IMPACT OF SOIL BIOLOGY ON NITROGEN CYCLING AND WEED SUPPRESSION UNDER NEWLY ESTABLISHED ORGANIC ORCHARD FLOOR MANAGEMENT SYSTEMS

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of LORI A. HOAGLAND find it satisfactory and recommend that it be accepted.

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(Chair)

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Abstract

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Sustainable methods of weed control and nitrogen fertility are a significant challenge in organic orchard production systems. These studies were conducted to determine whether soil tillage, living cover mulch establishment, or amendment of soil with Brassicaceae seed meal (BSM), clove oil herbicide, or wood chip mulch, could be used to suppress weeds and positively impact soil quality to result in enhanced nitrogen (N) supply and tree health. Orchard floor management treatments were established in a newly planted apple orchard and tree health was assessed based upon increase in tree circumference and leaf nutrient status. Soil cores were periodically collected to evaluate soil quality and N supply using biological and chemical indicator analyses. Compost amendment was enriched with ^{15}N fertilizer to track N partitioning among soil, living cover and wood chip mulch residues, and tree leaf components. In greenhouse experiments, weed emergence, stimulation of *Pythium* spp. populations and root infection by these oomycetes, were monitored in orchard soils amended with BSM's that varied in

glucosinolate content. None of the orchard floor treatments produced an ideal combination of weed control, maximum tree growth, sufficient leaf nutrient content, and improved soil quality. Rather, soil quality improvements tended to be achieved at the expense of tree performance. Living cover mulch and BSM amendment resulted in soil quality improvement, but tree health was negatively impacted. Wood chip mulch and clove oil herbicide did not positively impact soil quality and resulted in lower tree health. Soil tillage negatively impacted soil quality but tree health was greatest in this treatment. Weed suppression by BSM amendment was correlated with soil enrichment and root infection by resident pathogenic *Pythium* spp and not glucosinolate content or composition. These studies indicate that in the short-term, the standard practice of soil tillage is the most effective way to control weeds and meet tree N needs, but it may not be desirable in the long-term. Brassicaceae seed meal amendment can be used to meet tree N needs and selectively enhance resident pathogenic *Pythium* spp. for the purpose of weed control, but further research is necessary to determine ideal timing and application rates.

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INTRODUCTION

Over the past century, agricultural systems have undergone extensive transformation. Innovative research has led to development of high-yielding plant cultivars and extremely efficient fertilizers and pesticides, resulting in substantial crop yield increase. However, these new practices have also resulted in a number of unforeseen costs to human health, environmental stability and viability of rural communities. This industrialized system of agriculture is not sustainable. New production strategies are needed that can maintain production efficiency without negative impact. To accomplish this objective we must broaden our perspectives and consider multiple dimensions when designing agricultural research projects. Research conducted using a reductionist approach where each aspect of production is examined individually is successful, but it often ignores emergent properties of agricultural systems that are only observed when the system is viewed as a whole.

Use of a holistic approach to study agricultural systems will require change in the way that scientists are trained. Individual scientists must continue to become experts in their own individual field, but they must also develop the necessary skills to work within broad multi-disciplinary groups. These scientists must become familiar with the language and strategies of several different, but related disciplines. Scientists trained in this context will be able to accomplish broad research objectives and have the capacity to bring new insight into their specific academic discipline. Janovy (1985) asserts that when a student seeks out breadth in their education and experience, they can begin to see how contributions from other areas can apply to problems they may not currently be able to

solve within their own limited disciplinary confines. "The ability to make big leaps of thought is a common denominator among the originators of breakthrough ideas. Usually this ability resides in people with very wide backgrounds, multidisciplinary minds, and a broad spectrum of experiences" (Negroponte, 2003).

Training new scientists to evaluate research questions using different levels of inquiry will also help in the design of productive, sustainable agricultural systems. Applied studies conducted in the field will continue to be fundamental for determination of strategies that maximize production efficiency and minimize negative impact. However, training scientists to probe deeper and use new molecular tools to examine research questions at the basic level will yield new insight into mechanisms that are responsible for reactions observed in the field. Finally, research scientists must be able to communicate with agricultural producers, both in an effort to direct their research towards real-world problems, and to disseminate findings in an easily understandable context.

A good place for this new generation of agricultural research scientists to begin is in the context of organic agricultural systems. In organic production systems, synthetic fertilizers and pesticides are not allowed causing managers to return to more traditional production strategies. A number of comparative studies have shown that organic production systems are generally more sustainable than their conventional counterparts, but crop yield tends to be lower (Drinkwater et al., 2004; Reganold et al., 2001). Price premiums on organic products currently allow organic growers to maintain economic profitability, but these premiums are not guaranteed to continue. In addition, as the human population continues to grow, we must continue to increase crop yield in an effort

to meet food needs. Research programs conducted within organic agricultural systems are urgently needed to develop strategies that maintain sustainability while reducing input costs and raising crop yield. Research scientists must operate within a broad disciplinary context and use new tools to understand and make use of natural mechanisms to increase productivity in organic production systems.

In Washington State, organic tree fruit orchards are a significant and growing component of the state's agricultural economy. The mild, dry climate east of the Cascades contributes to the absence of many disease problems native to other regions. New pheromone mating disrupters have helped to control insect pests, and abundant water resources are available because of local irrigation projects. As a result, Washington State leads the nation in organic tree fruit production. In 2005, there were 8,955 acres certified and 1,617 acres in transition (Granatstein et al., 2005). Comparison studies, have shown organic apple systems to be equally profitable yet produce higher quality fruit and are more environmentally sustainable than their conventional counterparts (Reganold et al., 2001; Peck et al., 2006). Despite this success, orchard managers continue to struggle with sustainable methods of weed control and nitrogen (N) fertility.

In organic orchards, extensive soil tillage is often used to control weeds. While effective, this practice has a negative impact on soil quality, an essential component of organic production systems. Alternative weed control strategies that reduce disturbance are urgently needed. One alternative with great potential for producers in the Pacific Northwest is soil application of Brassicaceae seed meal (BSM), a byproduct of the extraction process for bio-diesel production. Regional demand for bio-fuel has led to

construction of large processing facilities and resulted in an abundant local supply of BSM. Soil amendment of BSM has been found to supply plant available nitrogen and result in weed and disease suppression, but the effectiveness of weed suppression in the field has not been consistent. The mechanism responsible for weed suppression is unclear and must be better understood if producers are to rely on BSM to control weeds in organic systems.

Nitrogen (N) fertility is a significant impediment to sustainable organic orchard management. Composted animal manures are commonly used to supply N, but only a fraction is in immediately available forms with the remainder released slowly as a result of microbial driven processes. These amendments come at a high cost, and excess application may result in salt damage, nitrate loss and environmental degradation. Systems are needed that will help reduce N fertility costs, minimize loss, and enhance availability at times corresponding with critical tree uptake periods. Soil tillage can be used to incorporate organic amendments and enhance nutrient availability, but excessive soil tillage has a negative impact on maintenance of soil organic matter and soil organisms responsible for nutrient cycling. Orchard floor management strategies that reduce disturbance and contribute to soil organic matter enrichment may help to reduce N fertility costs, minimize loss, and result in more efficient release of plant available N over time.

As demand for organic fruit continues to grow, a number of orchard managers are considering transition to an organic production system. Nitrogen fertility practices in organic systems are fundamentally different from conventional systems and thus development of an organic N fertility plan can be challenging. Nitrogen availability in

organic systems is dependent on availability of locally produced amendments, their total and mineralizable N concentrations, and site-specific soil, environmental and management conditions. Orchard managers need practical advice on how on how to confront this challenge and develop their own site-specific organic N management plan.

The research projects summarized in this dissertation were conducted to address the needs of WA state agricultural producers and train a new scientist in the methods needed to answer complex research questions in order to develop sustainable, productive agroecosystems.

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ROLE OF NATIVE SOIL BIOLOGY IN BRASSICACEAE SEED MEAL INDUCED WEED SUPPRESSION

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Keywords: Brassicaceae, allelopathy, *Pythium*, glucosinolates, weed suppression

Abstract

Effective application of pest management strategies across production systems in organic agriculture requires in-depth knowledge of the operative mechanism(s). Brassicaceae seed meal (SM) residue is a byproduct of bio-diesel production with potential for weed control. Weeds were treated with SM in tree fruit orchards and the processes responsible for observed weed suppression were examined in field and greenhouse studies. Although weed control obtained in response to Brassicaceae amendments has been repeatedly attributed to release of allelopathic phytochemicals, multiple lines of evidence acquired in these studies indicate the involvement of a microbiological component. Reduced emergence and increased weed seedling mortality was not related to SM glucosinolate (GC) content, but was correlated with significant increases in resident populations of *Pythium* spp. in three different orchard soils. Seed meal of *Brassica juncea* did not amplify resident *Pythium* populations and did not suppress weed emergence. Application of *Glycine max* SM did stimulate *Pythium* spp. populations and likewise suppressed weed emergence. Application of a mefenoxam

drench to *Pythium* enriched-soil significantly reduced weed suppression. These studies indicate that a microbial mechanism is likely involved in SM induced weed suppression, and that selective enhancement of resident pathogenic *Pythium* spp. can be utilized for the purpose of weed control.

