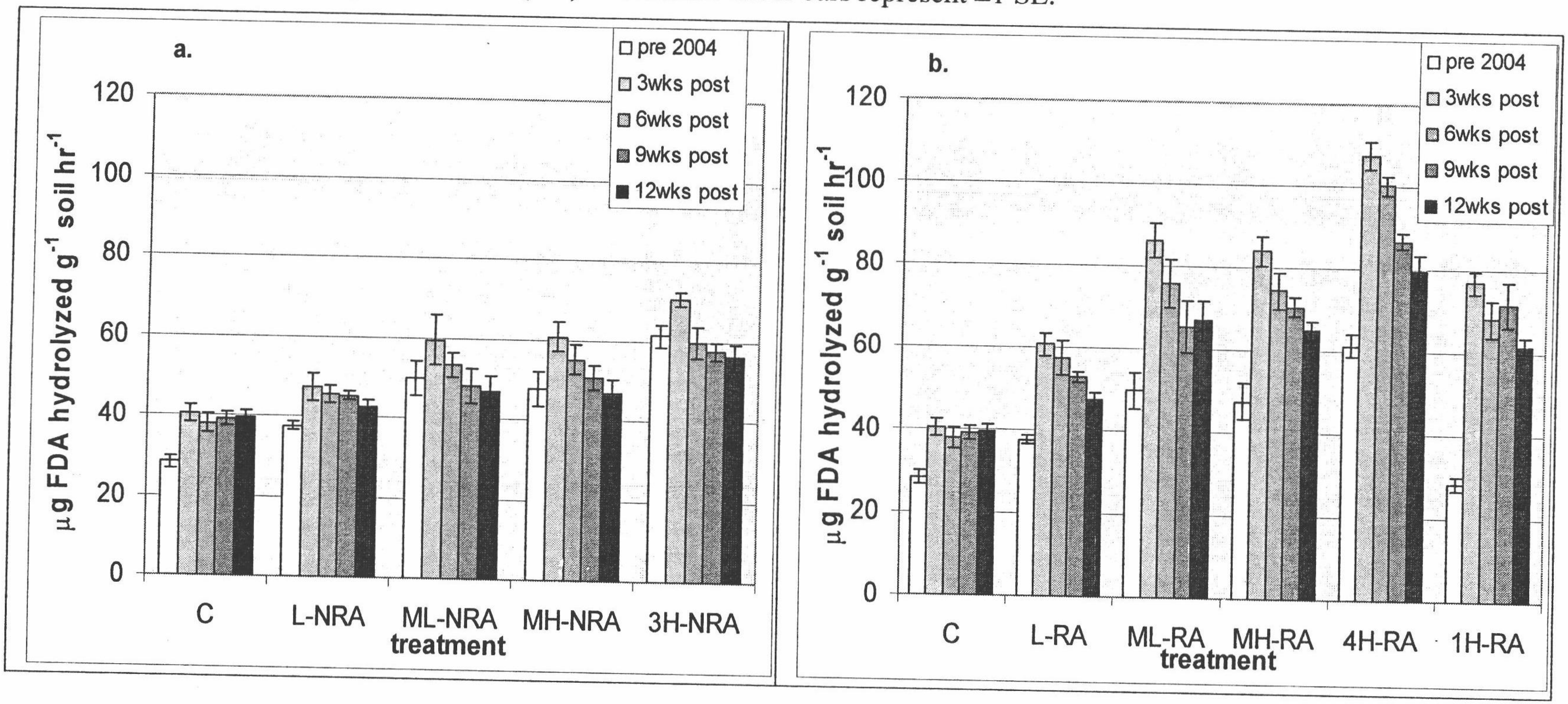
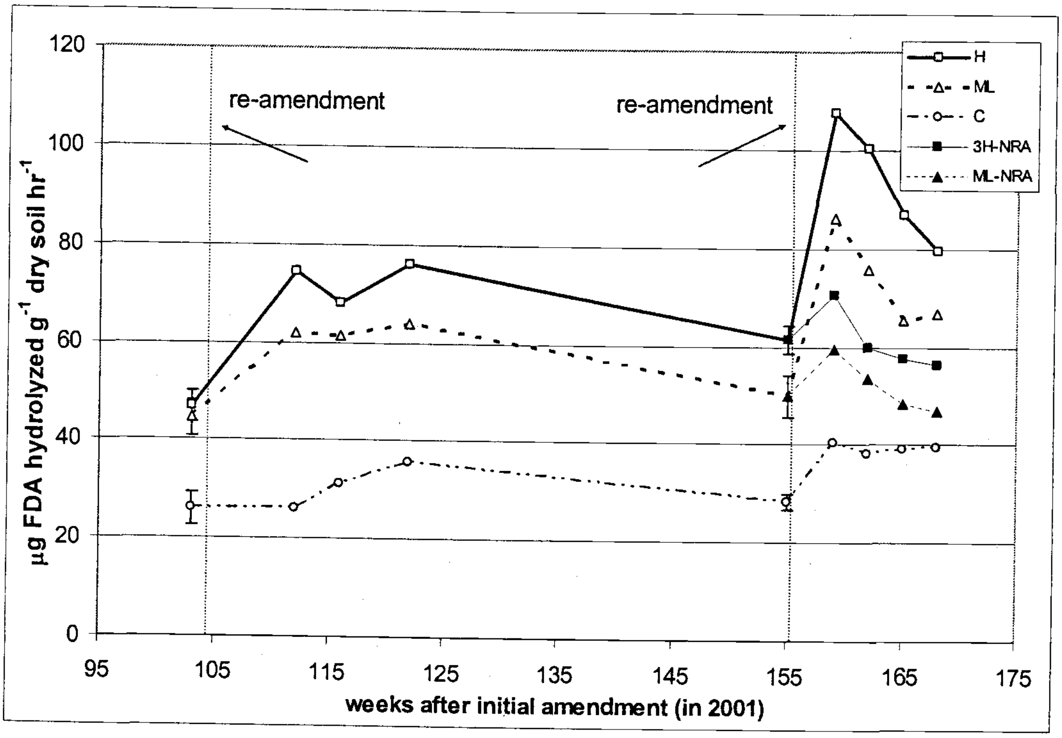


Figure 2.10 Soil microbial activity (as FDA activity) throughout the 2003 and 2004 seasons of the field trial for the high (H), medium-low (ML) and control (C) treatments. Solid symbols represent the not re-amended (NRA) splits of the described treatments. Vertical lines represent amendment applications. Error bars, when shown, represent ±1 SE.



Post-amendment—2003 and 2004 seasons

Eight weeks after amendment in 2003, FDA activity increased significantly above pre-amendment values in the MH and H treatments ($p<0.01$) (Figure 2.8).

In 2004, following the application of manure and the tillage of all treatments to incorporate amendments, FDA activity increased in all treatments, including a 43% increase in the control ($p=0.004$) (Figure 2.9). Three weeks after amending in 2004, FDA activity in all RA treatments was significantly higher than pre-amendment values ($p<0.01$). FDA activity in RA treatments increased between 61% (L treatment) and 75% (H treatment) between pre-amendment and 3 weeks post-amendment samplings. There was a trend of increased FDA activity in the NRA treatments three weeks after tillage compared to pre-amendment values, however, the NRA treatments lacked the post-amendment spike in FDA activity that was observed in the freshly RA treatments.

Microbial biomass Carbon (MBC)

Pre-amendment—2003 and 2004 seasons

Before amendment in 2003, all previously amended treatments had significantly higher MBC than the control ($p<0.05$) (Figure 2.11). MBC contents in the H, MH, ML, and L treatments were 114%, 45%, 70%, and 46% higher compared to the control, respectively.

Before amendment in 2004, again all previously amended treatments contained significantly higher MBC than the control ($p<0.05$) (Figure 2.12). MBC contents in the H, MH, ML, and L treatments were 135%, 70%, 62%, and 51% higher than the control, respectively.

In comparisons of baseline (pre-amendment) MBC values across 2001, 2002, and 2003, MBC increased nearly linearly in all of the amended treatments (Figure 2.13; H, ML, and C treatments). There was no change in MBC over time in the control treatment.

Post-amendment—2003 and 2004 seasons

After re-amendment, MBC increased in the RA treatments, but there was little change in MBC in the NRA treatments except in the 3H-NRA where a short-duration rise in MBC was observed three weeks after amendment (Figure 2.12). Eight weeks after re-amendment 2003, MBC was strongly positively related to amendment rate ($p<0.0001$, $r^2=0.77$). In 2004 nine weeks after re-amendment, MBC in the treatments was again strongly positively related to amendment rate ($p<0.0001$, $r^2=0.71$). In the L treatment in 2003, the highest MBC value was not observed until 18 weeks after amendment. All other amended treatments exhibited a post-amendment MBC increase by 8 weeks after amending. In the 1H-RA treatment, which had not been previously amended before 2004, the highest 2004 MBC value was from 12 weeks post-amendment.

Figure 2.11 Microbial biomass-C from all soil sampling dates in 2003. Error bars represent ±1 SE.

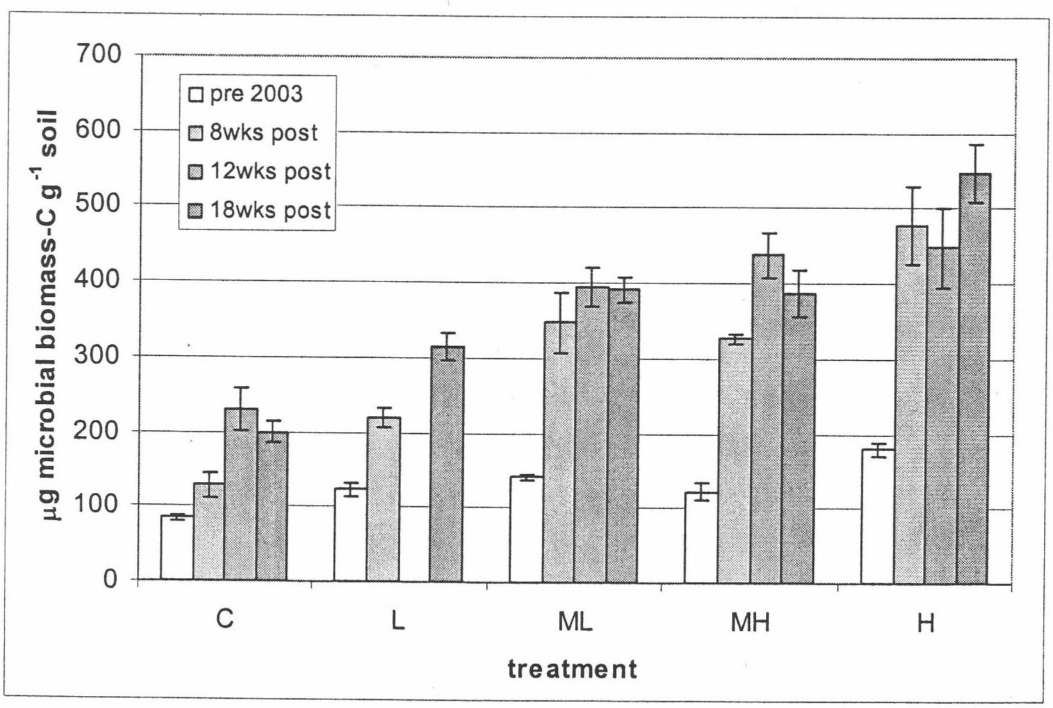


Figure 2.12. Microbial biomass-C from all soil sampling dates in 2004 season in the a) not re-amended (NRA) treatments and b) freshly re-amended (RA) treatments. Error bars represent ±1 SE.

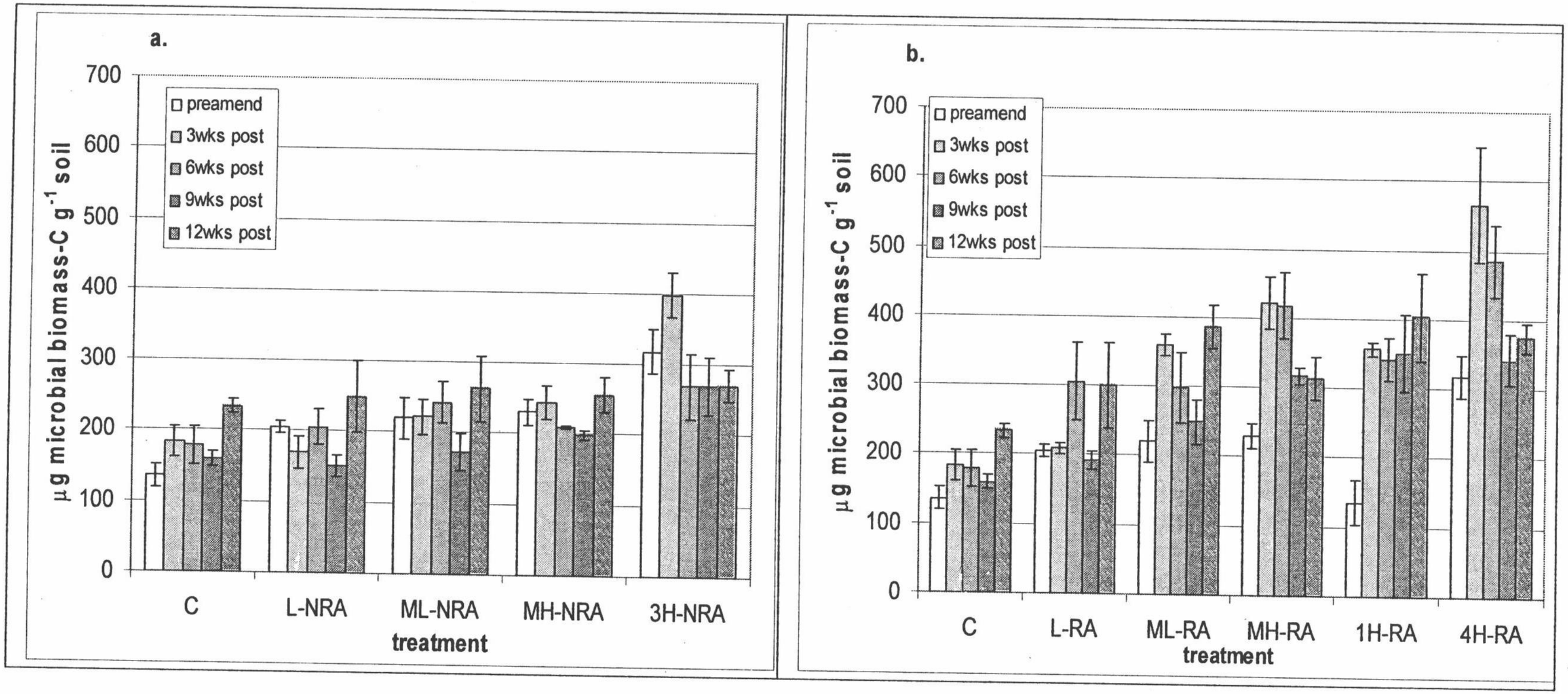
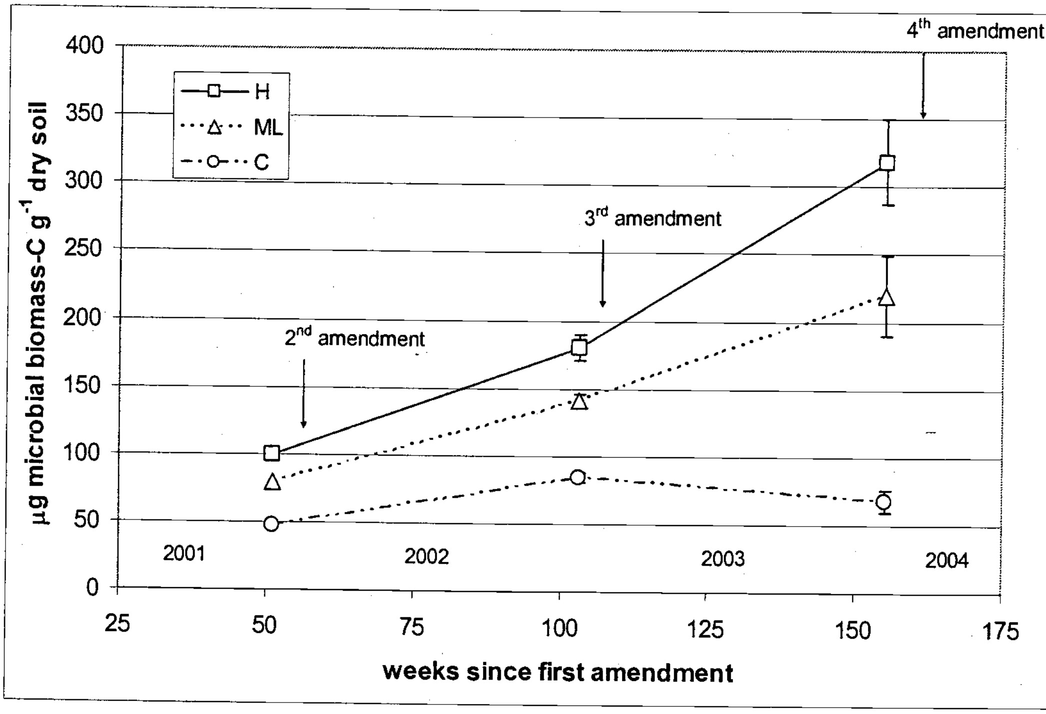


Figure 2.13 Baseline (pre-amendment) values of soil microbial biomass-carbon 12 months after amendment in 2001, 2002, and 2003. Only the high (H), medium-low (ML), and control (C) treatment levels are shown. Error bars represent ±1 SE.