1. Introduction

Expansive growth in organic agriculture production and sustainable agricultural systems, have focused attention on biologically based weed control strategies. Crops and their byproducts can be used or derivitized to produce renewable bioproducts with many potential uses in sustainable cropping systems. Brassicaceae plants are increasingly grown for bio-diesel production, green manures, or break crops to disrupt pest cycles and improve subsequent wheat (Kirkegaard et al., 1994) Brown and Morra, 1997; Roe, 2006) and potato (Boydston and Hang, 1995) yields. Residual Brassicaceae SM, a waste product of the oil extraction process, can provide a local resource for supplemental nutrients, disease control; (Lazzeri and Manici, 2001); (Mazzola et al., 2001), (Zasada and Ferris, 2004), and/or weed suppression (Brown and Morra, 1997) in high-value fruit and vegetable production systems. However, the mechanisms contributing to the observed Brassicaceae SM weed control remain unclear (Boydston and Hang, 1995; Brown and Morra, 1997) and must be better understood if producers are to realize the benefits of SM use for weed control.

Decreased weed emergence has been repeatedly documented following soil incorporation of Brassicaceae crop and SM residues (Boydston and Hang, 1995;

AlKhatib et al., 1997; Brown and Morra, 1997). The mechanism of weed suppression has been attributed to allelopathy, which is defined as the inhibitory effect of one plant or microorganism on another through chemical release from the donor to the environment (Kobayashi, 2004). Glucosinolate hydrolysis products are thought to be responsible for the allelopathic weed suppression by Brassicaceae residues (Brown and Morra, 1997). The hydrolytic enzyme myrosinase and water are required for GC hydrolysis. The type, total concentration, and functionality of GC hydrolysis products vary among Brassicaceae species. Glucosinolates are present in all Brassicaceae plant parts, but are most concentrated in seed, with GC content accounting for up to 10% of seed dry weight (NTNU, 2005). Seed meal, a residue resulting from the process of oil extraction, retains high GC content and viable myrosinase (Borek and Morra, 2005). Therefore, it is reasonable to hypothesize that GC hydrolysis products have a role in the weed suppression resulting from application of Brassicaceae SM.

Although weed suppression by Brassicaceae residues has long been attributed to GC induced allelopathy, there has not been a consistent correlation between observed weed suppression and calculated GC. For example, significant plant suppression is often observed with low GC content *B. napus* residues (Boydston and Hang, 1995; Brown and Morra, 1996; AlKhatib et al., 1997). These authors suggest either effective action by a relatively small amount of a specific but unidentified GC hydrolysis product, or that microbial degradation results in production of other inhibitory compounds. Additional evidence supporting GC mediated weed suppression involves commercial herbicides and fumigants that contain ionic thiocyanate, a common GC hydrolysis product (Borek and Morra 2005; and Teasdale and Taylorson, 1986). However, these pesticides control

weeds at effective concentrations much higher than that found in SM residue (137 – 1366 kg SCN^-/ha) (Borek and Morra, 2005); low fumigant rates yield only variable inhibition (Teasdale and Taylorson, 1986). In contrast, consistent weed control has been observed at SM amendment rates of 1000-4000 kg SM/ha, with 8.8 - 35.3 kg SCN^-/ha, assuming complete conversion to hydrolysis products (Borek and Morra, 2005). In addition, soil physical, chemical, and biological characteristics influence expression and longevity of allelochemicals under field conditions (Inderjit et al., 2001). Schmidt and Ley (1999) concluded that most purported allelochemicals are unlikely to build up to phytotoxic levels under natural conditions due to slow diffusion in soil, complexation and sorptive reactions, and microbial destruction by carbon limited organisms that are able to rapidly mineralize aromatic compounds. Rapid decay is supported by the finding that less than 1 – 5 % of total predicted hydrolysis products are present in soil shortly after incorporation of brassicaceae residues (Gardiner et al., 1999; Morra and Kirkegaard, 2002).

Incorporation of fresh green manure residue, including *Brassica* spp., is also commonly associated with rapid increases in total microbial activity, which can include plant pathogenic soil fungi and oomycetes (Grunwald et al., 2000; Manici et al., 2004; Cohen et al., 2005), often associated with root rot of subsequent crop and weed seeds (Pitty et al., 1987). Many members of the genus *Pythium* incite both pre- and post-emergent damping-off of plants. *Pythium* spp. populations are amplified in response to organic matter addition, survive in competition with other microorganisms (Chen et al., 1988) and withstand frequent cultivation (Grunwald et al., 2000; Mazzola and Gu, 2000).

In organic orchard systems, managers often supply nutrients with organic matter inputs and control weeds with extensive cultivation, a practice that can degrade soil

structure. Both of these practices can stimulate soil *Pythium* populations. Application of Brassicaceae amendments may help to control problem weed species and reduce soil disturbance, but the mechanism of action must be better understood in order to generate guidelines and recommendations for use of this practice as a management tool. These studies were performed in or with multiple orchard soils to test the hypothesis that induced amplification of resident *Pythium* spp. contributes to the weed suppression observed in response to Brassicaceae SM amendments.

2. Materials and Methods

2.1 Soils and soil treatments

Studies were conducted at or in soils collected from three experimental orchards; the Columbia View Experimental (CV) orchard, Orondo, WA; the Wenatchee Valley College-Auvil Teaching and Demonstration (WVC) orchard, East Wenatchee, WA; and the Tukey Horticulture Research and Experimental (TU) orchard, Pullman, WA. Soils at these sites are characterized as Adkins Very Fine Sandy Loam with 1.3% organic matter (OM) and pH 7.6, Pogue Sandy Loam (1-2% OM and pH 6.1-7.3), and Thatuna Silt Loam (4-5% OM and pH 6.8), respectively. Plots at WVC and TU orchards are under organic management.

Amendments used in field and greenhouse studies included a low glucosinolate (GC = 21.8 umol g^{-1}) commercial rapeseed, *Brassica napus* cv. Dwarf Essex (Montana Specialty Mills, Great Fall, MT), and two high glucosinolate mustard varieties, *Brassica juncea* cv. Pacific Gold (GC = 303 umol g^{-1}), and *Sinapis alba* cv. Ida Gold (GC = 244

umol g^{-1}) (Brassica Breeding Program, University of Idaho). Nitrogen content of the SM was 5.57, 6.09, and 6.84%, respectively. Greenhouse experiments also included a soybean (*Glycine max*) seed meal (3% N) treatment and a pasteurized soil treatment. All amendments were applied to soil at a rate of 0.3% vol/vol. In 2005, the field experiment carried out at CV included a 1,3-dichloropropene-chloropicrin (TeloneC17; DowElanco, Indianapolis, IN) soil fumigation treatment at 282 L ha^{-1}. All field and greenhouse experiments included a non-treated control. A mefenoxam (Ridomil Gold EC 49% ai) soil drench was used in the 2006 field experiment and all greenhouse experiments to selectively reduce plant infection by resident populations of *Pythium* spp.

2.2 Greenhouse Experiments

Composite soil samples were collected at WVC in spring 2005 (WVC1), fall 2005 (WVC2), and at TU orchards in spring 2006 for use in greenhouse assays. Soil was also collected from an experimental plot at CV orchard and an area immediately adjacent with native (uncultivated) vegetation. Soil samples were collected from within the root zone of random trees in established orchard sites to a depth of 10-30 cm, approximately 1-2 m from the tree base. Soil was stored in five gallon buckets at ambient conditions in the greenhouse until experiments were initiated. Three replicate soil samples from each site/date were pooled and stored at 4 C for subsequent laboratory analysis. For each experiment, soil was pre-mixed using a cement mixer and allocated to tubs (2 per treatment) in 5 L increments. Seed meal amendments were applied to soil at a rate 2.3 g L^{-1}, hand mixed and covered for incubation. After four days, a composite soil sample was collected from each treatment for laboratory analysis. At the same time, mefenoxam

was diluted to 0.635 ml L^{-1} and 116.7 ml was applied to 2.5 L soil representing each treatment. Soil was placed in conical tubes (21 cm deep X 4 cm top diameter) and seeded using rates based upon results from previous laboratory germination assays. An individual tube was planted with either 5 *Triticum aestivum* (cv. Madsen) seeds, 10 *Vicia villosa* seeds, 10 *Amaranthus retroflexus* seeds, or 7 *Echinochloa crusgalli* seeds. Each seed type x soil treatment combination was replicated in ten growth tubes. Plants were individually watered when a dry soil surface was observed. Plant emergence was recorded at 5 d and again at harvest 21 d after planting. Twelve days after planting, three cones per seed type/soil treatment were randomly selected for determination of *Pythium* soil populations and root infection.

2.3 Experimental field plots

Field plots were established at CV orchard in spring 2005 and 2006 in a randomized complete block design with split-plots and five replicates. Seed meal amendments were surface applied at a rate of 8,533 kg/ha, and subsequently incorporated to a depth of 15 cm using a rotovator. Forty-eight hours after SM amendment, mefenoxam was diluted (0.635 ml L^{-1}) and the diluted formula was applied to half of each plot at a rate of 1.48 ml m^{-2}. In 2005, SM was applied on 21 April and half of each split-plot was covered with a 152-µm thick clear plastic sheet (Sunbelt Plastics, Monroe, LA). Plastic was removed on 23 May (32 d). Experimental plots measured 3.048 m^2. In 2005, approximately 90 d after amendment application, all shoot and root biomass was collected from each plot, divided into grass and broadleaf species, oven-dried at 50 C for 48 h and weighed. Above ground weed biomass was also collected from a newly

established orchard study employing the same soil treatments and samples were analyzed using the same method. In 2006, 3 d following SM amendment, five *T. aestivum* seeds (cv. Madsen) were planted into each split-plot and germination was recorded after 14 days. Forty d after amendment application, 4 sub samples (0.1 m^2 each) of aboveground weed biomass were cut and pooled for analyses within each split-plot. Samples were oven-dried as above prior to determination of plant biomass.

In 2005, four soil samples were randomly collected from each plot to a depth of 10-30 cm and pooled for laboratory analysis**.** In 2006, soil from each split-plot was sampled using a 2-cm diameter probe to a depth of 10-cm, replicated three times and pooled for laboratory analysis. Soil samples were collected at 0, 3, 8 and 15 d post-SM amendment. All soil samples were stored at 4 C until analysis.

2.4 Characterization of soil and plant colonizing Pythium populations

Three separate 5-gram soil sub samples from each field and greenhouse treatment were suspended individually in 25 ml sterile distilled water, vortexed 60 s and serial dilutions were plated on a *Pythium* semi-selective growth medium (Mazzola et al., 2001). After 48 hours, adhering soil was washed from plates under running water, and colonies exhibiting typical *Pythium* morphology were enumerated. Hyphal plugs from representative *Pythium* colonies from each plate were excised and transferred to new agar plates. Three 0.4 cm diameter plugs were excised from the growing margin of individual cultures, transferred to 5 ml $1/5^{th}$-strength potato dextrose broth, and cultures were grown with aeration (150 rpm) at ambient laboratory conditions. DNA was extracted from *Pythium* mycelium using a MoBIO Ultraclean Soil DNA kit (Carlsbad, CA), and stored

at –20 C until analysis. Polymerase chain reaction amplification of *Pythium* DNA was conducted using the primer set internal transcribed spacer (ITS) 4 and ITS5 (White et al., 1990). Reactions were conducted in a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA) using reactions conditions as previously described (Tewoldemedhin et al., 2006). Amplification products were confirmed by visual comparison to a 100 bp ladder following electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Resulting amplicons were directly sequenced by use of a Dye Terminator Cycle Sequencing Quick Start Kit and a CEQ 8000, Genetic Analysis System capillary-based DNA sequencer (Beckman Coulter, Fullerton, CA) with ITS1 (White et al., 1990) as the sequencing primer. Sequences obtained were compared with the online NCBI BLAST database.

Plants from each tube were individually removed and roots were rinsed with tap water. Six root segments of approximately 3 cm in length were plated onto *Pythium* semi-selective growth medium. In the event that plant emergence and growth did not occur, large weed seeds (*T. aestivum* and *V. villosa*) were extracted from the pot and plated in the absence of root material. *Pythium* infection of each root/seed was recorded after 48 hours. Isolation, culture, DNA extraction, amplification, and product confirmation for individual *Pythium* colonies recovered from each root/seed segment was performed as above.

Pythium isolates were characterized using restriction fragment length polymorphism (RFLP) analysis. ITS amplicons from each individual *Pythium* colony were digested individually in single enzyme reactions using *Hae*III, *Hpa*I, *Rsa*I or *Taq*I. Each reaction contained 8 ul PCR product, 1 ul restriction enzyme, and 1 ul of the

appropriate 10x digestion buffer. All digests were incubated at ambient conditions overnight except *Taq*I, which was incubated overnight at 65 C. Digest patterns for each *Pythium* isolate were visualized by comparison to a 100 bp ladder following electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Restriction patterns were compared to a library of RFLP patterns generated from representative *Pythium* isolates, which had been identified by sequence analysis and morphological characterization in this and previous studies (Mazzola et al., 2002).

2.5 Quantification of soil Pythium populations by real-time PCR

Pythium species in SM-amended and unamended WVC2 and Tukey soils were quantified by real-time PCR (Schroeder et al., 2006). Briefly, DNA was extracted from 0.5 g soil using a MoBIO Ultraclean Soil DNA Isolation kit. DNA was isolated from two separate 0.5 g samples per treatment. The resulting DNA was employed in individual amplification reactions, conducted in duplicate using FastStart DNA Master SYBR Green I and one of ten primer pairs (Schroeder et al., 2006) each designed to amplify a specific *Pythium* species. Reactions were conducted in a total volume of 20 ul using a Roche Light Cycler. After initial analyses, *P. paroecandrum*, *P. echinulatum*, *P. irregulare*, *P. ultimum, P. heterothallicum*, and *P. attrantheridium* primers were selected for use on all soil treatments.

2.6 Statistical analysis

All statistical analyses were conducted with SAS 9.1 software (SAS Institute Inc., Cary, North Carolina). Data were subjected to analysis of variance and mean separation was based upon Fisher Protected LSD. Results were considered significant at $P \leq 0.05$.

3. Results

3.1 Weed emergence and biomass in the greenhouse

T. aestivum emergence and survival were significantly reduced by amendment of soil with *B. napus*, *G. max* or *S. alba* SM, relative to the control (Table 1). In contrast, pasteurization, *B. juncea* amendment, and mefenoxam treatments typically induced significant increases in plant emergence (Table 1). *V. villosa* had low emergence overall and no significant treatment effects were observed, although *V. villosa* emergence exhibited trends similar to those of *T. aestivum* in response to soil treatments (Table 1). *G. max* and *S. alba* SM amendments significantly reduced emergence of *E. crusgalli* compared to the control. Most pasteurization and mefenoxam treatments, and certain *B. juncea* amendments significantly increased *E. crusgalli* emergence relative to the control (Table 1). Soil amendment with *S. alba* and *G. max* SM significantly reduced *A. retroflexus* emergence in most cases, with most other treatments increasing *A. retroflexus* emergence (Table 1). Biomass followed similar trends to emergence data (data not shown).

3.2 Weed emergence and biomass in the field

In the 2005 new orchard planting, *B. napus* amendment resulted in significantly greater yield of grass biomass in comparison to all treatments except fumigation. (Figure 1). Broadleaf biomass was elevated in both *B. napus* amended and fumigated plots, and a small reduction was observed in *S. alba* amended plots although broadleaf biomass in these treatments were not significantly different from the control (Figure 1). In the 2005 experimental plots, broadleaf weed biomass was significantly reduced in all SM amended plots covered with plastic relative to uncovered plots (Figure 2). In contrast, without plastic cover, biomass from S. *alba* amended plots was still lower than the non-treated control, while biomass recovered from B. *napus* and *B. jun*cea amended plots were significantly greater than their respective plastic covered plots and control (Figure 2). Grass biomass followed a similar trend but there were no statistically significant differences among treatments (Figure 2). In 2006 there were no significant differences in weed biomass production among treatments (Figure 3). However, *T. aestivum* emergence was significantly reduced in both *B. napus* and *S. alba* treated plots relative to the control (Figure 4).

3.3 Pythium soil populations

In greenhouse experiments all soils exhibited significant increases in *Pythium* populations in response to *B. napus*, *S. alba*, and *G. max* SM, with the exception of CV orchard native soil (Table 2). Resident *Pythium* spp. were not detected in initial samples of CV orchard native soil, and there was no response in the population to any SM amendment (Table 2). *Pythium* populations reached similar levels in all orchard soils

tested, but relative increases were much lower in TU orchard soil (Table 2). In all soils, *B. juncea* amendment resulted in a reduction of *Pythium* to near the limit of detection. In field studies conducted at CV orchard, *B. napus* and *S. alba* amendments significantly elevated soil populations of *Pythium* relative to the control in both 2005 and 2006 (Table 3 and Figure 5). In both years, *Pythium* spp. numbers in *B. juncea* amended plots were reduced to near zero (Table 3 and Figure 5). Numbers of *Pythium* recovered from covered *B. napus* and *S. alba* SM amended plots in 2005 were higher relative to their adjacent uncovered plots, but were not significantly different (Table 3). Time series data from 2006 revealed an initial *Pythium* decrease in all SM amended plots. *Pythium* levels in *B. juncea* and *S. alba* SM amended plots were significantly reduced. Soil *Pythium* levels increased rapidly in *B. napus* and *S. alba* SM amended plots after the initial decline, with populations reaching their highest in *B. napus* amended plots (Figure 5). For all soil treatments, *Pythium* populations peaked approximately eight days (8 d) post-amendment and then declined (Figure 5).