DISCUSSION

Soil Physical Properties

Bulk density

The control treatment consistently had the highest bulk density at all sampling times, as would be expected of a soil under intensive cultivation with little return of OM. Before re-amendment in 2003 and 2004, there were trends of decreasing bulk density with increasing amendment rate but the differences from the control (11% to 12% lower in the H treatment) were not statistically significant. Martens and Frankenberger (1992) reported bulk density decreases of 10% and 11% following three serial amendments of 25 Mg ha^{-1} sewage sludge and barley straw, respectively. Similar decreases in soil bulk density were induced by serial amendment with 4 years of urban waste compost at rates of 10, 30, or 90 t ha^{-1} yr^{-1} (Giusquiani et al., 1995), 4 years of sewage sludge compost at rates of 33-268 Mg ha^{-1} yr^{-1} (dry wt basis) (Tester, 1990), or 4 years of secondary paper mill sludge applied at rates of 8, 16 and 24 t ha^{-1} yr^{-1} (dry wt basis) (Nemati et al., 2000).

Interestingly, after cultivation in 2004 bulk density of the 3H-NRA treatment was 13% lower than the control, even though it was not re-amended. This suggests that the soil factors associated with bulk density are not destroyed by tillage. H-treatment improvements in the proportion of water stable aggregates were reported by Darby (2003) in the first two years of this trial, but water stable aggregation was not measured in 2003 or 2004. In two other studies of soils serially amended with cattle manure, 2 years of amendment induced increases in the proportion of water stable aggregates greater than 4 mm (Whalen et al., 2003), and 5 years of amendment decreased bulk density and increased the proportion of aggregates greater than 1 mm (Sommerfeldt and Chang, 1985).

The magnitude of treatment differences in bulk density was greatest just after re-amending in 2003 and in 2004. Eight weeks after amending in 2003, bulk density in the H treatment was 24% lower than the control, the greatest treatment difference observed during the study. Soon after amending, the lower density of OM particles compared to mineral soil contributes to a decrease in bulk density (Martens and Frankenberger, 1992; Franzluebbers et al., 2001). As OM decomposition proceeds, the relative importance of OM particle density decreases while the impact of OM-induced changes on soil aggregation and structure continues to affect bulk density. Martens and Frankenberger (1992) reported an inverse relationship between aggregate stability and bulk density in soils serially amended with either poultry manure, sewage sludge, barley straw, or alfalfa additions. The high quantities of fPOM recovered just after amending in the high-rate treatments support the idea that OM particles directly reduce bulk density, but the lasting trend of decreasing bulk density 12 months after amendment (once fPOM levels have declined) suggests that improved aggregation also contributes to decreases in bulk density. Additionally, soil fauna (earthworms, insects) can contribute to reductions in bulk density in serially amended treatments. Zaller and

Kopke (2004) reported a 20% increase in earthworm cast production in soils amended for 9 years with cattle manure, compared to un-amended treatments. While soil faunal activity was not assessed in this study, earthworm burrows were consistently observed in soil samples. There was potential for an impact from soil fauna on bulk density.

The effect of serial amendment was apparent in the H treatments in 2004. Three weeks after amending, the 4H-RA treatment had the lowest bulk density. The 3H-NRA and 1H-RA treatments had a slightly higher bulk density, and were not different from each other. The control had the highest bulk density of all treatments. The difference between the 4H-RA and 1H-RA treatments reflects the effect of serial OM addition; although the two treatments received the same rate of fresh amendment in 2004, bulk densities were not the same. This indicates a persistent effect of OM addition on bulk density, and is further supported by observed differences in pore size distributions between the 4H-RA and 1H-RA treatments (Figure 2.7). In these serially amended soils, increased fPOM contents and greater total porosity both contributed to decreases in bulk density.

Particulate organic matter (POM)

Baseline (pre-amendment) changes in POM

The pre-amendment treatment differences in the quantity of fPOM and oPOM indicate the residual effect of previous amendments on POM. Interestingly, an examination of POM values throughout the 4 years of the field study indicated little additional accumulation of fPOM in the H treatment after the first season of amendment, and small increases in fPOM in the lower-rate treatments only through the second season of amendment. Following amendment in 2001, the H treatment had significantly more fPOM than the control even 12 months after amending. However, after the second and third years of amendment, there was little increase in baseline (pre-amendment) fPOM contents. All of the increase in pre-amendment fPOM contents in the H-treatment (compared to the control) occurred during the first year of amendment. It is difficult to make direct fPOM comparisons between years because the amendment rate changed in each year. Previous work has shown that as cultivation intensity and time increase, aggregate-associated OM is lost through mineralization (Stevenson and Elliott, 1989). Large declines in SOM have been associated with tillage in soils (Duxbury et al., 1989). In this system, however, SOM degradation does not explain the lack of fPOM accumulation in the amended treatments because no decline in fPOM was observed over time in the unamended control. An increase in SOM decomposition rate after fresh organic matter input, termed the "priming effect," has been described in soils receiving organic amendments (Fontaine et al., 2003). This increased SOM mineralization, generally attributed to the stimulation of soil microbial activity (Fontaine et al., 2003; De Nobili et al., 2001), could explain the lack of accumulation of fPOM in highly amended treatments. In this case, a direct comparison between the H and C treatments would not help us understand the lack of H-treatment fPOM accumulation because the H treatment was "primed" by its history of OM amendment while the control treatment was not. An alternate explanation is that in these serially amended soils, fPOM may be shifting into the oPOM pool. A suggestive trend of increasing total POM (fPOM + oPOM) over time was observed in the amended treatments, but also in the control treatment (data not shown).

Meaningful shifts in the oPOM pool did not occur until after the second amendment, and the strongest treatment effects on oPOM were consistently in the 2003 and 2004 pre-amendment samplings. These samplings occurred after a long period without soil disturbance (just before spring tillage), and after the decomposition of OM that occurred throughout the growing season, winter, and early spring. Microorganisms decomposing organic material in the soil are largely responsible for the formation of soil aggregates (Angers and Chenu, 1997). Aggregation generates the occluded POM pool as POM becomes protected within aggregates (Golchin et al., 1994). Occluded POM is comprised of both POM and mineral-associated organic matter that are physically protected within soil aggregates from microbial degradation (Herrick and Wander, 1997). There was no clear trend of increasing oPOM over time with serial amendment, but POM comparisons across years are confounded by changes to amendment rate and quality. In the H treatment the history of CMS in 2001 and 2002 most likely affected POM dynamics as CMS would decompose more slowly than MS. In spite of careful rate re-assignment when the CMS treatments were switched to high-rate MS amendments in 2003, the different quality of these materials as well as the change in application rate likely had a residual effect on accumulation of fPOM and oPOM pools over time. This, coupled with the changes to the actual amendment rates in each year makes it more difficult to generalize across the four years. For instance, the amount of OM added under the H treatment decreased from 2002 (33.6 dry Mg ha^{-1} CMS) to 2003 (22.4 dry Mg ha^{-1} MS). This likely explains the decreases in POM observed in 2003.

Pre-amendment and post-amendment changes in POM

Throughout the study there was a strong, positive relationship between amendment rate and POM (free and occluded). Free POM and oPOM were consistently the highest in the H treatment, with lower amendment rates generating POM values between that of the H treatment and the control.

Interestingly, 12 months after the 2002 amendment, and just before treatments were changed to include only MS, the ML treatment (formerly high MS treatment) had a slightly higher residual fPOM value than the MH treatment (formerly low CMS treatment). The amendment rates had been reassigned based on residual fPOM from samples taken 6 months after the 2002 amendment. At this time the MH treatment had more fPOM than the ML (Darby et al., in press). Differences in the quality of CMS compared to MS explain the similarities observed in 2001 and 2002 between the low CMS and high MS treatments. Similarly, residual effects of the CMS amendments also explain why the ML treatment, compared to the MH treatment, frequently had the same or greater amounts of fPOM and oPOM throughout the 2003 season, in spite of having received a lower rate of MS.

Re-amendment increased fPOM in all amended treatments. Three weeks after amendment in 2004, fPOM values rose 150% above pre-amendment values in the H treatment. In 2004, all of the RA splits had more fPOM than their NRA counterparts, even 12 weeks after amendment. Frequent sampling in 2004 demonstrated that fPOM decomposed rapidly after RA, as evidenced by the steep decline in fPOM contents between 3 and 9 weeks post-amendment. In 2002 and 2003 these fPOM "peaks" were likely missed because the first post-amendment sampling occurred at 8 weeks after amendment.

The greatest treatment differences in oPOM were seen at the pre-amendment sampling times in 2003 and 2004, which occur after 11 months without soil disturbance and after POM had decomposed for 12 months. By 8 weeks after amendment in 2003 and by 6 weeks after amendment in 2004, oPOM treatment differences were lost; they began to reemerge 18 (2003) and 9 (2004) weeks after amending.

There was a trend of decreasing oPOM in the short-term following RA. In the 4H-RA treatment, mean oPOM values were lower than the 3H-NRA treatment in 3 of 4 post-amendment sampling times. In lower-rate treatments, mean oPOM values were frequently lower in RA treatments than in NRA treatments. These differences, however, were not statistically significant. There was suggestive evidence that oPOM contents decreased after fresh re-amendment but then reaccumulated over a period of months as decomposition and transformation proceeds. At least some of the short-term oPOM loss is likely due to the impact of field preparation and amendment incorporation, as a slight decrease in oPOM was also observed in the control treatment in 2003 and 2004 following incorporation. However the differences between the 3H-NRA and 4H-RA treatments suggest that not just tillage, but also high-rate OM addition, impacted the quantity of oPOM following RA.

This transient loss of oPOM suggests that addition of fresh OM, especially at very high rates, increases aggregate turnover, reducing the quantity of oPOM in the short-term. Rates of aggregate turnover vary. In a restored prairie system, Jastrow and Miller (1997) calculated an average turnover time of 2.3 yr for macroaggregates (>212μm) compared with 83 yr for total soil organic carbon. Over the mid- to long-term (> 3 months), in this sweet corn cropping system, OM amendment plays a role in increasing soil aggregation by serving as a binding agent for soil particles (Tisdall and Oades, 1982). As POM is decomposed, microorganisms produce extracellular polysaccharides and fungal hyphae grow through the soil binding particles together; the sum of these activities stabilizes soil aggregates (Six et al., 1998). From a study of soils that received 18 years of cattle manure amendments, Aoyama et al. (1999 a, 1999 b) reported that manure application increased the formation of water-stable aggregates. This manure-derived OM preferentially accumulated in macroaggregates, and resulted in increases in the protected pools of C and N following long-term manure application (Aoyama et al., 1999 b).

Interestingly, during the frequent post-amendment samplings in 2004 (3, 6, 9, and 12 wks post-amendment) no significant oPOM increases above pre-amendment values occurred. However it is likely that OM-induced increases in oPOM would be apparent in a sampling 12 months after 2004 amendment, after the labile OM had been transformed into more stable and moderately decomposed material.

Effect of serial high-rate amendment on POM

A timecourse of only the control and H treatment RA and NRA split-plots during 2004 provides a snapshot of the impact of serial, high-rate amendment on both free and occluded POM pools (Figure 2.4). Before RA, the H treatment had significantly higher fPOM and oPOM values than the control. When half of each plot was amended at the high rate (33.6 dry Mg ha^{-1} MS) fPOM increased at the same rate in both RA treatments. However, free POM in the 4H-RA treatment appeared to decompose more quickly than in the 1H-RA (between 3 and 12 weeks post-amendment), although it is not possible with this data set to determine if the rates were significantly different. It is possible that this is evidence of a "priming effect," an example of increased SOM mineralization in soils that previously received organic amendments (Fontaine et al., 2003). However, results are not conclusive with this limited data set. In the 3H-NRA treatment, fPOM content changed little across sampling times, indicating that the residual fPOM remaining after serial amendment was not decomposing rapidly, even following tillage. The lack of short-term increase in the 1H-RA oPOM pool indicates that the benefits of improved aggregation in OM-amended soils accrue over the mid- to long-term, and not as soon as 12 weeks after amending. This is in agreement with results from 2002, in which oPOM increases were not observed until after the second amendment (Darby et al., in press).

Soil moisture characteristics

In-field gravimetric moisture content

Throughout years 2003 and 2004 there was a consistent, positive relationship observed between in-field soil gravimetric moisture content and amendment rate. In 2004, the magnitude of the treatment differences in soil moisture was greatest just after RA (Figure 2.5). A comparison of soil moisture contents in the NRA and RA treatments shows the effect of re-amendment on the magnitude of increase in soil moisture. While soil moisture content in the NRA treatments became more similar to the control, high-rate RA treatments had even greater moisture content differences from the control than at the pre-amendment sampling.

The effect of serial H amendment on soil moisture content was apparent after plots were split in 2004. Three weeks after amendment soil moisture content in the 1H-RA treatment was 21% higher than the control, but soil moisture content in the 4H-RA treatment was 36% higher than the control. This relationship held true in the 6, 9, and 12 week post-amendment samplings where soil moisture in the 1H-RA treatment ranged from 11-16% greater than the control, and soil moisture in the 4H-RA treatment ranged from 22-26% greater than the control. Martens and Frankenberger (1992) reported increases in gravimetric moisture content following 3 additions each of four different types of OM. Organic amendments of poultry manure, sewage sludge, barley straw, and alfalfa increased mean gravimetric moisture content by 3, 9, 25, and 4%, respectively, but the increase was statistically significant only in the barley straw treatment. In plots that had received 20 years of farmyard manure additions, Benbi et al. (1998) attributed increased water retention to increases in both soil organic matter content and aggregation. This agrees with results from this study in which the moisture increase in the 1H-RA treatment demonstrated the direct effect of OM on moisture. The even higher soil moisture contents of the serially amended 4H-RA treatment resulted from both higher OM contents and greater aggregation. One could then infer that in this system, roughly half of the amendment-induced increase in soil moisture results directly from fresh POM content and half results from both residual POM content and improvements in aggregation. Following a single irrigation, the lack of difference in moisture content between the 1H-RA and 3H-NRA treatments also supports this conclusion and is discussed below.

Greenhouse (GH) cone-tube gravimetric moisture content

Gravimetric moisture content in the GH was consistently higher overall than moisture measured in the field. Although moisture varies somewhat (within a range) between sampling times depending on the point in the irrigation cycle at which measurements were made, GH moisture contents were always equal to or higher than their in-field counterparts. In-field mean moisture contents ranged from 14% (C treatment) to 24% (4H-RA treatment), while mean GH moisture values ranged from 20% (multiple treatments) to 29% (4H-RA and 1H-RA treatments).

In-field measurements consistently reflected the positive relationship between moisture and rate, but treatment effects on soil moisture were highly variable in the GH. This is most likely due to a combination of factors. The removal and processing of field soil and its placement into a cone-tube disturbed soil macrostructure. In addition, although care was taken to fill and lightly tamp cone-tubes consistently, the soil packed somewhat differently depending on the subsample selected (i.e. presence of a large aggregate vs. many smaller aggregates). The lack of connectivity of cone-tube soil with a larger body of soil prevented the lateral flow and deep upward/downward flow of water that would be present in undisturbed field conditions. Finally, although the cone-tube position (i.e. edge vs. center) on the GH bench was randomized, position likely had an effect on drying conditions of a given tube. All of these factors combine to explain both the increased moisture observed in the GH compared to the field, and the greater variability observed within treatments.

Soil moisture following an irrigation event

At all sampling times, during the period just before and for 5 days following a single irrigation, the 4H-RA soils had the highest soil moisture contents, up to 28% higher than the control. Interestingly, 1H-RA and 3H-NRA treatments had soil moisture contents consistently higher than the control (6.5-17.7% higher), and consistently similar to each other. This further supports the conclusion that within this system the effect of one fresh, high-rate amendment on soil moisture content is similar to the residual effect of three years of annual amendment.

Soil moisture release curves

Moisture release curves generated for NRA treatments indicated two distinct groups—a high-moisture-retention (HMR) group (H, MH, and ML treatments) and a low-moisture-retention (LMR) group (L and C treatments). The three highest-rate serially amended treatments (H, MH, and ML) held more moisture than the LMR group, at all pressures tested (0 to 500 kPa). This is in agreement with Schjonning et al. (1994) who reported greater water retention at all potentials (0.6 kPa to 1500 kPa) in soil that had received 90 years of farmyard manure amendment, compared to an unfertilized control. Also compared to an un-amended control, Rose (1991) reported increases in water retention at all tensions measured (between 0 and -3 MPa) in a soil that had received 112 years of farmyard manure. Interestingly, although the L treatment had been serially amended for three years, the OM additions were apparently not large enough to significantly alter moisture retention or total porosity of this soil compared to the control.