3.4 Pythium root and seed infection

Recovery of *Pythium* from roots and seeds of all plant types established in WVC2 and TU orchard soils amended with *S. alba* or *G. max* was significantly greater than the control and pasteurized treatments, as well as the respective SM amended soils treated with mefenoxam (Table 4). In *B. napus* SM amended soil, plant infection by *Pythium* spp. was significantly increased in five of eight analyses (Table 4). All *B. juncea* SM amendments uniformly reduced *Pythium* plant infection to levels less than the control and pasteurization treatments. Seed and root samples from TU amended soils were infected

by *P. ultimum, P. attrantheridium* and *P. heterothallicum*, while plant tissues established in WVC2 amended soils were infected primarily by *P. irregulare*, and *P. ultimum*. There was no preference for a particular *Pythium* spp. to infect one plant spp. over another.

3.5 Soil Pythium population characterization using real-time PCR

Pythium populations in WVC2 and TU orchard soils amended with *S. alba*, *G.max,* or *B. napus* SM were significantly greater than in the control, pasteurized, and *B. juncea* SM treated soils (Figures 5 and 7). In both soils, amendment with *B. napus* SM resulted in *Pythium* numbers that were lower relative to *S. alba* or *G. max* treatment based upon estimates obtained from plate counts (Figures 6 and 7). *Pythium* species enrichment varied between the two soil types and between SM's. For example, *P. irregulare* was prominent in WVC2 soil, but absent in TU soil. In contrast, TU soil amended with *S. alba*, *G. max*, or *B. napus* SM was highly enriched with *P. attrantheridium,* whereas this species was only slightly enriched by *G. max* amendment in WVC soil. Both soils treated with either *B. napus* or *G. max* SM were enriched with *P. echinulatum*, whereas this species was nearly absent when soil was amended with *S. alba*.

4. Discussion

4.1 Influence of seed meal on weed emergence and biomass

In both field and greenhouse experiments, *S. alba* SM amendment consistently resulted in the greatest and most consistent weed suppression, although field results were

not always statistically significant. Lack of significance may be the result of highly variable conditions in the field in both weed seed distribution and distribution of *Pythium* spp. in field soil environments. In contrast, amendment with *G. max* or *B. napus* SM also resulted in weed suppression, but results were not as consistent, and seedling recovery was sometimes observed during final assessment of plant emergence. Correspondingly, soil amendment with *S. alba*, *G. max* or *B. napus* SM's significantly increased *Pythium* populations. Influence of seed and root cuttings by spp. of *Pythium* was detected by culture plating on a semi-selective medium and identification of the individuals by RFLP analysis. Pasteurization and treatment of SM amended soils with mefenoxam almost consistently increased plant emergence and biomass. *S. alba* amended plots treated with mefenoxam still exhibited some reduction in plant emergence. These results confirm our hypothesis that plant-pathogenic *Pythium* spp. mediate, at least in part, the weed suppression observed in response to Brassicaceae SM amendments.

In the instance of *S. alba* SM amendment, the data indicate that multiple mechanisms contributed to the weed suppression observed in these studies. Consistent with previous research, we believe that the high 4-hydroxybenzyl GC content in *S. alba* SM results in the production of phytotoxic, water-soluble hydrolysis products that injure seeds and seedlings (Borek and Morra, 2005). However, *S. alba* SM also enhances pathogenic *Pythium* spp. that cause pre- and post-emergence damping-off, which ultimately was responsible for seedling death. In contrast, weed suppression following *G. max* or *B. napus* SM amendment, with zero and low GC content respectively, occurs solely in response to enrichment of and infection by resident pathogenic *Pythium* spp. Dependence of weed suppression on enhancement of resident *Pythium* spp. may help to

explain the inconsistency in weed control that has been reported in previous research with Brassicaceae plant residues. If pathogenic *Pythium* spp. are not present, then weed suppression will not likely occur.

Covering *B. napus* and *B. juncea* SM amended plots with clear plastic resulted in significantly reduced weed biomass relative to the non-treated control, a response that was not achieved in the absence of covering treated soils. This finding supports the hypothesis that weed suppression results in part from release of toxic volatile hydrolysis compounds derived from p-propenyl (allyl) GC products, present to a high degree in *B. juncea* and to small extent in *B. napus* (Brown and Morra, 1997). However, application of the plastic covering could also have raised soil temperature creating optimal conditions for growth of *Pythium* spp., which exhibit greatest activity in terms of plant infection during the spring of the year (Mazzola et al., 2002). This premise is supported by the trend of increased *Pythium* numbers in *B. napus* and *S. alba* SM amended soils when covered relative to the corresponding non-covered treatments. It could be argued that covering the soil with plastic may have resulted in solarization, which inhibited weed emergence. However, this occurred in early spring when temperatures were not very high, and if temperatures had reached levels high enough for solarization, it would have also been too hot for *Pythium* growth.

B. napus SM and 1,3-dichloropropene-chloropicrin fumigation treatments increased weed biomass in some cases. This may be the result of the high availability of nitrogen associated with *B. napus* SM (Snyder et al., 2006) amendment, and the lower enrichment, of specific pathogenic *Pythium* spp. relative to other SM as observed using real-time PCR analysis. Greater weed biomass in response to 1,3-dichloropropene-

chloropicrin fumigation is likely due to control of resident *Pythium* spp. as well as reduced competition from soil microorganisms for available nutrients. Mefenoxam application to most SM amended and control plots either stimulated weed emergence or resulted in an increase in weed biomass relative to the control. Again, these data support our hypothesis that the enrichment of resident *Pythium* spp. in response to SM amendments plays a significant role in the observed weed suppression.

T. aestivum and *A. retroflexus* were generally more susceptible to SM treatments than were *V. villosa* and *E. crusgalli*. Liebman and Davis (2000) speculated that small weed seeds, like *A. retroflexus* may suffer greater allelopathic susceptibility in comparison to large seeds due to their small store of nutrient and energy reserves, and a greater root length per unit mass, which increases their relative absorptive surface area. However, Haramoto and Gallandt (2005) found monocots to be more susceptible to allelochemicals than dicots, regardless of seed size. Greater nutrient and energy reserves may enable large dicot seeds to tolerate *Pythium* spp. enrichment and may explain the reduced inhibition and later recovery observed with the large *V. villosa* seeds. In addition, *V. villosa* seeds have hard coats, which may help to reduce infection by *Pythium* spp. In contrast, the relatively large *T. aestivum* seed used in our studies exhibited high susceptibility, which may result from greater sensitivity as a monocot. Differences in rooting patterns and seed exudates could also be a factor in the differential response to weed suppression by *Pythium* species.

4.2 Pythium response to seed meal amendments

Under field conditions, resident soil *Pythium* populations were dramatically enhanced by application of *B. napus* or *S. alba* SM, possibly in response to availability of carbon and nitrogen compounds as a substrate source. In contrast, soil amendment with *B. juncea* suppressed *Pythium* populations to near or below the limit of detection, confirming its potential as an alternative treatment for the control of *Pythium* spp. (Brown and Morra, 1997). Covering *B. napus* and *S. alba* SM amended plots with plastic increased *Pythium* spp. populations, which is likely a result of better growth conditions (temperature, moisture). While it could be suggested that the response resulted from reduced microbial competition due to the activity of GC hydrolysis products, total soil fungal and bacterial populations consistently increase in response to these SM amendments (Cohen and Mazzola, 2006). The initial decline of *Pythium* in response to *S. alba* and *B. juncea* SM amendments observed at 0 d in the time series experiment may be a soil fumigation response due to an initial release of toxic metabolites. However, *Pythium* spp. rapidly increased in *B. napus* and *S. alba* SM amended plots by 3 d revealing the ability to quickly recover and out compete other soil microorganisms in order to utilize available substrates from the SM as a food source. The decline in *Pythium* numbers after 8 d likely occurs as available substrate is exhausted. In contrast, the metabolites of *B. juncea* SM amendment may inhibit *Pythium* spp. and/or encourage rapid growth of a different soil organism that is able to out compete soil inhabiting *Pythium* spp. (Izzo and Mazzola, unpublished data). Overall *Pythium* spp. populations in the field were greatest in *B. napus* amended soil, which may result from its high degree of N availability. For example, equivalent amendment of *B. napus* or *S. alba* SM resulted in

58 and 18% available N respectively, over the course of one growing season (Snyder et al., 2006). Recovery of *Pythium* spp. was consistently reduced after mefenoxam application, however low populations were still obtained from suspensions of soil treated with mefenoxam. This result is due to the fact that mefenoxam chemistry acts by killing actively growing hyphae and does not impact dormant spores.

Similar overall trends were observed in greenhouse trials however; some differences were detected among the different orchard soils. In TU soil, initial *Pythium* spp. populations were greater, which may explain why application of *B. juncea* did not reduce *Pythium* populations to near zero, as observed in CV or WVC soil. Based upon plate count estimates, amendment of all soils with *S. alba, B. napus* or *G. max* SM resulted in *Pythium* enrichment to around 1500 colony forming units (CFU) g^{-1}, an increase from initial populations of 20 to 40X in CV and WVC soil, but only 2X in TU soil. Since equivalent amounts of SM were added to each soil, this could indicate that soils attained the maximum *Pythium* populations capable of being sustained by the available substrate. Alternatively, the higher clay and OM contents in TU soil may have exerted a buffering influence that limited population expansion and/or reduced the effective available substrate. Similarly, higher clay and OM contents minimize soil acidification that results from nitrification reactions, favoring bacterial rather than fungal community enrichment in high clay and OM soils (Stotsky, 1986). In addition, recovery of allelopathic phenolic compounds varies with soil type (Dalton et al., 1989) and pretreatment of soil to remove organic matter and free metal oxides has been found to decrease sorption of phenolic compounds (Cecchi et al., 2004). These different responses

to SM amendment in different soils may help to explain the variability in weed suppression observed under field conditions.

4.3 Pythium community response to SM amendment

Results from these studies demonstrate that SM amendments induce amplification of soil *Pythium* spp. populations, but enhancement is dependent on resident community profiles. *Pythium* spp. were not initially detected in CV orchard native soil and this community did not respond to SM amendment. In contrast, WVC and TU orchard soils showed a differential response to SM amendments given initial communities, and community enrichment also varied among the different SM amendment types. Total *Pythium* spp. population estimates were higher using real-time PCR analyses as compared to plate counts. The disagreement in these data is likely the result of the fact that plate counts only account for live, active cells. In contrast, real-time PCR is a gene-based approach whose estimates are based upon total DNA, which could include that from spores and dead cells. Interestingly, *Pythium* populations were much lower in response to *B. napus* populations in comparison to *S. alba* and *G. max* SM amendments using the real-time approach, which is likely due to the primer sets used. The ten original primer sets selected and designed based on the most prevalent *Pythium* spp. resident in these soils. However, many soil *Pythium* spp., are non-pathogenic to most plant species and can even be beneficial (Mazzola et al., 2002). It is plausible that soil amendment with *B. napus* SM results in enrichment of a variety of *Pythium* species, many of which are non-pathogenic, and could have contributed to the lower level of weed inhibition

obtained with this SM relative to *G. max* or *S. alba* amendment despite their similar impact on total *Pythium* spp. populations.

4.4 Conclusion

Findings from these studies demonstrate that weed suppression in response to certain Brassicaceae SM amendments involves a microbial mechanism. Likewise, the role, if any, of GC hydrolysis products, as implied by allelopathy, is species dependent. Independent of GC concentration, with the exception of *B. juncea* SM amendments in both field and greenhouse studies resulted in significant increases in *Pythium* spp. soil populations, which corresponded with reduced emergence and increased weed seedling mortality. Application of the oomycete-selective chemical mefenoxam as a drench to *Pythium* enriched soil significantly reduced recovery of *Pythium* and increased weed emergence. Weed suppression was greatest with a high-4-hydroxybenzyl glucosinolate *S. alba* SM, indicating that hydrolysis products may weaken seeds or seedlings while *Pythium* induced damping-off may be responsible for plant death. RFLP analyses were used to confirm that seed and seedlings were infected with a subset of the elevated *Pythium* species. The differential response in quantitative and qualitative attributes of the *Pythium* community in different soils may explain, in part, the inconsistent performance of SM amendments when used for the purpose of weed control. Our data imply that SM amendments can be used to selectively enhance resident pathogenic *Pythium* spp. for the purpose of weed control, however management caution is advised to prevent damage to target crops.

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Table 1a. *T. aestivum* emergence in greenhouse experiments using two different orchard soils.

		WVC1	WVC2		Tukey	
Seed	**Treatment**	**Initial**	**Initial**	**Final**	**Initial**	**Final**
T. aestivum	Control	3.1 a	3.1 c	3.1 bc	1 e	1.6 d
T. aestivum	Control+Ridomil	4.1 ab	4.1 ab	4.5 ab	4.7 a	4.9 a
T. aestivum	Pasteurized	4.7 a	4.7 a	4.8 a	4.8 a	4.6 a
T. aestivum	Past.+Ridomil	4.4 a	4.4 a	4.3 ab	3.2 cd	3.6 de
T. aestivum	*B.napus*	0.4 e	0.4 d	3.5 abc	0.2 f	0.9 de
T. aestivum	*B.napus*+Ridomil	4.6 a	4.6 a	4 abc	4.6 ab	4.8 a
T. aestivum	*B.juncea*	3.4 bc	3.4 bc	3.2 bc	2.7 d	3.1 c
T. aestivum	*B.juncea*+Ridomil	4.5 a	4.5 a	3.9 abc	3.9 cd	4.2 ab
T. aestivum	*S.alba*	1.5 d	0.6 d	1 d	0.2 f	0.3 e
T. aestivum	*S.alba*+Ridomil	4.5 a	4.5 a	3.5 abc	4.7 a	4.7 a
T. aestivum	*G.max*	0.6 e	0.6 d	2.7 c	0.1 f	0.4 e
T. aestivum	*G.max*+Ridomil	4.7 a	4.7 a	3.7 abc	4.4 ab	4.3 ab

* Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005. Means in the same column followed by the same letter are not significantly different (P > 0.05; n=10).

Table 1b. *V. villosa* emergence in greenhouse experiments using two different orchard soils.

		WVC1	WVC2		Tukey	
Seed	**Treatment**	**Initial**	**Initial**	**Final**	**Initial**	**Final**
V. villosa	Control	1.3 bcd	1.3 bcd	3 c	1.2 c	2.2 d
V. villosa	Control+Ridomil	2.3 ab	2.3 ab	4.9 a	2.3 abc	3.8 bcd
V. villosa	Pasteurized	1 cd	1 cd	4.7 ab	3.2 ab	6.4 a
V. villosa	Past.+Ridomil	2 abc	2 abc	4.5 ab	1.7 bc	4.9 ab
V. villosa	*B.napus*	1.1 cd	1.1 cd	4.1 b	1.8 abc	3.7 bcd
V. villosa	*B.napus*+Ridomil	2 abc	2 abc	4.8 ab	2.2 abc	3.8 bcd
V. villosa	*B.juncea*	2 abc	2 abc	1.8 d	1.1 c	2.6 d
V. villosa	*B.juncea*+Ridomil	2.5 a	2.5 a	4.5 ab	2.0 abc	4.0 bc
V. villosa	*S.alba*	1.1 cd	1.1 cd	0.4 e	1.3 c	2.9 cd
V. villosa	*S.alba*+Ridomil	2.3 ab	2.3 ab	4.6 ab	2.8 abc	4.3 bc
V. villosa	*G.max*	0.5 d	0.5 d	1.3 d	1.7 bc	2.9 cd
V. villosa	*G.max*+Ridomil	1.7 abc	1.7 abc	4.7 ab	3.3 a	4.1 bc

* Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005. Means in the same column followed by the same letter are not significantly different (P > 0.05; n=10).

Table 1c. *E. crusgalli* emergence in greenhouse experiments using two different orchard soils.

		WVC1	WVC2		Tukey	
Seed	**Treatment**	**Initial**	**Initial**	**Final**	**Initial**	**Final**
E. crusgalli	Control	4.8 bc	4.8 bc	3.8 cd	2.7 c	2.8 ef
E. crusgalli	Control+Ridomil	5.3 abc	5.3 bc	5.4 ab	4.3 ab	4.3 abcd
E. crusgalli	Pasteurized	5.6 ab	5.6 ab	4.3 bcd	4.9 a	5.4 a
E. crusgalli	Past.+Ridomil	5.6 ab	5.6 ab	5.3 ab	4.4 ab	4.7 abc
E. crusgalli	*B.napus*	5.1 abc	5.1 bc	5.4 ab	4.1 ab	3.3 de
E. crusgalli	*B.napus*+Ridomil	4.4 c	4.4 c	6.1 a	4.2 ab	4.6 abcd
E. crusgalli	*B.juncea*	6.5 a	6.5 a	3.3 de	3.3 bc	3.3 cde
E. crusgalli	*B.juncea*+Ridomil	5.5 ab	5.5 ab	5.3 ab	4.8 a	4.0 bcde
E. crusgalli	*S.alba*	3.1 d	3.1 d	1.9 f	3.3 bc	3.7 bcde
E. crusgalli	*S.alba*+Ridomil	4.4 c	4.4 c	4.6 bc	4.4 a	5.1 ab
E. crusgalli	*G.max*	5.7 ab	5.7 ab	2.4 ef	2.3 c	1.6 f
E. crusgalli	*G.max*+Ridomil	5.2 abc	5.2 bc	4.7 bc	4.4 ab	3.8 bcde

* Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005. Means in the same column followed by the same letter are not significantly different (P > 0.05; n=10).

Table 1d. *A. retroflexus* emergence in greenhouse experiments using two different orchard soils.