Moisture release curves generated for RA treatments also formed two distinct groups. The grouping was similar to the NRA curve with the exception of the MH-RA treatment, which fell into the LMR group. This may be an artifact of the amendment history of the MH treatment, which received CMS in 2001 and 2002, but is difficult to explain. Interestingly, the single-year H treatment (1H-RA) grouped with the low-rate treatments (LMR). Although it was amended in 2004 at the same rate as the 4H-RA treatment, water holding characteristics of the 1H-RA treatment were different. This indicates that not only amendment rate, but also the number of serial applications of OM, affect total porosity and soil moisture release characteristics. Fourteen weeks after a single-year high-rate amendment was not sufficient time for these OM-induced changes to develop. It was also interesting that there were few differences between the RA and NRA moisture release curves, again giving support to the idea that these soil characteristics are affected over the mid-term as opposed to immediately after RA. Zibilske et al. (2000) described the cumulative effect of 5 years of serial amendment with paper mill sludge on plant available water (PAW). Regressions of PAW against total C added to the system resulted in steeper slopes in years 4 and 5 of the experiment, suggesting a long-term effect of added C.

In a moisture release curve, each pressure tested corresponds to a particular size class of soil pores, and the volumetric moisture content at saturation represents the total porosity of each soil (Danielson and Sutherland, 1986). The treatments in the HMR group had greater contents of all pore sizes. The widening distance between the two curves at lower pressures indicated that the treatments in the HMR group gained more large-size pores than any other size pore, as compared to the LMR group. Because of the increase in total porosity, high-amendment treatments had more air-filled pore space at a given moisture content than the C or L-NRA treatments. This is in agreement with the findings of Giusquiani et al. (1995) that 4 annual amendments of urban waste compost increased water retention and total porosity in the high rate treatments. The increased water retention was ascribed to both the water holding capacity of OM and to improvements in the soil porosity. In addition, the increased porosity, and especially the observed improvement in the quality of the pore system, had positive implications for crop health. These conditions resulted in more plant available water and more pores to ease root growth (Giusquiani et al., 1995).

Soil Biological Properties

Rate of hydrolysis of fluorescein diacetate (FDA)

Before re-amendment in 2003 and 2004, there were clear residual effects of serial amendment on FDA activity. In both years, at 12 months after amendment all but the L treatment had significantly higher FDA activity levels than the control.

At all sampling times, FDA activity was positively related to amendment rate. This is in agreement with the relationship between FDA and amendment rate observed in 2001 and 2002 (Darby et al., in press). In 2004 when the plots were split, the fresh amendment resulted in a significant increase in FDA activity in RA treatments compared to the NRA split-plots. Following re-amendment, FDA activity consistently increased in all treatments, including the control. Increases in FDA activity in the amended treatments were of greater magnitude than in the control treatment and can be attributed to the addition of OM. The rate of hydrolysis of FDA has been shown to be a good indirect measure of OM decomposition level and of the disease suppressive potential of soil-less container mixes (Hoitink and Boehm, 1999; Chen et al., 1988). Interestingly, with the disturbance from tillage but no fresh RA in 2004, the NRA treatments all exhibited a similar, small increase in FDA activity between the pre-amendment and 3 week post-amendment sampling times. This suggests that although the disturbance could have stimulated further mineralization of amendment-derived soil POM, none was labile enough 12 months after amending to generate a rise in FDA activity.

The earlier post-amendment sampling in 2004 (3 weeks post-amendment) compared to 2003 (8 weeks post-amendment) revealed a higher than expected increase in FDA activity following re-amendment, particularly in the higher amendment rates. This is in agreement with the findings of Grünwald et al. (2000) that FDA activity was generally highest 1 week after cover crop incorporation. This peak in microbial activity was likely missed in 2003, as FDA activity declined rapidly between 3 and 8 weeks post-amendment. In 2003, the lack of difference between pre- and post-amendment FDA activity in the L and ML treatments was probably due to this later first sampling date.

The effect of serial manure amendment on FDA activity was apparent in comparisons of the 4H-RA and 1H-RA treatments. Although the two treatments received the same rate of fresh manure in 2004, the 1H-RA treatment had 28% lower FDA activity than the 4H-RA, 3 weeks after amendment. This indicates that both recent and historical amendments affect the microbial activity of an amended soil. It has been shown that microbial biomass and activity are almost always significantly higher in soils managed organically than those managed conventionally (Gunapala and Scow, 1998), and this effect is likely due to serial OM additions.

Microbial biomass-C (MBC)

A comparison of baseline (pre-amendment) MBC values across the first three years of amendment showed steady increases each year in the serially amended treatments (Figure 2.13). MBC in the H treatment increased by 76-80% in each twelve month period between amendments, and MBC in the ML treatment increased by 55-76% between amendments. There was no significant increase in MBC in the control treatment.

Eight weeks after RA in 2003, MBC increased from pre-amendment values in all treatments except the control. In subsequent samplings 12 and 18 weeks post-amendment, MBC values changed little from eight week MBC values.

In 2004, RA again induced increases in MBC. Increases were observed as soon as 3 weeks after RA in the ML-RA, MH-RA and 4H-RA treatments. MBC in the L-RA treatment did not increase until 6 weeks after amendment, dropped 9 weeks after amendment, and then rose again 12 weeks after amendment. This increased variability may be a result of an uneven application of the low rate amendment as it is more difficult to evenly apply a small amount of manure. The variability may also indicate a delayed increase in MBC with low-rate amendments. The greatest increases in MBC were observed in the high-rate treatments (4H-RA and MH-RA). Following the 3 week sampling in 2004, MBC values in the 3H-NRA, MH-RA and 4H-RA treatments declined; in all other treatments, the MBC values that increased after RA did not decline in subsequent samplings. The lack of decline in MBC across post-amendment samplings more than 6 weeks after amendment suggests that MBC is a measure of the more stable response of the soil microorganisms. It appears that the enhancement of MBC occurs over time, and lags behind the rapid and transient response of FDA activity to fresh OM addition that other studies have shown (Grünwald et al., 2000; Darby et al., in press).

Relationships between soil properties

The relationships among five of the soil properties studied: FDA activity, MBC, fPOM, oPOM, and in-field gravimetric moisture, provide insight into the interconnectedness of the various soils processes that responded to OM addition (Figure 2.14, Table 2.11). Correlations between variables were based on treatment means for each variable grouped across all samplings in 2003 and 2004. Soil fPOM content was significantly, positively related to both FDA activity and MBC ($p<0.0001$) (Figure 2.14). Free POM content explained 77% of the variability in FDA activity, and 53% of the variability in MBC values (Table 2.11). In-field gravimetric moisture content was positively related to both MBC content and FDA activity ($p=0.008$, $p<0.0001$, respectively), however moisture explained less of the variability in soil biological properties (32% and 11%, for MBC and FDA, respectively), than was explained by fPOM content (Figure 2.14, Table 2.11). Interestingly, soil moisture content was positively related to oPOM content ($p=0.014$, $r^2=0.09$), and not related to fPOM content ($p=0.242$) (Figure 2.14). This is an indication that oPOM better describes OM-induced changes to soil moisture, perhaps because oPOM by definition relates to the degree of soil aggregation, and hence soil porosity. In addition, because of the collinearity among the soil properties, it will be difficult to identify individual effects of each variable on root rot severity.

Figure 2.14 Correlation plots for five key soil variables: rate of hydrolysis of fluroescein diacetate (FDA), microbial biomass-carbon (MBC), free and occluded particulate organic matter (fPOM and oPOM, respectively), and gravimetric soil moisture content (MST). Each point represents a treatment mean (n=4) for one sampling date. All sampling dates in 2003 and 2004 are shown.

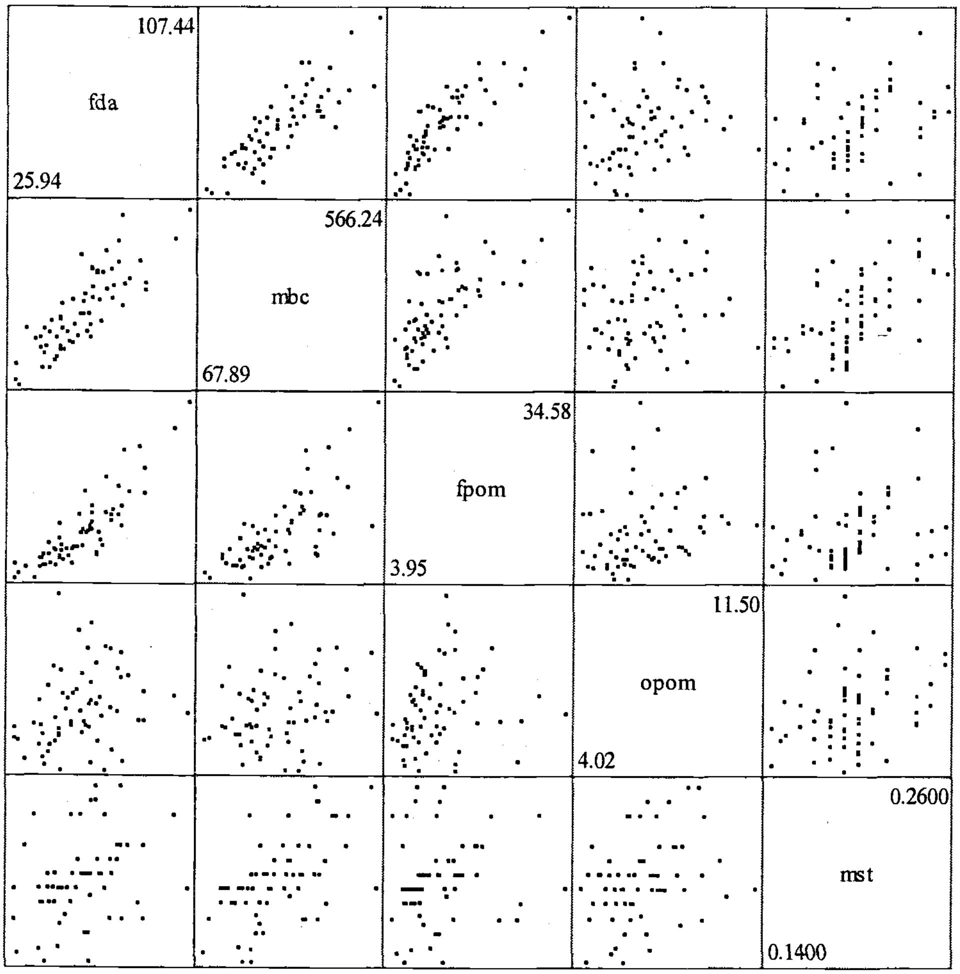


Table 2.11 Correlation coefficients (r) and associated p-values for the relationships between 5 key soil variables: microbial activity (FDA), microbial biomass-carbon (MBC), free and occluded particulate organic matter (fPOM and oPOM, respectively), and gravimetric moisture content. Relationships were determined among 64 treatment means (n=4).

		Pearson correlation coefficients (r) and associated p-values, based on treatment means for each sampling date.				
		FDA activity	MBC	fPOM	oPOM	grav moisture
FDA activity	r	1.0	0.8364	0.8776	0.256	0.3290
	p-value		<.0001	<.0001	0.0409	0.0079
MBC	r	0.8364	1.0	0.7293	0.2047	0.5689
	p-value	<.0001		<.0001	0.1046	<.0001
fPOM	r	0.8776	0.7293	1.0	0.1892	0.1483
	p-value	<.0001	<.0001		0.1343	0.2420
oPOM	r	0.2563	0.2047	0.1892	1.0	0.3044
	p-value	0.0409	0.1046	0.1343		0.0145
gravimetric moisture content	r	0.3290	0.5689	0.1483	0.3044	1.0
	p-value	0.0079	<.0001	0.2420	0.0145	

CONCLUSIONS

Four years of serial amendment with dairy manure solids had beneficial impacts on soil physical and biological properties, which serve as indicators of soil quality. However, increases in moisture retention in the highly amended treatments may have negative impacts on root rot diseases sensitive to soil moisture conditions. Both amendment rate and the number of serial amendments affected the magnitude of changes in soil conditions. With increasing amendment rate, bulk density decreased, quantities of fPOM and oPOM increased, water retention increased, total porosity increased, FDA activity increased, and MBC increased.

The negative relationship between rate and bulk density became stronger over time with repeated amendment. The presence of pre-amendment treatment differences in fPOM contents suggested two distinct pools of fPOM following amendment, a labile pool of fresh OM and a pool of residual OM from previous amendments that decomposed more slowly (Grünwald et al., 2000). Surprisingly, there was no clear increase in baseline fPOM contents between 2003 and 2004, but this may be due to the confounding effect of changes in amendment rates. Occluded POM contents were positively related to amendment rate, but these changes did not occur within the first year after amendment. The difference between the soil water retention in the serial high-rate and single year high-rate treatments indicated positive effects of both OM particles and OM-induced aggregation on soil moisture content.

Both microbial activity and microbial biomass at 12 months after amendment increased over time. FDA activity exhibited a strong and rapid response to re-amendment, and this rise in activity declined rapidly between 3 and 12 weeks after amendment. MBC increased more slowly and steadily following re-amendment, especially in the low-rate treatments which lagged behind the high-rate treatments. Free POM content was positively related to this rise in FDA activity, which is in agreement with the findings of Boehm and Hoitink (1992) that less decomposed sources of organic matter were positively related to microbial activity.

The comparison of single-year (1H-RA) and serial (4H-RA) high-rate amendments identified the soil properties that respond rapidly to RA and the properties that change over the period of 12 months following amendment. FDA activity and fPOM responded most rapidly to fresh amendment, while changes in oPOM, bulk density, water retention, and porosity developed over the mid- to long-term.

CHAPTER 3

IMPACTS OF SERIAL DAIRY MANURE AMENDMENT ON THE SEVERITY OF SWEET CORN ROOT ROT

Bonnie Hoffman Cox and Alexandra Stone

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ABSTRACT

Much of the work to date characterizing organic matter (OM) mediated disease suppression has been conducted in soil-less container mixes or in field soils receiving single-year amendments. Consequently, less is known about the impacts of serial organic amendments on the suppression of soil-borne diseases. The objective of this study was to describe the effects of the third and fourth years of annual, serial amendment with dairy manure solids on the severity of sweet corn root rot. A secondary objective was to identify the soil properties most strongly related to disease suppression in this serially amended system. Plots were amended with five rates of separated dairy manure solids annually for three years. In the fourth year, plots were split and only half of each plot was re-amended. Root rot severity was assessed *in situ* and in the greenhouse with multiple sweet corn (*Zea mays* L. cv Golden Jubilee) bioassays conducted in the amended field soils. Necrosis of the radicle and nodal roots was assessed when plants reached the 6-leaf stage. In the third year after amendment, weak suppression was observed in-field and was associated with increased FDA activity. By the fourth year of serial amendment this trend was no longer evident, however evidence from the high-rate treatment that was not re-amended (3H-NRA) pointed to an emerging suppressive mechanism that persisted up to 13 months after the third amendment. Factors that may be interacting over time to generate observed disease suppression include: short-term post-amendment microbiostasis, soil moisture retention, inoculum levels, and a novel suppressive mechanism.

INTRODUCTION

Much of the work to date characterizing OM-mediated disease suppression has been conducted in soil-less container mixes or in field soils receiving single-year amendments. The impact of serial organic amendment on soil-borne plant diseases in field soils is poorly understood. Recent reviews of this literature have identified this gap in knowledge (Stone et al., 2004; van Bruggen, 1995). Root rot diseases are profoundly affected by the physical, biological, and chemical properties of the soil environment (van Bruggen, 1995). The effects of mid- to long-term OM amendment on soil properties have been studied extensively (Table 1.1). Fewer studies, however, investigate the impacts of serial amendment on plant disease (Stone et al., 2004; van Bruggen, 1995).

Many pathogens associated with root rot diseases are facultative saprophytes, and fresh organic debris can increase pathogen populations by providing a food base for the pathogens (Lumsden et al., 1983; Agrios, 1997). In addition, increases in soil water retention, when observed in serially amended soils, could favor the development of root rot diseases sensitive to soil moisture conditions (Peachey et al., 2004; Kerr, 1964).

In contrast, soil biological properties generally improve with regular additions of OM (Drinkwater et al, 1995; Gunampala and Scow, 1998; Zaller and Kopke, 2004). Soil-borne disease suppression has been shown to be related to soil microbial activity [as hydrolysis of fluorescein diacetate (FDA)] in soil-less container mixes (Hoitink et al., 1991; Chen et al., 1988; Boehm and Hoitink, 1992) and field soils (Dissanayake and Hoy, 1999; Drinkwater et al, 1995; Davis et al, 1994). In situations of general suppression, competition among the total community of the soil microorganisms for nutrients and energy-sources (particularly carbon) contributes to reduced success of the pathogen (Cook and Baker, 1983; Hoitink and Boehm, 1999). It is generally accepted that microbial biomass (MBC) increases in soils receiving serial organic amendments (Gunampala and Scow, 1998). However it is less clear how FDA activity is affected over time and whether FDA activity is related to disease suppression in mid- to long-term amended field soils.