		WVC1	WVC2		Tukey	
Seed	**Treatment**	**Initial**	**Initial**	**Final**	**Initial**	**Final**
A. retroflexus	Control	1.8 ab	2.1 ab	1.4 de	1.8 ab	1.7 cdef
A. retroflexus	Control+Ridomil	1.7 abc	2.0 abc	2.0 abcd	1.7 abc	2.5 abcde
A. retroflexus	Pasteurized	1.5 abc	2.3 abc	3.0 a	1.5 a	3.7 ab
A. retroflexus	Past.+Ridomil	1.8 ab	1.7 ab	2.3 abcd	1.8 abcd	2.6 abcd
A. retroflexus	*B.napus*	1.1 bcd	0.8 bcd	1.0 bdc	1.1 bcd	2.2 cdef
A. retroflexus	*B.napus*+Ridomil	1.6 abc	3.0 abc	3.1 cde	1.6 a	1.9 abc
A. retroflexus	*B.juncea*	1.6 abc	1.8 abc	1.1 ef	1.6 abc	0.9 def
A. retroflexus	*B.juncea*+Ridomil	2.6 a	2.0 abc	2.2 ab	2.6 abc	3.3 abc
A. retroflexus	*S.alba*	0.2 d	0.3 d	0.4 f	0.2 d	0.5 f
A. retroflexus	*S.alba*+Ridomil	2.4 a	2.1 a	2.2 ab	2.4 ab	3 abcd
A. retroflexus	*G.max*	0.5 d	0.7 cd	0.7 ef	0.5 cd	0.9 ef
A. retroflexus	*G.max*+Ridomil	1.6 abc	1.8 abc	1.7 cd	1.6 abc	2.1 bcdef

* Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005. Means in the same column followed by the same letter are not significantly different (P > 0.05; n=10).

Table 2. *Pythium* enrichment (cfu) in three different orchard soils amended with SM in greenhouse experiments.

	WVC1	**WVC2**	**Tukey**	**CV**	**CV-Native**
Initial	150 c	150 c	616 cd	150 d	0 a
Control	33 c	67 c	833 c	50 d	0 a
B.napus	1300 b	1466 ab	1416 b	1216 a	0 a
B.juncea	0 c	0 c	483 d	0 d	0 a
S.alba	1350 b	1350 b	1916 a	617 c	0 a
G.max	1566 a	1583 a	1516 b	1033 b	0 a
Pasteurized	0 c	0 c	0 e	0 d	0 a

* Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA; Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA; and CV, Columbia View Orchard, Orono, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005. CV-native soil was collected in an uncropped area adjacent to the production orchard. Means in the same column followed by the same letter are not significantly different (P > 0.05; n=3).

Table 3. *Pythium* enrichment at CV in SM amended soil during 2005.

Treatment	*Pythium* (cfu)
Control	25 b
Fumigated	63 b
B.napus	675 a
B.napus-Plastic	937 a
B.juncea	25 b
B.juncea-Plastic	0 b
S.alba	175 b
S.alba-Plastic	262 b

* Orchard designation: CV, Columbia View Orchard, Orono, WA. Means in the same column followed by the same letter are not significantly different (P > 0.05; n=5).

Table 4. % *Pythium* infection of root and/or seed in greenhouse trials.

	T.aestivum		*V. villosa*		*A. retroflexus*		*E.crusgalli*	
	WVC2	Tukey	WVC2	Tukey	WVC2	Tukey	WVC2	Tukey
Control	0	17 c	22 c	45 b	6 cd	6 b	0 b	0 c
Control+Ridomil	0 c	0 d	6 d	17 c	0 d	0 b	0 b	0 c
Pasteurized	11 c	0 d	0 d	0 c	0 d	0 b	0 b	0 c
Past.+Ridomil	0 c	0 d	0 d	0 c	0 d	0 b	0 b	0 c
B.napus	50 b	100 a	67 b	72 ab	28 bc	46 a	9 b	90 a
B.napus+Ridomil	6 c	28 b	0 d	0 c	0 d	17 b	0 b	6 bc
B.juncea	17 c	0 d	28 c	68 ab	11 cd	11 b	0 b	0 c
B.juncea+Ridomil	0 c	0 d	0 d	0 c	0 d	0 b	0 b	0 c
S.alba	87 a	100 a	94 a	91 a	80 a	62 a	60 a	-
S.alba+Ridomil	6 c	0 d	0 d	6 c	0 d	6 b	0 b	11 bc
G.max	64 ab	100 a	94 a	83 a	39 b	45 a	75 a	17 b
G.max+Ridomil	6 c	0 d	0 d	0 c	0 d	0 b	0 b	0 c

* Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC2 represents soil collected in autumn 2005. Means in the same column followed by the same letter are not significantly different (P > 0.05; n=3).

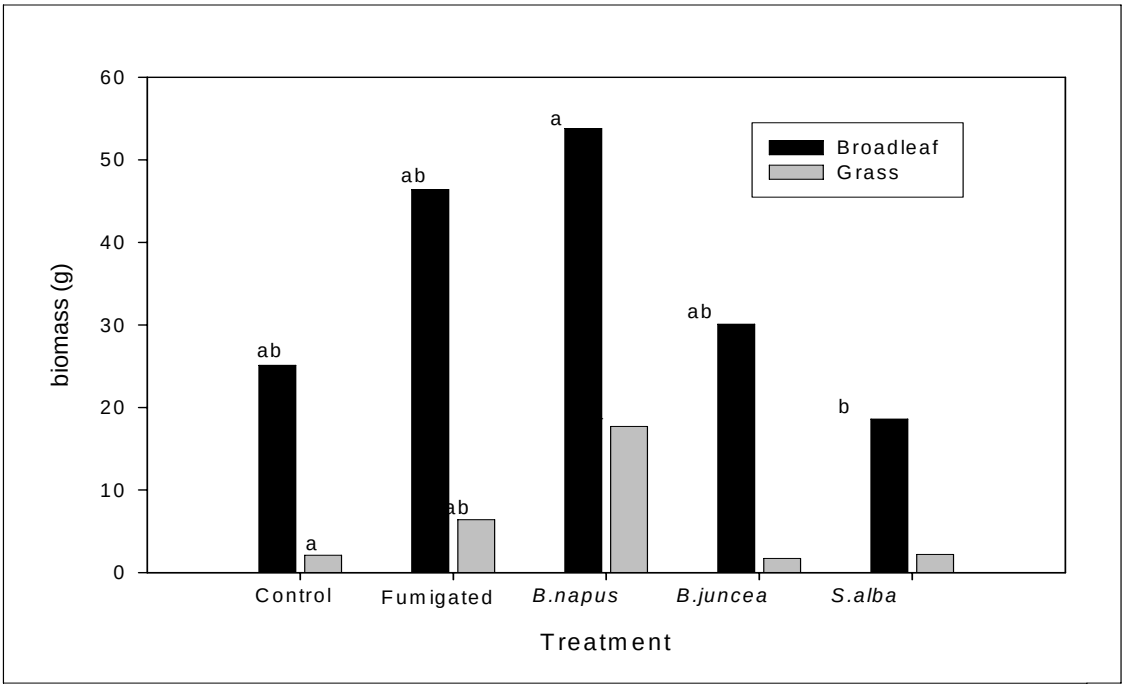

Figure 1. 2005 Aboveground weed biomass in CV newly established orchard plots.
* Bars with the same letter are not significantly different (P > 0.05; n=5).

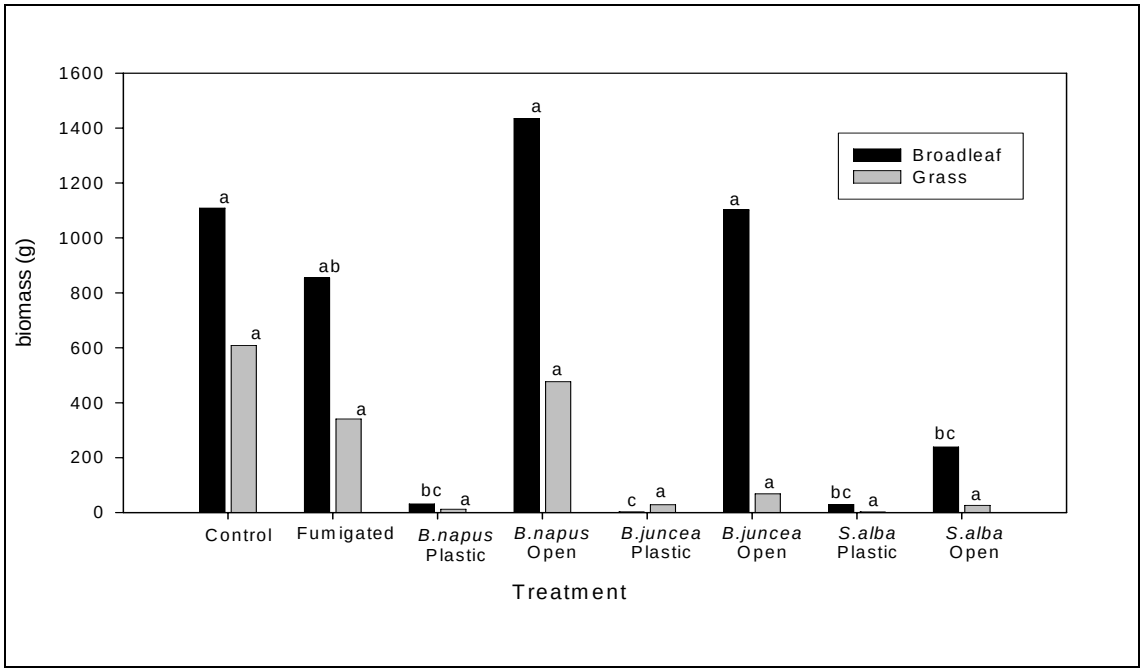

Figure 2. 2005 Above and belowground weed biomass in CV experimental field plots.
* Bars with the same letter are not significantly different (P > 0.05; n=5).

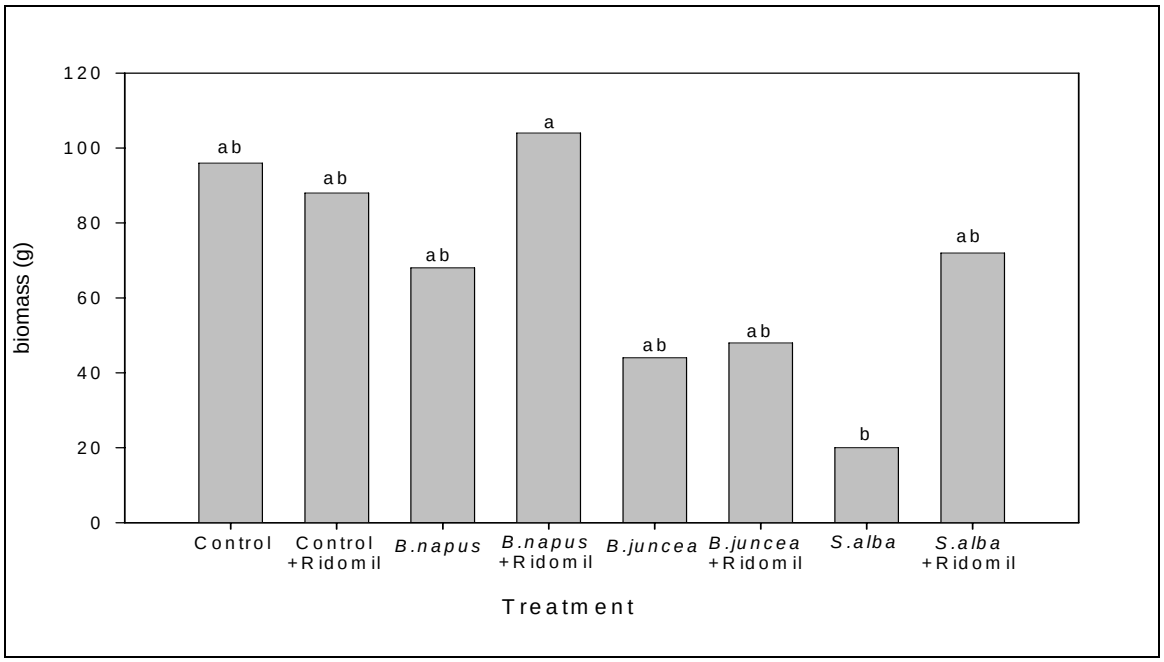

Figure 3. 2006 Aboveground weed biomass in CV experimental field plots.
* Bars with the same letter are not significantly different (P > 0.05; n=5).

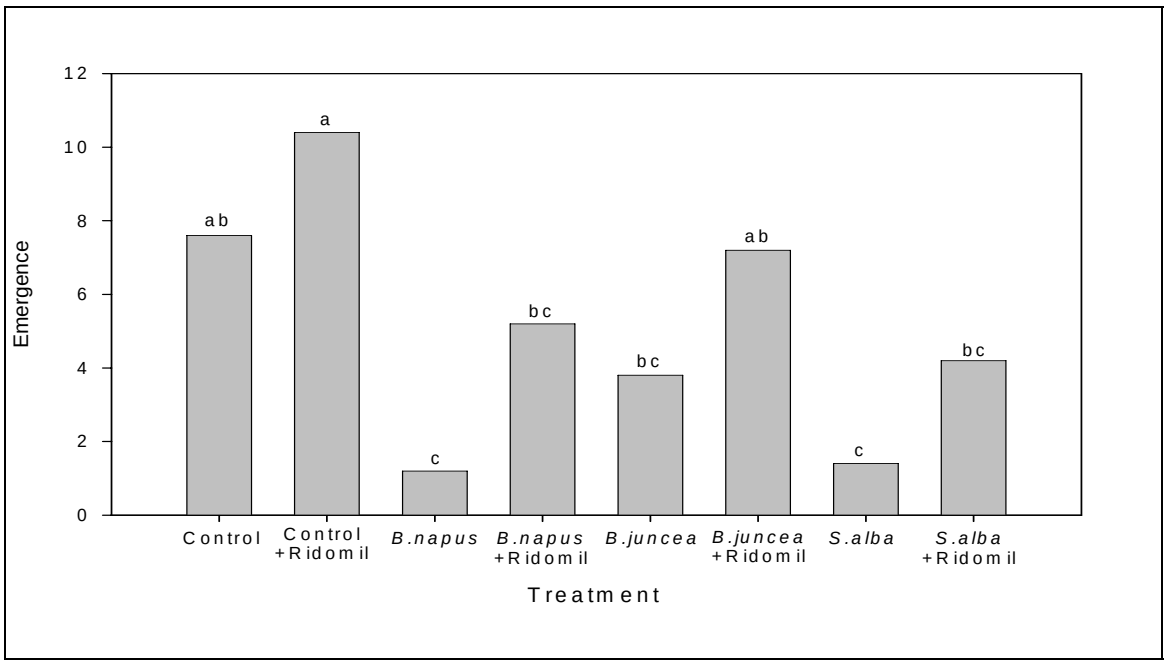

Figure 4. 2006 *T. aestivum* emergence in CV experimental field plots.
* Bars with the same letter are not significantly different (P > 0.05; n=5).

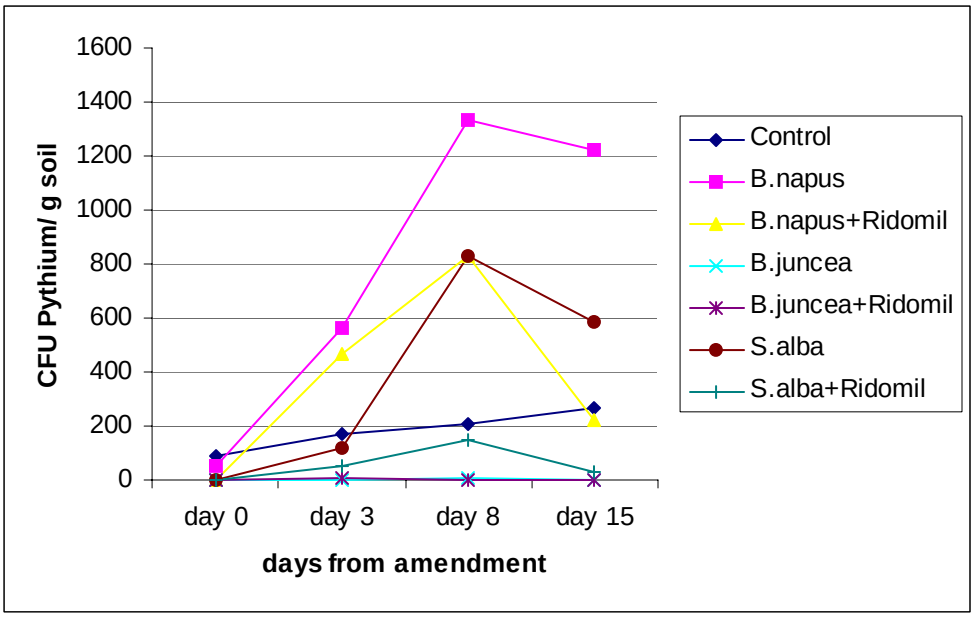

Figure 5. 2006 *Pythium* abundance over time in CV experimental field plots.

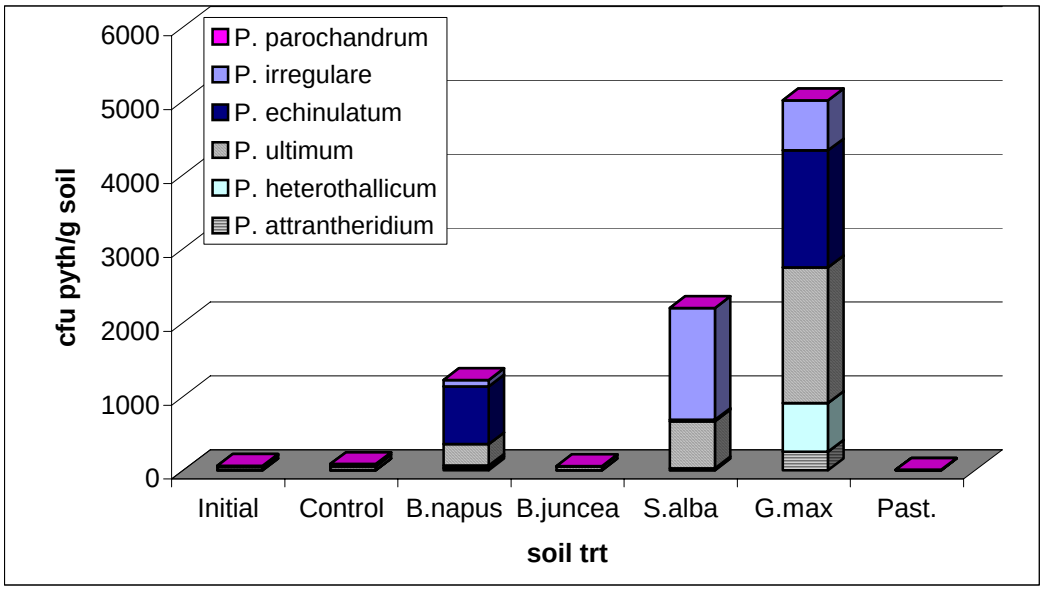

Figure 6. *Pythium* spp. enrichment in WVC soil as determined by real-time PCR.