Benefits to crop plants from serial amendment may be related to improvements in soil physical, biological, and chemical properties, as well as from direct effects on plant disease. Improvements in crop yield have been observed following long-term organic additions. Data from the Morrow plots, a well-known long-term study in Illinois, revealed that corn yields from manured, continuous-corn treatments were similar to corn yields from non-manured long-rotation treatments, indicating that either serial amendment or long rotation alone can sustain corn productivity over the long-term (Aref and Wander, 1998). Under a low-input farming system with regular animal manure additions, corn roots were more dense in soil pockets rich in organic matter (Pallant et al., 1997). There are many OM-related processes by which crop health might be enhanced in a soil receiving serial organic amendments, including improved nutrient availability, increased cation exchange capacity, and improved soil porosity (Zaller and Kopke, 2004; Giusquiani et al., 1995; Pernes-Debuyser and Tessier, 2004).

During the first two years of this field study, annual manure amendment generated suppression of radicle rot of sweet corn in each year, and radicle rot severity was strongly, negatively related to FDA activity (Darby et al., in press). Even following 3 and 4 years of serial amendment, we expected that FDA activity would continue to be a strong indicator of suppressiveness. FDA activity increases rapidly following OM amendment, and then decreases over a period of months as labile substrate is decomposed (Grünwald et al., 2000; Boehm and Hoitink, 1992). However, in this study baseline (pre-amendment) indices of microbial biomass and activity increased over time with repeated OM amendment (Darby et al., in press). Assuming that there is a suppressive threshold of FDA activity (as described by Boehm and Hoitink, 1992; Darby et al., in press; Dissanayake and Hoy, 1999) that could be reached via the gradual increase in baseline FDA activity, we expected that after several to many years of serial amendment, lower rate fresh manure additions would induce suppression.

The primary objective of this study was to assess the impacts of the third and fourth years of serial manure amendment on the severity of root rot of sweet corn. A secondary objective of this study was to identify the soil properties most strongly related to disease suppression in this serially amended system.

MATERIALS AND METHODS

Site description

In 2001, plots were established within a 30.5 m by 86.9 m parcel on the Oregon State University Vegetable Research Farm in the Willamette Valley of Oregon. The soil is classified as a Chehalis silt loam (fine-silty, mixed, mesic Cumulic Ultic Haploxeroll). The valley has a Mediterranean climate with cool, wet winters and warm, dry summers. The mean annual temperature is 11.5°C. The mean annual precipitation is 111 cm, the majority of which falls between November and March.

Cropping history

The cropping history for the field site was snap bean (*Phaseolus vulgaris* L.) in 2000 and 1999, fallow in 1998 and 1997, and sweet corn (*Zea mays* L.) in 1996 and 1995. Following the establishment of treatment plots in 2001, sweet corn (cv Golden Jubilee) was grown to harvest, and in 2002 snap bean (cv Oregon 91G) was grown to harvest.

Experimental design

Manure treatments were initially randomly assigned in 2001 within a complete block design (Table 3.1, Figure 3.1). Four blocks containing ten plots each were established within the study site. The blocking factor was an observed soil texture gradient between the east and west field edges. Plots originally measured 6.1 m by 9.1 m, and each treatment was initially replicated 8 times within the field site. In the summer of 2002, a sudangrass (*Sorghum bicolor)* cover crop treatment was applied to half of the field plots. In order to better understand the effects of only serial manure amendment, without the additional variability of the presence of a cover crop, these cover cropped plots have been excluded from this study. Therefore, beginning in 2003 the number of replicates was reduced to four per treatment. In 2004, plots were split and a randomly assigned half of each plot received a fresh manure re-amendment (RA), while the other half was not amended in 2004 (NRA) (Table 3.1). Once split, the plots measured 6.1 m by 4.6 m.

Table 3.1 Five different levels of dairy manure solids amendment were made annually over 4 years of the field trial conducted in Oregon. Amendment rates varied each year, but were consistently assigned by treatment level. Plots were split in 2004, and half of each plot was freshly re-amended (RA) while the other half was not-re-amended that year (NRA). In 2001 and 2002, amendments included composted dairy manure solids (CMS) and fresh dairy manure solids (MS).

Treat-ment	**Abbrev. pre-split**	**Abbrev. post-split in 2004**	**Manure amendment rate**				
			2001	**2002**	**2003**	**Plot split**	**2004**
			------------------ dry Mg ha^{-1}		------------------		dry Mg ha^{-1}
High	H	4H-RA	56 CMS	33.6 CMS	22.4 MS	RA	33.6 MS
		3H-NRA				NRA	no manure
Medium-high	MH	MH-RA	28 CMS	16.8 CMS	16.8 MS	RA	22.4 MS
		MH-NRA				NRA	no manure
Medium-low	ML	ML-RA	33.6MS	33.6 MS	11.2 MS	RA	16.8 MS
		ML-NRA				NRA	no manure
Low	L	L-RA	16.8 MS	16.8 MS	5.6 MS	RA	5.6 MS
		L-NRA				NRA	no manure
Un-amended control	C	1H-RA	no manure	no manure	no manure	RA	33.6 MS
		C				NRA	no manure

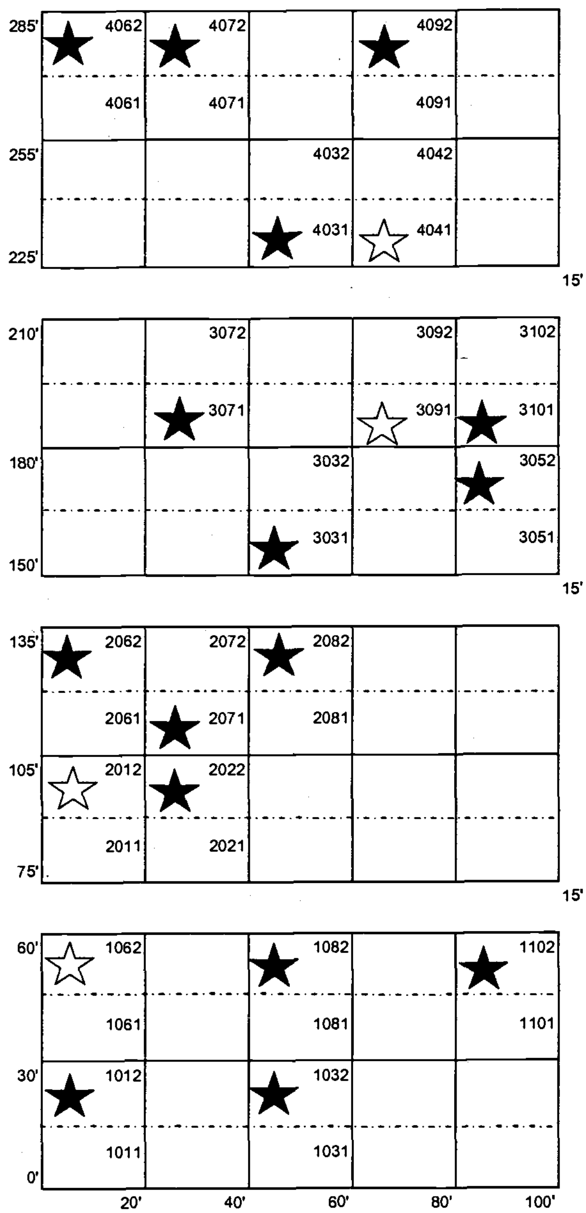
Figure 3.1 Schematic of the experimental design for the field trial examining effects of 5 levels of annual amendment with dairy manure solids on soil properties and severity of sweet corn root rot. In 2004, the original plots were split and half of each plot received a fresh re-amendment (RA) while half was not re-amended (NRA).

← North

	Treatment assignments		
	2003	2004	
		RA	NRA
Abbrev.	H	4H-RA	3H-NRA
Plot	101	1012	1011
	202	2022	2021
	307	3071	3072
	407	4072	4071
Abbrev.	MH	MH-RA	MH-NRA
Plot	103	1032	1031
	208	2082	2081
	303	3031	3032
	406	4062	4061
Abbrev.	ML	ML-RA	ML-NRA
Plot	108	1082	1081
	207	2071	2072
	305	3052	3051
	409	4092	4091
Abbrev.	L	L-RA	L-NRA
Plot	110	1102	1101
	206	2062	2061
	310	3101	3102
	403	4031	4032
Abbrev.	C	1H-RA	C
Plot	106	1062	1061
	201	2012	2011
	309	3091	3092
	404	4041	4042

★ serial RA

☆ 1st year RA



In 2003 one planting of sweet corn (*Zea mays* L. cv Golden Jubilee) was grown to harvest, and in 2004 sweet corn was grown to the 6-leaf stage in four staggered plantings three weeks apart.

The field site was irrigated weekly throughout the dry season with 2.54 cm of water delivered via overhead sprinklers.

Corn emergence was evaluated in 2003 as number of plants row-meter^{-1} to assess any treatment effects on seedling emergence. Yield data were taken at harvest in 2003, including ear weight, length, and width; kernel depth; and aboveground plant biomass. In 2004, no plants matured to harvest and no yield data were taken.

Treatments

Organic amendments were applied each spring over 4 years (Table 3.1). On May 29, 2003 and May 4, 2004, raw dairy manure solids (MS) were manually applied on a weight basis, and incorporated with a rotovator and a Roterra to a depth of 15 cm. Amendments were applied at different rates to give five levels of manure treatment: high (H), medium-high (MH), medium-low (ML), low (L), and no manure (control; C). The MS were purchased from a local dairy and consisted of the solid fraction of fresh dairy manure that had been separated using a screen separator.

In each year, the MS was analyzed for nutrient content and chemical characteristics. On the day the MS was received, 10 shovelfuls were taken from randomly selected locations within the MS pile. The sample was composited and mixed, and a subsample was sent to Agri-Check Inc. (Umatilla, OR) for nutrient analysis. Six subsamples of approximately 30 g each were oven-dried to determine MS moisture content. This was used to calibrate actual MS application rates from treatment assignments based on dry MS. In addition, two approximately 500 g subsamples were frozen as reference material.

Actual manure application rates varied in each of the study's four years. Amendments added in 2001 and 2002 included two treatments of composted dairy manure solids (CMS) instead of MS (Table 3.1). In determining the placement of 2003 rates, the new high rate was matched to the previous high rate based on free particulate OM (fPOM) remaining 6 months after 2002 amendment (data not shown). Composted manure solids were not included in the 2003 treatments because 2002 results indicated no benefit in the use of compost over fresh manure for inducing disease suppression. Rates in 2004 were increased from 2003 rates because of similarity between low rate treatments, and to widen the range of amendment rates.

In the spring of 2004, plots were split and manure amendments were applied to a randomly selected half of each plot. This permitted a comparison of disease severity throughout the season between freshly re-amended (RA) and not re-amended (NRA) split-plots. RA and NRA treatments received the same tillage management following re-amendment. When plots were split, half of the control treatment (C) was re-amended for the first time at the high rate (H); these treatments were given the designation 1H-RA, to represent the first year of high-rate amendment applied in 2004. When the H treatment was split, the re-amended half was designated 4H-RA (4 years of serial, high-rate amendment) while the not re-amended half was designated 3H-NRA (3 years of residual, serial, high-rate amendment).

Sweet corn was planted in all treatments on June 16, 2003 and simultaneously fertilized with a 12-29-10 starter fertilizer applied at a rate of 67 kg N ha^{-1}. At the 6-leaf stage, all control plots were sidedressed with an additional 112 kg N ha^{-1} (Darby, 2003). In 2004, sweet corn was planted in each plot two rows at a time on May 21, June 21, July 9, and August 3. Fertilizer was applied at planting as described above when rows were marked at the first planting date. The August 3 planting received additional fertilizer because of the late planting date.

Root rot bioassays

Corn root rot severity was determined using both *in situ* (in-field) assessments and greenhouse cone-tube bioassays except at the pre-amendment sampling dates in 2003 and 2004 when field operations prevented the planting of in-field bioassays.

In-field root rot bioassays

In-field root rot bioassays were planted on June 16, 2003 and on May 21, June 21, July 9, and August 3 in 2004. Sweet corn (cv Golden Jubilee) seeds treated with captan were planted approximately 3.8 cm deep and 20 cm apart in rows spaced 0.76 m apart. When plants reached the six-leaf stage, 5 plants were randomly selected from each of two rows designated for disease-assessment.

Radicle rot severity at the six-leaf stage is positively related to nodal root rot severity at harvest, and negatively related to yield (Stone, 2004). Radicle rot severity was rated by evaluating percent radicle necrosis. In 2003 a 5-point rating scale was used (Table 3.2), however for more precise estimations of disease severity a modified 9-point scale was used in 2004 (Table 3.3). In 2004 nodal root rot severity was also assessed using the new rating scale. In 2004, all plants in a bioassay were removed after assessment to prevent shading of adjacent bioassays.

Table 3.2 Rating scale to assess root rot severity of sweet corn from in-field and greenhouse bioassays conducted in 2003.

Scale for assessing radicle root rot severity		
Rating	Midpoint percent	Radicle description
0	0	healthy
1	5	lesion present
2	30	10-50% necrotic
3	75	51-99% necrotic
4	100	100% necrotic

Table 3.3 Rating scale to assess root rot severity of sweet corn from in-field and greenhouse bioassays conducted in 2004.

Scale for assessing radicle and nodal root rot severity		
Rating	Midpoint percent	Radicle or nodal root description
0	0	healthy
1	5	1-10% necrotic
2	15	11-20% necrotic
3	30	21-40% necrotic
4	50	41-60% necrotic
5	70	61-80% necrotic
6	85	81-90% necrotic
7	95	91-99% necrotic
8	100	100% necrotic

Greenhouse cone-tube bioassays

Greenhouse bioassays were conducted on soils sampled in 2003 on May 7, July 25, and September 1, and in 2004 on May 2, May 24, June 17, July 12, and July 28. During soil sampling, 10 wedges (approximately 15 cm by 15 cm by 5 cm) were collected from each plot with an AMS SharpShooter sampling shovel (AMS Inc., American Falls, ID) and composited. The sample was passed through a 2.54 cm mesh sieve and used to fill five 550 mL cone-tubes for each plot. During filling, the cone-tubes were gently tapped to settle the soil within the tube consistently across all tubes. Just before planting, captan-treated sweet corn seeds (cv Golden Jubilee) were surface disinfected with a 10% sodium hypochlorite solution for 5 minutes and rinsed with DI water. Two seeds per tube were planted 2.54 cm deep and then thinned to one plant per tube after emergence. Plants grew in a greenhouse (24°C day, 18°C night), with a 16 h photoperiod. Bioassays were fertilized weekly with 0.058 g N, 0.038 g P, and 0.049 g K cone-tube^{-1} using a water soluble fertilizer mix (Schultz Co., St. Louis, MO) and irrigated with overhead watering. Plants were harvested when they reached the 6-leaf stage. Root rot severity was evaluated as described previously for in-field bioassays.

Statistical Analyses

Treatment effects on root rot severity was determined using analysis of variance (ANOVA) procedures, computed for individual sampling times. Treatment means were separated using the Dunnett method to conduct multiple comparisons against the control (SAS Inst., 1999). Differences were considered significant at $p<0.05$ (unless otherwise noted). Linear regression analyses using PROC GLM were conducted in SAS to describe relationships between amendment rate and various soil properties (SAS Inst., 1999). Regression analyses were performed for individual sampling dates because relationships were not the same throughout the year. Paired two-sample t-tests were performed using S-PLUS (2001), and p-values for multiple comparisons were corrected using the Bonferonni adjustment.

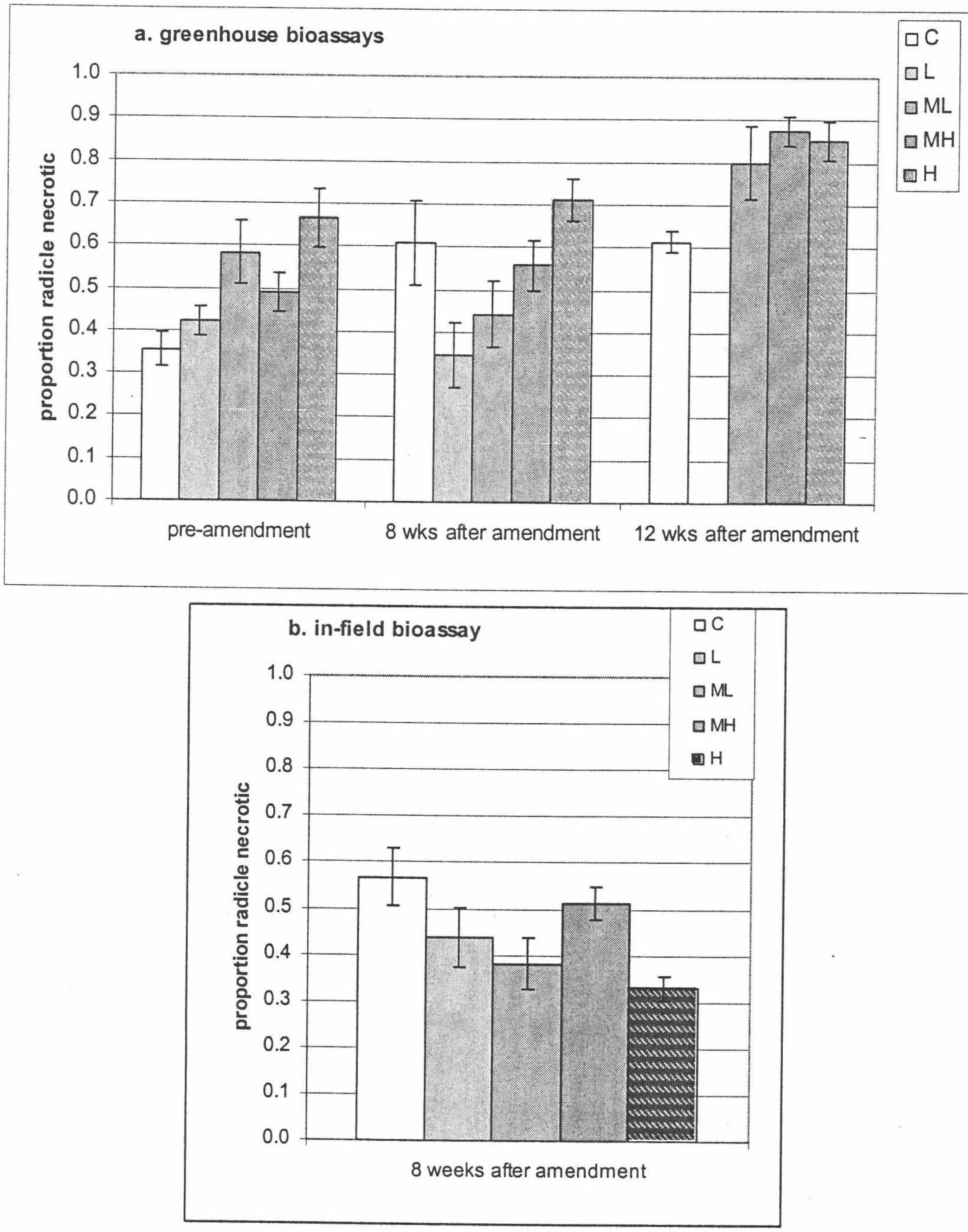
RESULTS

Pre-amendment 2003

Greenhouse bioassays

In 2003 manure amendment rate was positively related to disease severity 12 months after amendment across all rates ($p=0.001$, $r^2=0.45$). Corn grown in the H and ML treatment soils had 86 and 61% greater radicle necrosis ($p=0.003$ and $p=0.024$, respectively) than corn grown in the control soils (Figure 3.2). Corn grown in the MH and L treatments also had numerically higher disease severity than corn grown in the control soils, but the differences were not statistically significant.

Figure 3.2 Radicle rot severity from sweet corn grown in a) greenhouse bioassays and b) the in-field bioassay conducted in serially amended field soils in 2003. Error bars represent ±1 SE.

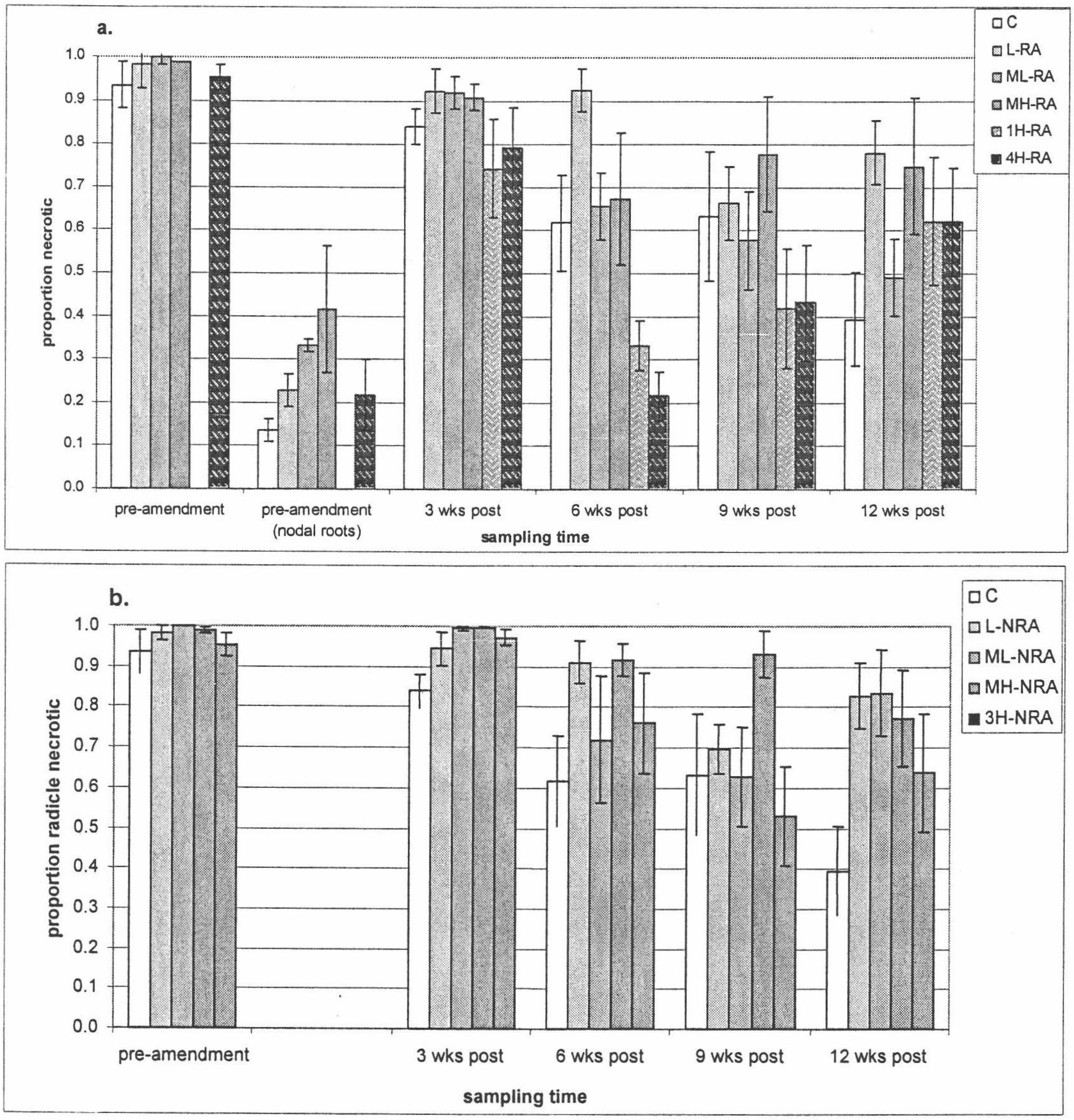


Pre-amendment 2004

Greenhouse bioassays

Radicle necrosis was nearly 100% in all treatments (Figure 3.3); therefore, nodal root rot severity was evaluated in addition to radicle rot severity. Amendment rate was positively related to radicle rot severity with the exception of the H treatment; corn grown in the H treatment had noticeably lower nodal rot severity than would be expected based on the trend observed in the other amended treatments (Fig. 3.3). With the H treatment excluded, there was a strong positive relationship between manure rate and root rot severity ($p=0.007$, $r^2=0.48$). Corn grown in the MH treatment had 287% greater nodal root rot severity than corn grown in the control ($p=0.078$).

Figure 3.3 Root rot severity (radicle and nodal, as specified) from sweet corn grown in serially amended field soils in 2004. Greenhouse bioassays were conducted on soils from a) freshly re-amended (RA) and b) not re-amended (NRA) treatments. Error bars represent ±1 SE.



Post-amendment 2003

In-field bioassays

Eight weeks after amendment corn grown in the H and ML treatments had 42% and 32% lower radicle rot severity, respectively, than corn grown in the control soils ($p=0.010$ and $p=0.044$, respectively) (Figure 3.2). Radicle rot severity in corn grown in the MH and L treatments was not different from the control. There was a negative relationship between disease severity and amendment rate ($p=0.04$, $r^2=0.21$).

Greenhouse bioassays

Eight weeks after amendment, there were no significant treatment effects on radicle rot severity (Figure 3.2). However, radicle rot severity in corn grown in the L treatment was 43% lower numerically than corn grown in the control soils ($p=0.102$). There was a positive relationship between disease severity and amendment rate ($p<0.001$, $r^2=0.57$) among the four amended treatments (when control was taken out of the analysis).

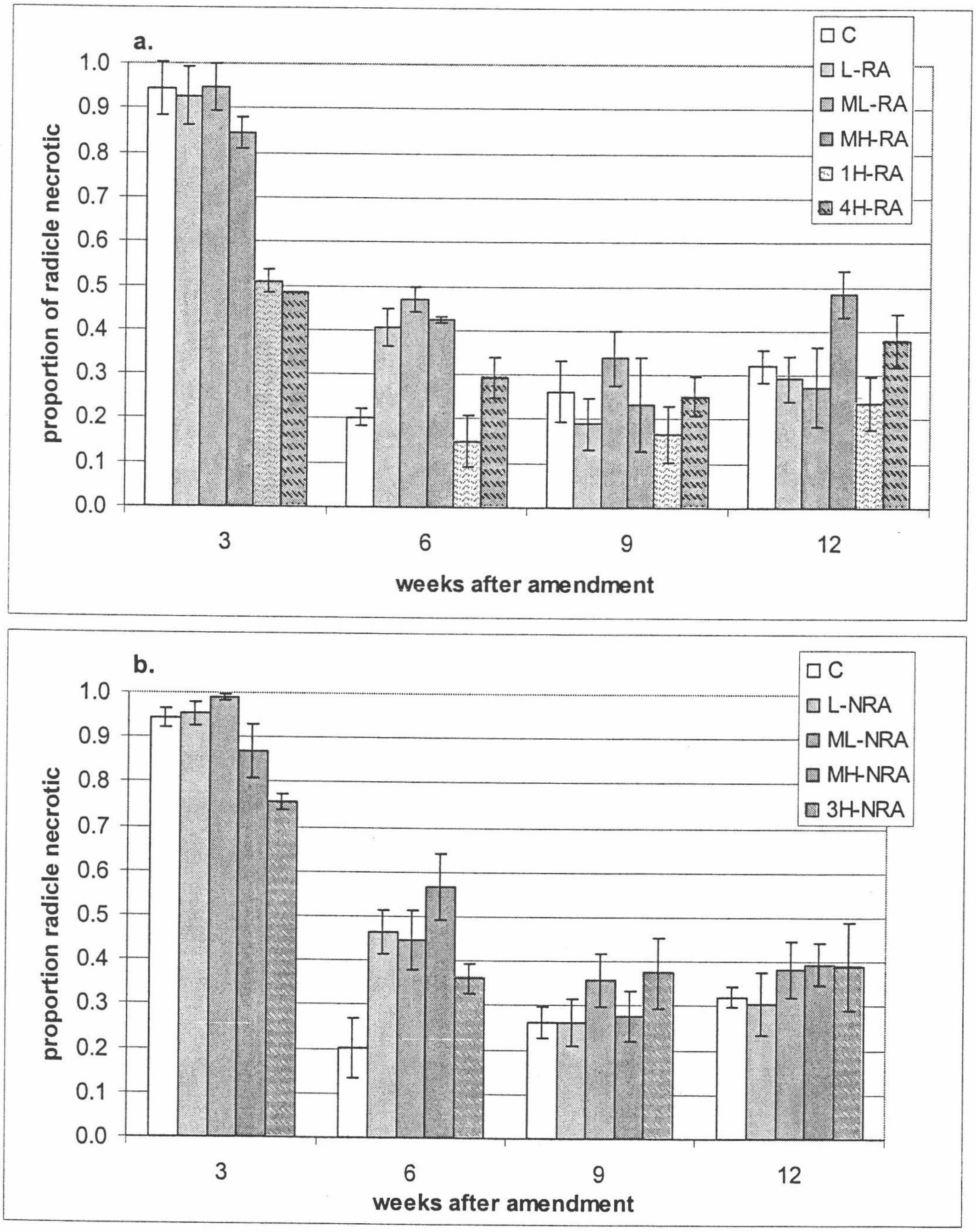
Twelve weeks post amendment, there was a positive linear relationship between radicle rot severity and amendment rate ($p=0.003$, $r^2=0.49$) (Figure 3.2). Radicle rot severity was 43% and 39% higher in corn grown in the MH and H treatments, respectively, than in corn grown in the control soils ($p\leq0.05$). Radicle rot severity in corn grown in the ML treatment was 31% greater numerically compared to corn grown in the control, but the difference was not statistically significant ($p=0.120$). The L treatment was not sampled at this time.

Post-amendment 2004

In-field bioassays

Three weeks after amendment all of the H treatments, including the 3H-NRA treatment, suppressed radicle rot of corn compared to the control (Figure 3.4). Radicle rot severity was reduced by 49%, 46%, and 20% in corn grown in the 4H-RA, 1H-RA, and 3H-NRA treatments, respectively, compared to corn grown in the control soils ($p<0.05$).

Figure 3.4 Radicle rot severity from sweet corn grown in serially amended field soils in 2004. In-field bioassays were conducted on soils from a) freshly re-amended (RA) and b) not re-amended (NRA) treatments. Error bars represent ±1 SE.



Six weeks after amendment in-field radicle rot severity in the control dropped significantly compared to the disease assessment 3 weeks earlier; disease severity in the control was 4.7 times lower than three weeks prior (Figure 3.4). Six weeks after amendment, radicle rot severity was significantly higher in corn grown in many of the amended treatments when compared to corn grown in the control: MH-NRA (p=0.071), ML-NRA (p=0.041), L-NRA (p=0.023), MH-RA (p=0.071), and ML-RA (p=0.024) (Figures 3.4). Disease severity was not different from the control in any of the H treatments (4H-RA p=0.816, 3H-NRA p=0.295, 1H-RA p=0.987).

Nine and twelve weeks after amendment there were no treatment effects on radicle rot severity (Figures 3.4). Radicle rot severity in corn grown in the control treatment was 3.6 times lower and 3.7 times lower at 9 and 12 weeks post-amendment, respectively, than control-grown corn from the 3 week post-amendment bioassay.

Greenhouse bioassays

There were no treatment differences in radicle rot severity 3 weeks after amendment in 2004. Radicle rot severity was numerically lower in corn grown in the 1H-RA and 4H-RA treatments compared to the control, but the difference was not statistically significant (Figure 3.3).

Six weeks after amendment, radicle rot severity was 65% lower in corn grown in the 4H-RA treatment compared to the control (p=0.026) (Figure 3.3).

Disease severity in the 1H-RA treatment was 53% lower than the control, but the difference was not statistically significant ($p=0.161$).

There were no treatment differences in radicle rot severity nine weeks post-amendment. Radicle rot severity was numerically lower in corn grown in the 4H-RA and 1H-RA treatments compared to corn grown in the control, but the differences were not statistically significant (Figure 3.3).

Twelve weeks after amendment, radicle rot severity was greater than the control in all NRA treatments ($p \leq 0.02$), except the 3H-NRA (Figure 3.3). Among RA treatments, radicle rot severity was greater than the control in the L-RA and MH-RA ($p \leq 0.03$) treatments only (Figure 3.3).

Comparisons of radicle rot severity in NRA and RA treatments

Radicle rot severity in corn grown in the NRA treatments was, in general, slightly numerically higher than that of corn grown in the RA treatments (Figures 3.3, 3.4). The differences were more pronounced in greenhouse bioassays than from in-field assessments, and in instances when RA treatments were suppressive. Statistically significant suppression was observed once in an NRA treatment; three weeks after amendment, radicle rot severity was 20% lower in field-grown corn in the 3H-NRA treatment compared to the control (Figure 3.4). The magnitude of disease suppression in the 3H-NRA treatment was roughly half that observed in the freshly amended H treatments (4H-RA and 1H-RA). At this time, corn grown in the MH-NRA treatment had numerically lower radicle rot severity compared to corn grown in the control, but the difference was not statistically significant (Figure 3.4).

Comparisons of radicle rot severity in the single-year and serially amended high-rate treatments

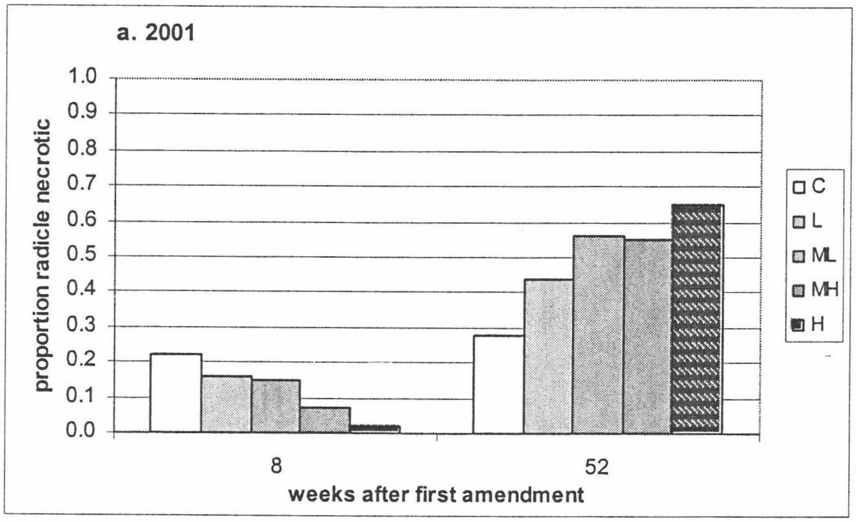
There was no difference in radicle rot severity in the 1H-RA and 4H-RA treatments as determined by in-field bioassays 3 weeks after amendment, and at this time both treatments were suppressive (Figure 3.4). Six, 9, and 12 weeks after amendment, radicle rot severity of corn grown in the control was not different from corn grown in either the 1H-RA or 4H-RA treatments (Figure 3.4). However, radicle rot severity of corn grown in the 1H-RA treatment was numerically lower than in the 4H-RA and control treatments at 6, 9, and 12 weeks after amendment (Figure 3.4).

In greenhouse bioassays, the magnitude of suppression observed 6 weeks after amendment was not significantly different between the 1H-RA and 4H-RA treatments, although root rot severity from corn grown in the 4H-RA treatment was numerically lower (Figure 3.3). At the 3, 9, and 12 week samplings, there were no differences in radicle rot severity of corn grown in the 1H-RA and 4H-RA treatments (Figure 3.3).

Trends in root rot severity over 4 years

There were general trends in root rot severity 8 weeks after amendment and pre-amendment in 2001 - 2003. Eight weeks after amendment, radicle rot severity was negatively related to amendment rate; at pre-amendment samplings, radicle rot severity was positively related to amendment rate (Figure 3.5; Darby et al., in press). In 2003, radicle rot of field-grown corn at the 6-leaf stage was similar to the previously observed trend, but the trend for radicle rot 8 weeks after amendment in the greenhouse bioassay was very different (Figures 3.2, 3.5). The 2004 pre-amendment trend was similar to the trends following the first and second amendments, with the exception of the high treatment. Nodal root rot severity in corn grown in the H treatment was much lower than expected, and was not different from corn grown in the control treatment (Figures 3.3, 3.4, 3.5). There was no apparent trend in radicle rot at 6 and 9 weeks after amendment in 2004 although radicle rot severity was significantly lower in the H treatment than the other treatments at 3 weeks and 6 weeks, and numerically lower at 9 weeks (from in-field, greenhouse, and greenhouse bioassays, respectively) (Figs. 3.3 and 3.5).

Figure 3.5 Summary of results from assessments of radicle rot severity of sweet corn throughout the four years of the study. Effects of five levels of serial manure amendment on root rot severity are shown for a) 2001, b) 2002, c) and d) 2003, and e) 2004. All disease severity ratings are from greenhouse bioassays except d) which represents in-field radicle rot severity. Panel c) includes one assessment of nodal root rot severity.



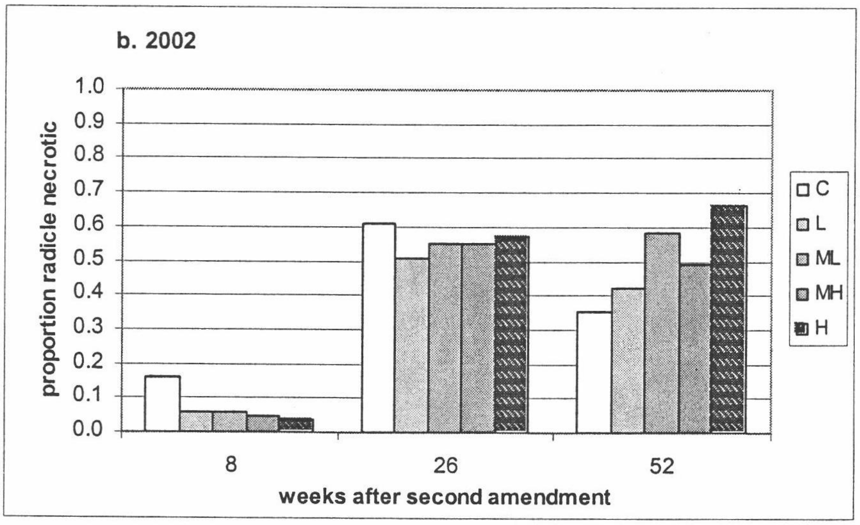
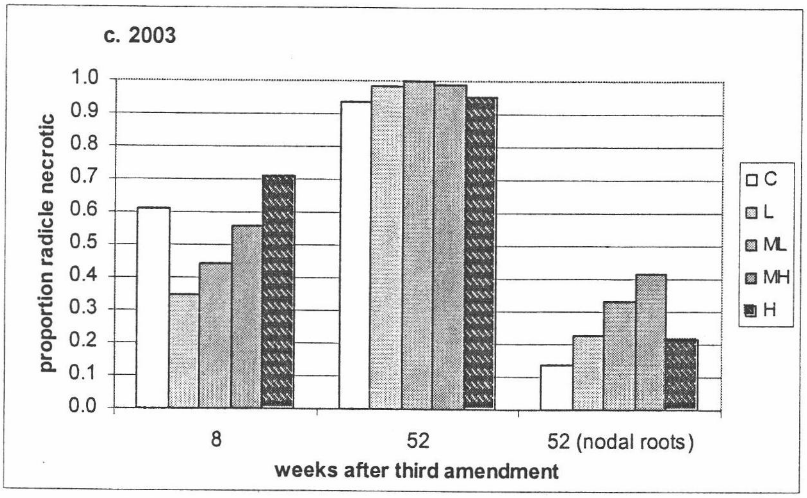
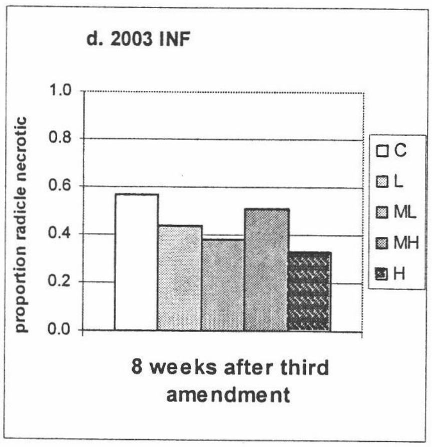
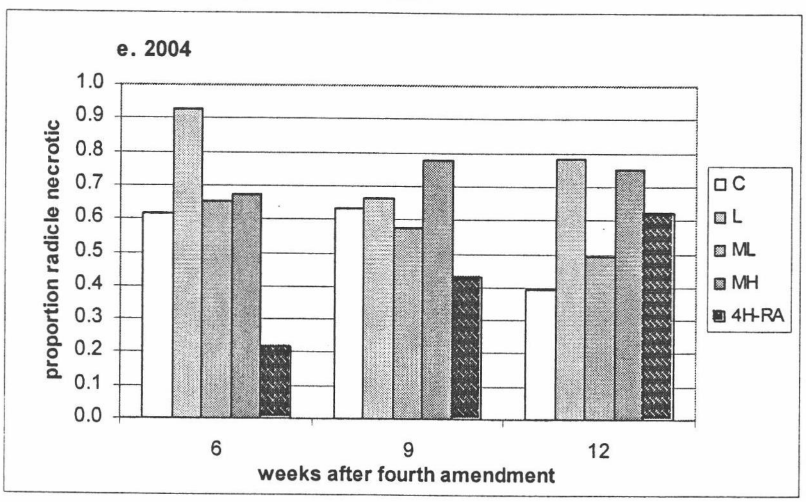


Figure 3.5 (Continued)







Relationships between root rot severity and soil properties

2003

Pre-amendment, gravimetric soil moisture content (Figure 3.6), FDA activity (Figure 3.7), and fPOM contents (Figure 3.8) were all positively related to greenhouse radicle rot severity. Eight weeks after amendment, all were negatively related to in-field radicle rot severity (Figures 3.6, 3.7, and 3.9).

2004

Pre-amendment, gravimetric soil moisture content (Figure 3.10), FDA activity (Figure 3.11), and fPOM contents (Figure 3.8) were all positively related to greenhouse nodal root rot severity (when 4H-RA was removed from the analysis). At 3 weeks after amendment, gravimetric moisture content and FDA activity were negatively related to in-field radicle rot severity (Figures 3.10, 3.11). Six, 9 and 12 weeks after amendment, there were no relationships between these soil properties and radicle rot severity.

Figure 3.6 Relationships between in-field soil moisture content and radicle rot severity of sweet corn in 2003 a) before re-amendment with manure solids, and b) 8 weeks and c) 12 weeks after amendment. Graphs a) and c) represent greenhouse bioassays, b) represents an in-field assessment.

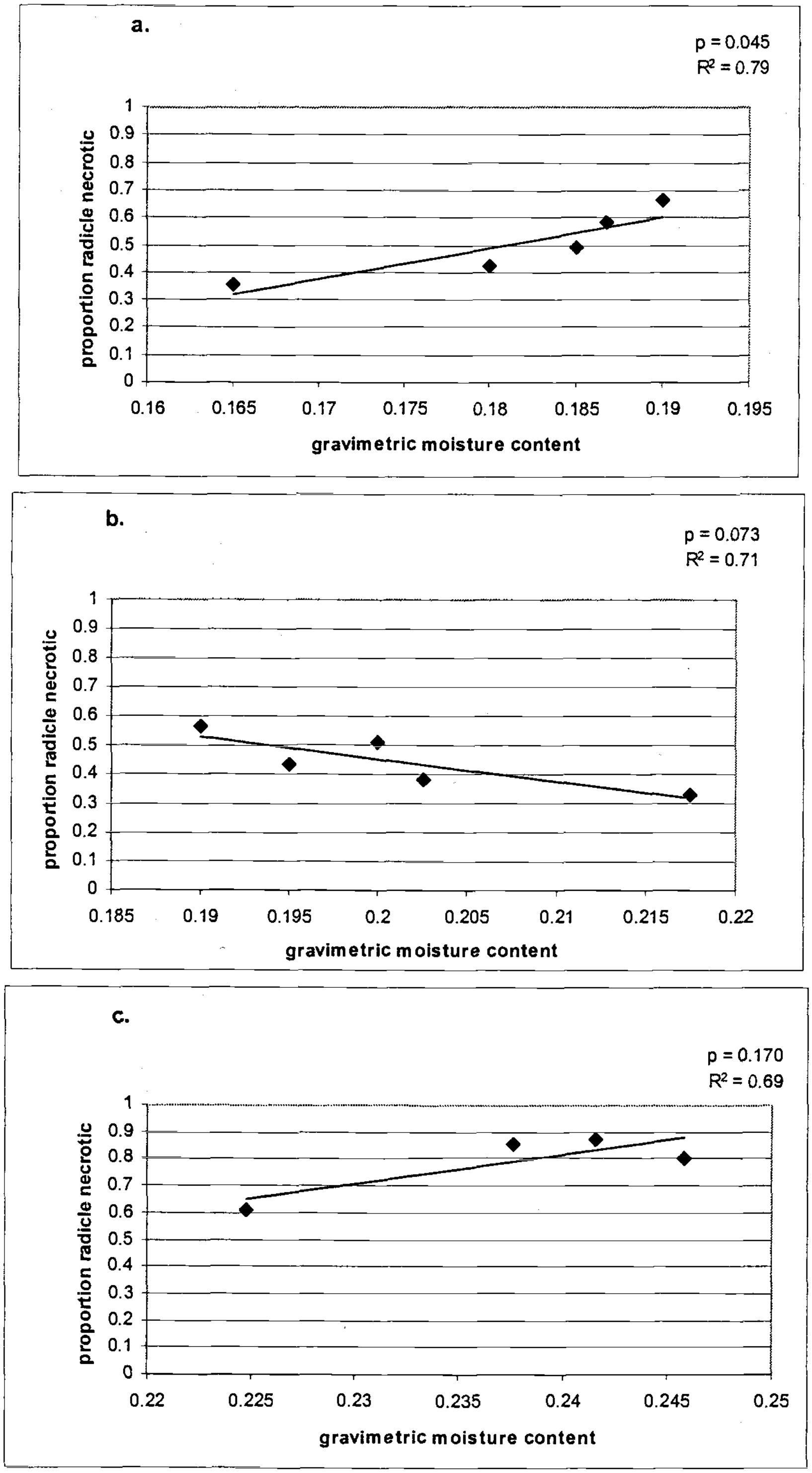


Figure 3.7 Relationships between soil microbial activity (as FDA activity) and radicle rot severity of sweet corn in 2003 a) before re-amendment with manure solids, and b) 8 weeks and c) 12 weeks after amendment. Graphs a) and c) represent greenhouse bioassays, b) represents an in-field assessment.

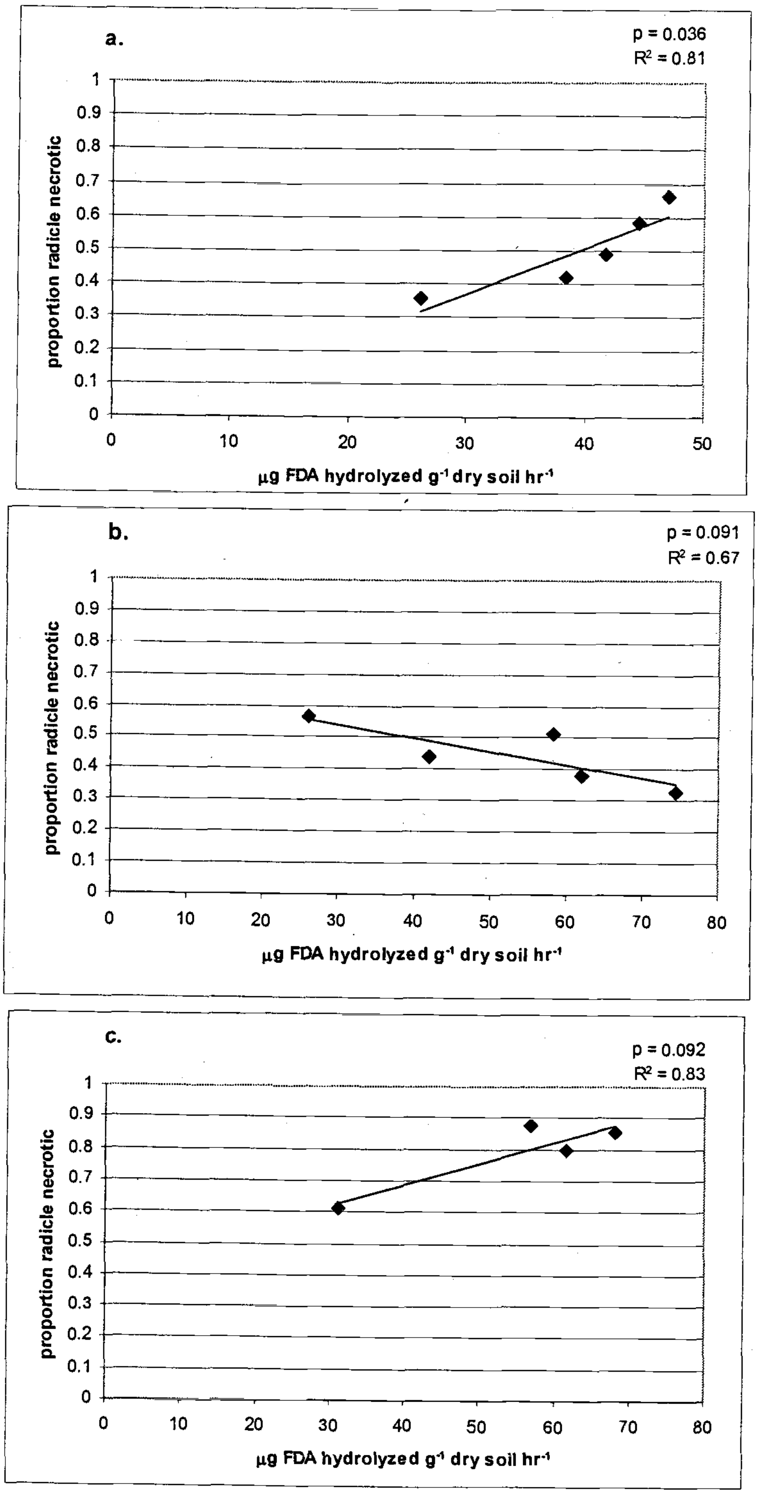


Figure 3.8 Pre-amendment relationships between greenhouse root rot severity of sweet corn and free particulate organic matter (fPOM) were positive across all years of the study. Samplings occurred 12 months after amendment of dairy manure solids in 2001, 2002, and 2003. Radicle rot severity is shown for 2001 and 2002. 2003 shows nodal root rot severity and excludes the high (H) treatment from the regression.

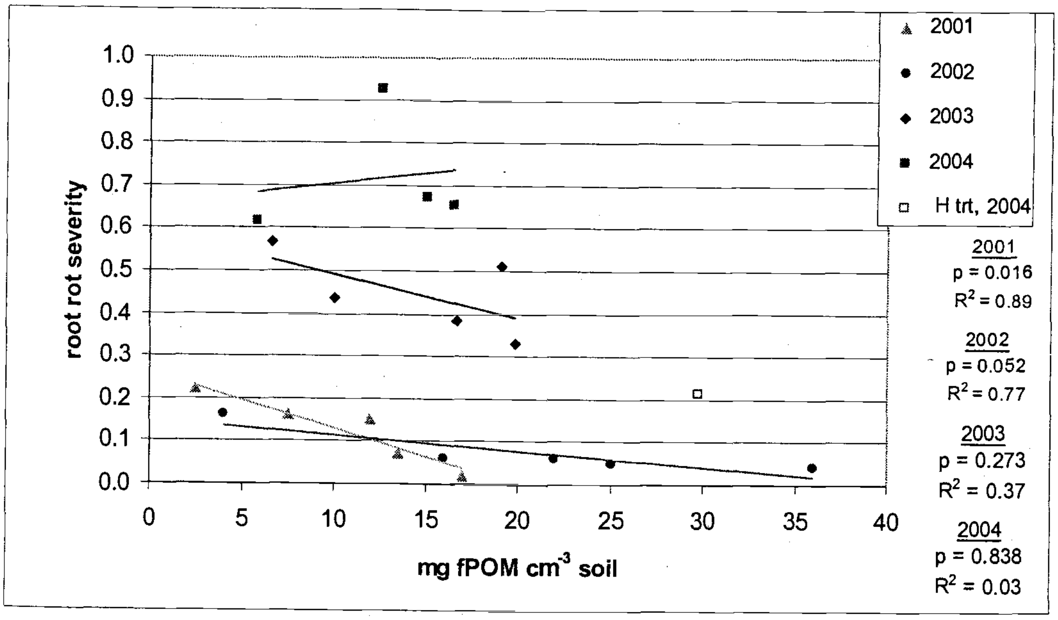


Figure 3.9 Eight weeks after amendment with dairy manure solids, relationships between root rot severity of sweet corn and free particulate organic matter (fPOM) changed with serial amendment. 2001 and 2002 data represent radicle rot severity from greenhouse bioassays. 2003 data represent in-field radicle rot severity. 2004 data represent radicle rot severity from greenhouse bioassays 6 weeks after amendment with the 4H-RA treatment excluded from the regression.

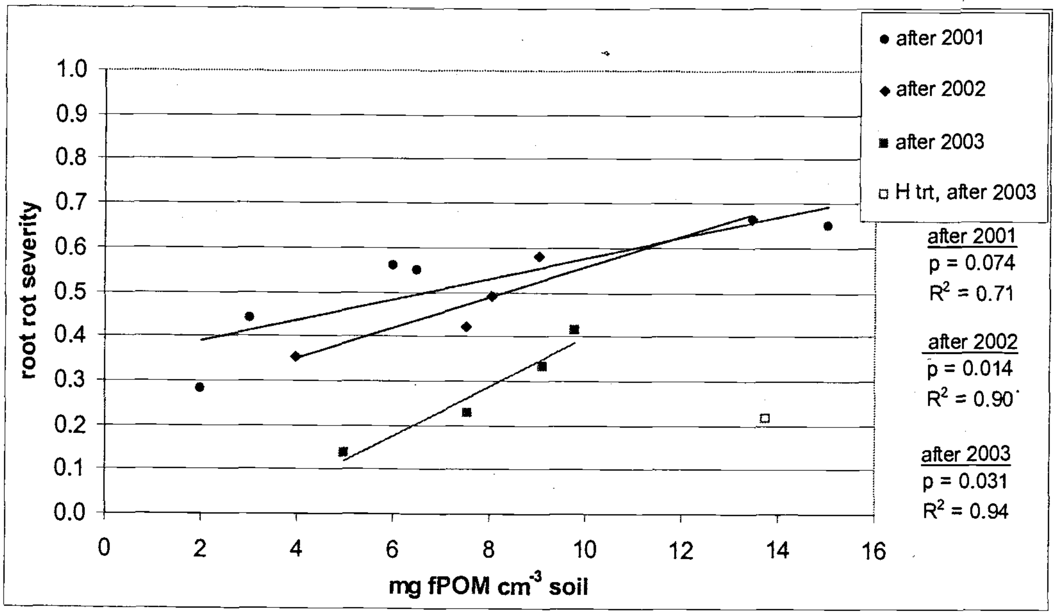


Figure 3.10 Relationships between in-field soil moisture content and root rot severity of sweet corn in 2004 a) before amendment and b) 3 weeks, c) 6 weeks, d) 9 weeks, and e) 12 weeks after re-amendment. Pre-amendment (a.) greenhouse nodal root rot severity is shown. In-field radicle rot severity is shown in all other panels. In (a.) the H treatment (○) was excluded from the regression.

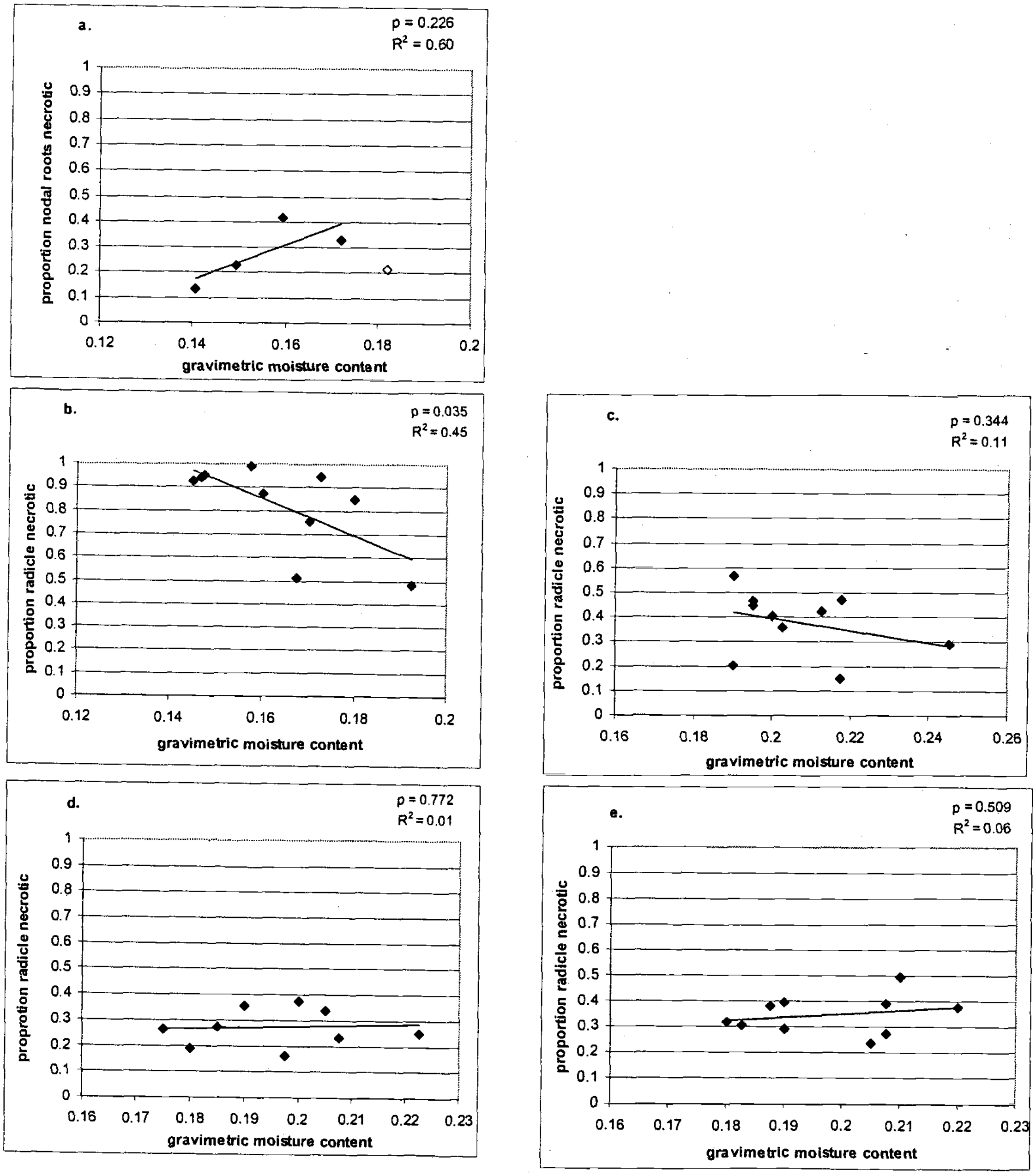
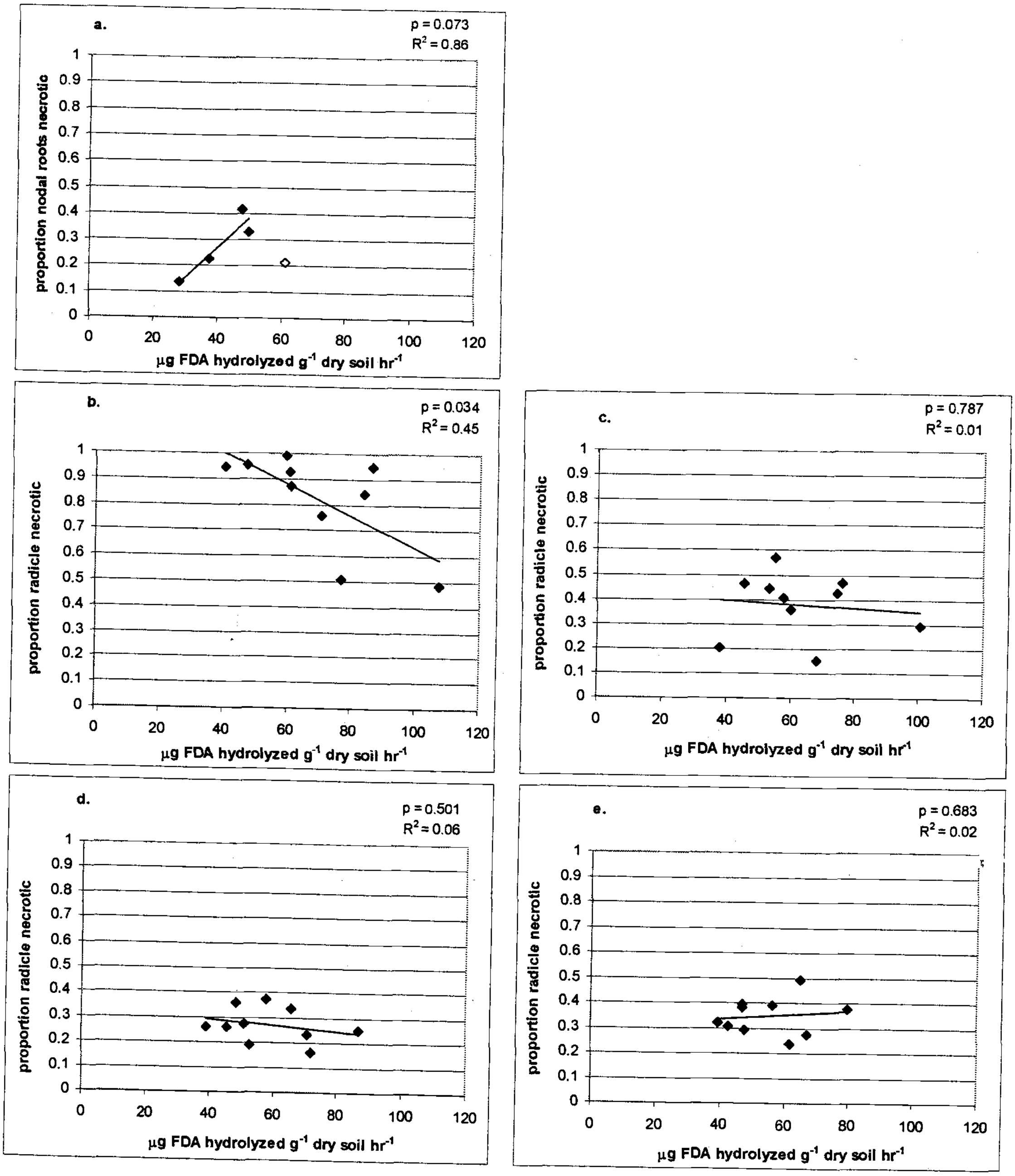


Figure 3.11 Relationships between soil microbial activity (as FDA activity) and root rot severity of sweet corn in 2004 a) before amendment and b) 3 weeks, c) 6 weeks, d) 9 weeks, and e) 12 weeks after re-amendment. Pre-amendment, (a.) greenhouse nodal root rot severity is shown. In-field radicle rot severity is shown in all other panels. In (a.) the H treatment (o) was excluded from the regression.



DISCUSSION

Trends in observed root rot severity

Four major trends were observed over the 4 years of this study: 1) short-term root rot suppression immediately after amendment (except in 2004) 2) a positive relationship between root rot severity and amendment rate at 12 months post-amendment samplings, 3) unusually low root rot severity in the H treatments at 2004 pre- and post-amendment samplings, and 4) little difference between root rot severity ratings in NRA treatments compared to RA treatments in 2004.

Short-term root rot suppression immediately after amendment

Eight weeks after the first manure application in 2001 all treatments except the low MS (now L) were suppressive to corn root rot (Figure 3.5). Eight weeks after the second amendment in 2002, root rot suppression was observed in all amended treatments (Figure 3.5). Suppression was lost at some point before the 12 month post-amendment sampling in the first year, and before the 6 month post-amendment sampling in the second year (Darby, 2003). The precise duration of suppression in 2001 and 2002 is not known, as soil samplings were widely separated in time in these years. Root rot severity 8 weeks after amendment in the first two years of the study was strongly negatively related to FDA activity ($p=0.002$, $r^2=0.56$) (Darby et al., in press). Root rot severity was less strongly related to soil fPOM ($p=0.06$, $r^2=0.24$) and MBC contents ($p=0.09$, $r^2=0.21$) (Darby et al., in press). FDA activity was the factor most strongly associated with disease suppression in 2001 and 2002 (Darby et al., in press). FDA activity has been shown to be a reliable indicator of disease suppressive potential of soils and peat or compost-amended soil-less container mixes (Stone et al., 2004; Dissanayake and Hoy, 1999; Boehm and Hoitink, 1992).

Because suppression in the low and high MS treatments (now L and ML, respectively) was of greater magnitude following the second year of amendment and because MBC aggrades over time under serial amendment (Gunampala and Scow, 1998; Fraser et al., 1988; Zaller and Kopke, 2004), we hypothesized that in subsequent years of the study it would take less labile organic matter (lower rates of organic amendment) to raise FDA levels above the proposed "suppressive FDA threshold" and to generate suppression of radicle rot.

However, that hypothesis was refuted by the data in years 2003 and 2004. Baseline (pre-amendment) levels of soil MBC and FDA activity increased following each year of amendment (Figures 2.10, 2.13) but overall, the level and consistency of suppression decreased. Interestingly in 2003 only the ML and H treatments were suppressive, and by 2004 only the H treatments (4H-RA, 3H-RA and 1H-RA) were suppressive. Comparisons between years are somewhat confounded by changes in the actual amendment rates in each year, although treatment levels (L, ML, etc.) were maintained by re-assigning rates in the same order every year. In addition, both fresh and composted MS were applied in 2001 and 2002. Composted MS has already gone through the early stages of rapid manure decomposition and therefore decomposes more slowly than MS after soil application (Hoitink et al., 1991); this difference in composition and lability could have residual effects on soil properties and suppressiveness. However, in a greenhouse study of radish damping-off (causal agent *Rhizoctonia solani)*, composting dairy manure did not significantly enhance effectiveness of manure for inducing suppression (Voland and Epstein, 1994). Composted manure did not appear to be more suppressive than MS in this field trial in 2001 and 2002; the lack of significant suppression in the low MS treatment in 2001 was considered to be due to the low amendment rate (Darby et al., in press).

Positive relationship between root rot severity and amendment rate at pre-amendment (12 months post-amendment) samplings

Twelve months after the first manure amendment there was a positive relationship between disease severity and amendment rate (Figure 3.5) (Darby et al., in press). This same trend was observed at each subsequent pre-amendment sampling time (Figures 3.5). It was expected that suppression would be lost before re-amendment, as OM-mediated disease suppression immediately after high-rate amendment is typically short-term (several weeks to a year in duration) (Stone et al., 2001; Boehm and Hoitink, 1992).

It was interesting, however, that disease severity did not simply return to pre-amendment levels but increased to levels significantly higher than in the control treatments, resulting in a positive relationship between amendment rate and root rot severity. Possible explanations for this effect are explored below in the discussion of factors affecting root rot severity.

Unusually low root rot severity in the H treatments at 2004 pre- and post-amendment samplings

Just before re-amending in 2004, the positive relationship between disease severity and amendment rate was again observed, except for the H treatment. Nodal root rot severity in the H treatment was lower than would be expected based on the trend observed in the other amended treatments (Figure 3.5). In addition, three weeks after re-amendment to half of each treatment, the 3H-NRA treatment suppressed disease compared to the control, in spite of not receiving a fresh amendment in 2004 (Figure 3.4). In both the pre-amendment and 3H-NRA samplings, FDA activity levels were relatively low compared to the periods of amendment-induced FDA increase that had been associated with disease suppression previously. Taken together, these suggest the emergence of a new mechanism of disease suppression in serially amended high-rate treatments that is of longer duration and not related to the post-amendment flush of microbial activity. Many mechanisms for disease suppression have been identified, including competitive root colonization, induced systemic resistance, and pathogen destruction, any one of which may explain this phenomenon (Alabouvette, 1990; Vallad et al., 2003; Hointink and Boehm, 1999; Stone et al., 2004; Lockwood, 1990).

Little difference between root rot severity ratings in NRA compared to RA treatments in 2004

The hypothesis going into 2004, when plots were split, was that root rot severity in NRA treatments would increase with amendment rate as occurred at each pre-amendment sampling. However, the relationship between root rot severity and amendment rate in corn grown in NRA treatments during the 2004 growing season did not show that positive trend. On the other hand, NRA treatments (except 3H-NRA, 3 weeks after amendment) were not suppressive compared to control plots. Overall, there were no obvious trends in root rot severity in NRA treatments post-amendment in 2004, and there was also little difference overall in NRA and RA treatments.

The pre-amendment sampling occurs before any spring tillage operations. Pre-amendment soils have not been disturbed since planting almost 12 months previously, as crop residues were never fall-incorporated. Following manure application to RA treatments, all treatments were rotovated to incorporate manure (if present) and to keep soil disturbance consistent across all treatments. Therefore, the 2004 NRA samples experienced a significant disturbance (tillage) event, as well as soil warming, before sampling, even though they did not receive a manure amendment. There was no overall treatment effect on root rot severity in NRA treatments, which were sampled post-tillage. This is very different than at pre-amendment, when root rot severity increased with amendment rate (with the exception of the H treatment). Tillage breaks soil aggregates and also larger particles of organic matter, exposing particles and surfaces of particles to microbial attack that were previously physically protected. Increased microbial availability of this material after tillage may account for the relative "suppressiveness" of the NRA treatments when compared to the trend observed at pre-amendment. At any amendment rate, there appears to be some level of residual suppressiveness in the NRA treatments which had a history of serial amendment but no fresh amendment in 2004. While increased availability of residual POM after tillage may account for the short-duration suppression that emerged in the 3H-NRA treatment 3 weeks after amendment, that would not explain the lower-than-expected nodal root rot severity observed pre-amendment in the H treatment. This suggests the presence of an alternative suppressive mechanism that has developed over time in the H treatment, and that is less related to OM decomposition level and post-amendment FDA activity.

Overall, there were no obvious trends in root rot severity in NRA or RA treatments post-amendment in 2004, although in general, corn grown in NRA treatments exhibited higher root rot severity than corn grown in corresponding RA treatments (although not statistically significant). The lack of strong differences between the RA and NRA treatments in 2004 is not easily explained; some combination of soil factors described in the next section might interact to generate the observed root rot severity in 2004 treatments soils. In summary, there may be factors contributing to residual suppressiveness, as well as factors negating short-term suppression, in serially amended soils.

Other confounding factors

To add to the complexity, direct comparisons of in-field root rot severity between sampling times is difficult because environmental conditions (soil T, day-length, light intensity) vary throughout the season. If soils with high residual OM contents (such as serially amended H treatment) dry more slowly than the control, then treatment differences in soil moisture content may be exacerbated by hot, dry conditions that are more commonly observed in the mid- to late-season. This could explain the significantly lower root rot severity of corn grown in the in-field control treatment 6, 9, and 12 weeks post-amendment compared to the pre-amendment and 3 week post-amendment samplings.

Possible factors affecting root rot severity

There are several factors that may be interacting to generate observed root rot severity: 1) microbiostasis (associated with short-term fluxes in FDA activity), 2) soil moisture retention, 3) inoculum potential, and 4) suppressive mechanisms other than short-term "microbiostasis."

Microbiostasis associated with short-term fluxes in FDA activity

Soil microorganisms regularly exist in an energy-limited environment (De Nobili et al., 2001; Fontaine et al., 2003; Lockwood, 1990). In the absence of organic inputs from plants or animals, microbial biomass maintains only basal metabolism and lacks the capacity to undergo exponential growth. The addition of a fresh manure amendment, however, provides a labile energy source for microbial growth and can dramatically increase microbial biomass (Fontaine et al., 2003). Short-term disease suppression following a high-rate organic amendment is associated with "microbiostasis." General suppression, a frequently described suppressive mechanism, is generated by the total activities of the soil microbial biomass (Cook and Baker, 1983). It is equivalent in a sense to "microbiostasis" because the success of individual soil microorganisms is limited by competition for energy sources (Cook and Baker, 1983).

Organic-matter mediated "microbiostasis" has frequently been correlated with periods of elevated FDA activity (Boehm and Hoitink, 1992; Stone et al., 2003; Dissanayake and Hoy, 1999; Chen et al., 1988). FDA is hydrolysed by microbially-produced extracellular esterases, lipases, and proteases, and is considered a general indicator of soil microbial activity (Dick, 1997). FDA activity has been shown to be better related to disease suppression after organic amendment than other microbial indicators such as β-glucosidase, aryl sulfatase, or MBC (Darby et al, in press), and has been related to soil borne disease suppression in soil-less container mixes (Hoitink et al., 1991; Chen et al., 1988; Boehm and Hoitink, 1992) and field soils (Dissanayake and Hoy, 1999; Drinkwater et al, 1995; Davis et al, 1994). It is likely that this phenomenon explains the suppression of root rot that was observed shortly after amendment in years 2001-2003.

Residual FDA activity

In this field study of serially amended soils, when disease suppression was observed (8 weeks after 2003 amendment, and 3 and 6 weeks after 2004 amendment) root rot severity was negatively related to FDA activity (Figure 3.7, 3.11). This is in agreement with the findings from the first 2 years of this field trial (Darby et al., in press). It was unexpected, however, that the relationship between disease severity and FDA activity would change throughout each season as suppression was lost; at the pre-amendment sampling dates there was a strong positive relationship between disease severity and FDA activity (Figure 3.7, 3.11). FDA activity that aggrades over years, or "residual FDA activity" is of a different quality than the flush of FDA activity observed just after fresh amendment, and its relationship to disease severity is unknown.

Soil moisture retention

A number of long-term studies have shown that annual organic amendments increase soil water retention (Arriaga and Lowery, 2003; Benbi et al., 1998; Pernes-Debuyser and Tessier, 2004; Zibilske et al., 2000; Giusquiani et al., 1995; Schjonning et al., 1994; Tester, 1990). This effect is likely due in part to the impact of OM on soil structure, and in part to the water holding ability of POM. Repeated OM amendment induces improvements in macroaggregate formation (Aoyama et al., 1999 a; Whalen et al., 2003; Sommerfeldt and Chang, 1985) and contributes to increased moisture retention across a wide range of soil water potentials (Rose, 1991; Giusquiani et al., 1995).

Soil moisture is positively related to the severity of some soil-borne root rots (Kerr, 1964; Bhatti and Kraft, 1992), and soil moisture content was positively linearly related to severity of root rot of sweet corn in irrigation management field trials conducted in 2003 and 2004 at the OSU Vegetable Research Farm (Peachey et al., 2004). High soil moisture increased severity of root rot and wilt of chickpea (causal agents *Fusarium oxysporum* f. sp. *ciceri*, *F. solani* f. sp. *pisi, Pythium ultimum*, and *Thielaviopsis basicola*) and increased rhizosphere populations of the pathogens (Bhatti and Kraft, 1992). Similarly, infection of peas by *Pythium ultimum* increased with increasing soil moisture content (Kerr, 1964). Rao et al. (1978) reported decreased recovery of *Pythium spp.* from corn roots during periods of low moisture.

It is possible that the high water retention of the amended soils exacerbated root rot severity when the POM had decomposed to the point where biologically-driven disease suppression (related to FDA activity) was weak or lost. However, we are unable with this data set to determine with certainty which, if any, of the measured soil properties are causally related to the observed increase in root rot severity that occurred at each pre-amendment sampling date, as all of the measured properties are interrelated and tied closely to fPOM contents and generalized amendment rate (Figure 2.14).

In both 2003 and 2004, the relationships between disease severity and soil moisture content changed between sampling times throughout each season. Pre-amendment, there was a positive relationship between disease severity and soil moisture (Figures 3.6, 3.10), as would be expected from other work showing that moist conditions increase root rot severity (Peachey et al., 2004; Kerr, 1964; Bhatti and Kraft, 1992). Interestingly, in both years there was a negative relationship between disease severity and soil moisture just after re-amendment, which corresponded with periods of disease suppression (Figures 3.6, 3.10). Because soil moisture can not contribute to both increases and decreases in disease severity, the shifting relationships suggest that an additional factor (presumably soil biological activity) affects disease severity after soils have been re-amended. When disease severity was negatively related to soil moisture, levels of FDA activity peaked. During these periods, microbial activity is the likely factor driving the decrease in disease severity. As suppression subsided, the relationship between disease and moisture dissolved, and eventually re-emerged as a positive relationship between disease severity and soil moisture (Figures 3.6, 3.10).

The idea that observed disease severity is the product of two opposing forces—soil biological activity and soil moisture—is supported by results from greenhouse bioassays in 2003. When in-field bioassays indicated weak suppression in 2003, greenhouse bioassays at this time showed a positive relationship between disease severity and amendment rate in the amended treatments (Figure 3.2). Measurements of greenhouse cone-tube soil moisture in 2004 revealed that moisture at all sampling times was consistently higher in the cone-tubes than in the field. The wetter greenhouse conditions (coupled with the lower actual amendment rates applied in 2003) likely combined to negate suppression in the GH, while weak suppression was observed in-field.

It is noteworthy that GH disease bioassays did not always reflect patterns of disease severity observed in-field for a given sampling time. GH bioassays are a commonly used tool for assessing disease severity, in part because they can be conducted in a controlled environment and are highly repeatable. However, because of the soil disturbance created by moving soil into cone-tubes and the artificially small, moist environment created, GH cone-tube conditions seem to exacerbate soil moisture effects on root rot severity. In conditions of lower disease pressure, this may be useful for observing treatment effects (as in the first and second years of the study). However, following multiple years of serial amendment the treatment-induced increases in soil water retention may be amplified by the GH environment.

Inoculum potential

Overall root rot severity increased over the four years of the trial. The trial was planted to corn in each year except 2002, when it was planted to bean. Root rot of sweet corn is strongly associated with intensity of corn production (Stone, 2004), so this effect is not surprising. As inoculum levels increase, the degree of OM-induced suppression may decrease. This has been shown for compost-mediated suppression of cucumber damping-off (causal agent, *Pythium ultimum*) (H.A.J. Hoitink, personal communication).

Serial OM addition may have a stimulatory effect on saprophytic growth of pathogens in the root rot complex. Many soil-borne pathogens are classified as facultative saprophytes (Agrios, 1997), and OM increases in serially amended plots may stimulate pathogen growth. The three causal agents of root rot of sweet corn are suspected or confirmed saprophytes. *Phoma terrestris* is a widespread saprophyte (Mao et al., 1998), many *Pythium* spp. are common soil inhabitants and *Drechslera sp.* is a facultative saprophyte (Vargas, 2005). If soil populations of the root rot complex pathogens respond to residual OM, pathogen populations could be positively related to amendment rate. Therefore, inoculum potential might have changed differentially with amendment rate during the four years of the study. This is an important factor to assess in subsequent years of work on this field site.

While cultural practices that could enhance pathogen growth would not be thought of as beneficial, studies have shown that suppression can occur without a decrease of inoculum. In a 6-year field study of the effects of green manures on Verticillium wilt in potato, Davis et al. (1999 a) reported disease suppression following two consecutive years of a sweet corn green manure. After two years of potato and an additional year of green manure (sweet corn or barley), suppression of Verticillium wilt was observed again, even though inoculum density (ID) of *Verticillium dahliae* had increased 2-4 fold (Davis et al., 1994). Amendment of field plots with composted sewage sludge (7-10 t ha^{-1}) reduced incidence of pea damping-off (casual agents *Rhizoctonia solani* and *Pythium ultimum*); compost significantly reduced ID of *R. solani*, but the population of *P. ultimum* increased significantly in the compost-amended soil compared to the non-amended soil (Lewis et al., 1992).

In addition, amended treatments might support the activities of biocontrol organisms that could destroy pathogen propagules, as has been shown in other OM-mediated systems (Lumsden et al., 1983). Investigations of the microbial communities of soils in Australia that are either suppressive or conducive to *Phytophthora cinnamomi* revealed that suppressive soils had higher percentages of antagonistic bacteria and actinomycetes (Malajczuk, 1983). This increase was correlated with reduced pathogen sporulation and increased hyphal lysis (Malajczuk, 1983). In addition, in the suppression of *Phytophthora cinnamomi* observed in composted hardwood bark potting mix, inhibitors present in the compost leachates lysed zoospores and cysts, suggesting the presence of microbial antagonists in the suppressive media (Hoitink et al., 1977).

Suppressive mechanisms other than short-term "microbiostasis"

The persistent reductions in disease severity observed in the pre-amendment sampling in 2004, and continuing in the 3H-NRA treatment after tillage, suggests the development of an alternate suppressive mechanism that is not directly related to labile OM decomposition and FDA activity. In a study of the cumulative benefits of a sudangrass green manure grown for 2 and 3 years, Davis et al. (1994, 1999 b) suggested that competitive colonization of roots with the non-pathogenic *Fusarium equiseti* contributed to suppression of Verticillium wilt of potato. Verticillium wilt was reduced by 78% following 2 years of sudangrass green manure, and reduced by 86% following 3 years of sudangrass, compared to a fallow control. Of the multiple green manures tested, colonization of potato roots by *Fusarium equiseti* was significantly greater among the green manures that had the greatest impact on disease suppression.

Suppression of foliar diseases, lasting up to 18 months, was observed in soils amended with paper mill residuals composted with bark (Vallad et al., 2003). *Arabidopsis* plants grown in compost-amended soil displayed increased expression of pathogenesis-related defense genes, prior to pathogen inoculation (Vallad et al., 2003). It was concluded that this persistent disease suppression in plants grown in compost-amended soil could be attributed to the systemic induction of plant defenses (Vallad et al., 2003). Future work on this serially-amended field trial should help determine if the unusually low root rot severity observed in the H treatment soils in 2004 is a continuing trend, and if so, what mechanism or mechanisms might be at work.

CONCLUSIONS

In summary, in the first two years of the study, suppression was shown to be strongly related to the post-amendment flush in microbial activity and declined with decreasing FDA as OM decomposed (Darby et al., in press). In the third year weak suppression was observed INF only and was associated with FDA activity. By the fourth year of serial amendment that trend was no longer evident. Evidence from the 3H-NRA treatment pointed to an emerging suppressive mechanism that was not tied to the post-amendment rise in FDA and that persisted up to 13 months following the third amendment. Factors that may be interacting over time to generate observed disease suppression include: short term post-amendment microbiostasis, soil moisture retention, inoculum levels, and novel suppressive mechanisms.

SUMMARY

Four years of serial amendment with dairy manure solids (DMS) had beneficial impacts on soil biological and physical properties which serve as indicators of soil quality. However increases in moisture retention in the highly amended treatments may have negative impacts on root rot severity of corn, a disease that is sensitive to soil moisture conditions. Comparisons of treatments receiving single-year and serial high-rate amendments distinguished the soil properties that respond rapidly to fresh amendment from those that change over time following amendment. FDA activity and fPOM content responded most rapidly to fresh amendment, while changes in oPOM, bulk density, water retention, and porosity developed over the mid- to long-term.

The effect of each organic amendment on root rot severity of corn was not the same throughout the four years of the study. In the first two years of the study, disease suppression was shown to be strongly related to the post-amendment flush in microbial activity, and declined with decreasing FDA activity (Darby et al., in press). In the third year weak suppression that followed this trend was observed in-field only, and by the fourth year that trend was no longer evident. However, suppression observed in the 3H-NRA treatment which was not amended in 2004 suggests the presence of an emerging suppressive mechanism that was not tied to the post-amendment rise in FDA activity, and that persisted up to 13 months following the third amendment.

It is clear that the effects of serial organic amendments on root rot severity of corn do not exactly mimic the effects of a single-year amendment in this sweet corn cropping system. This is probably due to the effect of multiple interacting factors, including treatment effects on soil water retention and other soil properties, as well as the possibility of increased inoculum potential in serially amended soils. Future work on this serially-amended field trial should help determine if the unusually low root rot severity observed in the H treatment soils in 2004 is a continuing trend, and if so, what mechanism or mechanisms might be at work.

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