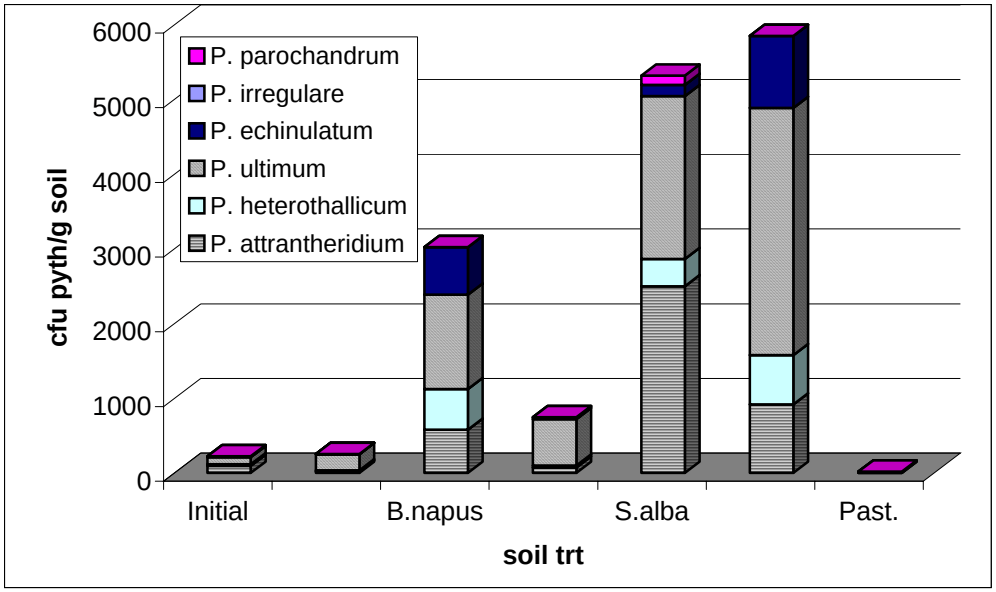

Figure 7. *Pythium* spp. enrichment in Tukey soil as determined by real-time PCR.

ORCHARD FLOOR MANAGEMENT FOR NITROGEN FERTILITY AND WEED CONTROL IN NEWLY ESTABLISHED ORGANIC APPLE ORCHARDS

(*to be submitted to Biology and Fertility of Soils*)

Keywords: organic agriculture, nitrogen and carbon cycling, nitrogen partitioning, living cover, wood chip mulch, organic herbicide, Brassicaceae seed meal, orchard legume

Abstract

Organic tree fruit production is thriving, and yet sustainable methods of nitrogen (N) fertility and weed management in organic orchards remain a challenge. Nutrient supply in organic systems is dependent on mineralization of organic matter; however, the intensive cultivation commonly used to control weeds in organic systems can disrupt biological processes and cause undue loss of organic matter. Here we address the often-competing goals of organic fertility and weed control by evaluating alternative orchard floor management strategies for their impact on N cycling, soil quality, and tree health in a newly established apple orchard. The standard practice of weed control using extensive tillage resulted in good tree growth with acceptable levels of leaf N and most other nutrients; conversely, soil quality declined relative to the control. Maintenance of a living cover understory increased soil N retention and availability and improved soil quality, but cover plants severely competed with young trees and reduced tree growth. Application of wood chip mulch in the tree understory enhanced soil moisture and

resulted in adequate tree growth, but it also facilitated N loss and correspondingly resulted in low tree leaf N. Clove oil herbicide provided poor weed control and resulted in lower leaf N and tree growth likely due to weed competition and did not improve soil quality. Although Brassicaceae seed meal (BSM) applications enhanced N availability and soil nematode abundance, leaf N and many other nutrients were below acceptable levels. None of the treatments applied produced an ideal combination of weed control, maximum tree growth, adequate leaf nutrient content, and improved soil quality. Rather, soil quality improvements tended to be achieved at the expense of tree performance.

1. Introduction

Consumer demand for organic fruit and advances in biologically based production strategies have led to a significant conversion of conventional to organic orchard production systems. Yet, sustainable methods of N fertility and weed control have remained a challenge. Extensive research has determined ideal timing and application rates for N fertilizer (Khemira et al., 1998; Ryugo, 1988; Stiles, 1994); and the negative consequences of excessive late-season N availability to fruit yield, quality and storage (Bramlage et al., 1980; Marsh et al., 1996; Sanchez et al., 2003). However, the majority of these studies used synthetic fertilizers that contain N in readily available plant accessible forms.

In contrast, organic systems generally rely on complex organic materials, such as composted animal manures to supply N. Only a fraction of the nutrients in these amendments are in immediately available forms with the remainder released slowly as a

function of decomposition and mineralization mediated by soil faunal communities (Laakso et al., 2000). As a result, producers generally rely on large amounts of compost to meet tree N needs, which may result in excessive salt levels in soil and plant damage. In addition, increased demand for compost has limited availability, resulting in long distance transport of bulky materials at high cost. Alternatives with greater N solubility are available, but these come at a high cost, ranging from $6.40-$11.70/kg N (I.F.M., 2005). In addition, if nutrient availability and uptake are not synchronized, excess nitrate-N is subject to leaching and subsequent environmental degradation (Stork and Jerie, 2003; Wagger et al., 1998). Organic practices are needed that will help reduce N fertility costs, mitigate N loss, and enhance N availability at times corresponding with critical tree uptake periods.

In the absence of reliable herbicides approved for use in organic systems, orchard managers often control weeds with intensive cultivation, a practice that can degrade soil structure (Six et al., 1998), negatively impact soil faunal communities (Fiscus and Neher, 2002) and accelerate nutrient cycling and organic matter loss (Cambardella and Elliott, 1993). In contrast, practices that reduce disturbance may help to ameliorate the negative impacts on soil quality and enhance N availability. For example, intensive cultivation prevents build-up of beneficial nematode communities (Neher, 2001), estimated to contribute 8 and 19% of mineralized N in conventional and integrated farming systems respectively (Beare, 1997). In contrast, maintenance of adequate soil moisture and labile C pools, generally associated with reduced disturbance systems, is correlated with greater microbial activity and promotion of nematode trophic groups that are associated with enhanced availability of mineral N to subsequent crops (Ferris et al., 2004).

Application of organic mulch materials, such as wood chips, is an effective weed control strategy shown to increase tree yield and improve soil physical, chemical and biological properties (Forge et al., 2003; Neilsen et al., 2003; Oliveira and Merwin, 2001; Sanchez et al., 2003; Yao et al., 2005). However, the effect of wood chip mulch on N fertility in organic orchard systems is less clear. Theoretically, the mulch will contribute recalcitrant carbon compounds that improve long-term soil nutrient and water-holding capacity. However, the high C:N ratio of a wood chip mulch may result in short-term N immobilization and reduced tree availability (Larsson et al., 1997).

Maintenance of a vegetative cover, or "living mulch", can reduce nutrient loss by acting as a 'catch crop', immobilizing and retaining available soil N, and/or contribute additional N via residue decomposition if leguminous (Marsh et al., 1996; Sanchez et al., 2003; Stork and Jerie, 2003; Yao et al., 2005). In addition, root exudates and decaying residues from cover crops contribute labile carbon compounds that stimulate microbial activity responsible for enhanced nutrient retention and cycling (Rovira et al., 1990; Wardle et al., 2001) and disease control (Forge et al., 2003; Gu and Mazzola, 2003). However, living cover crops also compete with trees for nutrients and water, reducing tree growth and yield, particularly in young trees or when N demanding grasses predominate (Marsh et al., 1996; Sanchez et al., 2003). In contrast, leguminous cover has been shown to increase tree growth and yield, but can also come at a cost in terms of delayed fruit maturity and quality if tree N levels become too high (Marsh et al., 1996). Additional research is needed to identify means or schedules that modulate the effects of living cover crops.

Organic herbicides, such as those derived from clove oil, will reduce soil disturbance, but their effect on N cycling and soil quality is unknown. Application of Brassicaceae seed meal, a byproduct of bio-fuel production, could reduce soil disturbance and contribute multiple benefits, potentially serving as a supplemental N fertilizer as well as providing weed and disease control through complex microbial interactions (Mazzola et al., 2006); nonetheless its effect on N availability and tree health is unclear.

To address the needs of organic orchardists for sustainable methods of N fertility and reduced disturbance weed control, we measured the effects of different organic orchard floor management strategies on N cycling, soil quality and apple tree health. We used varying levels of amendment to determine N-use efficiency in these organic systems. Identifying short-term treatment differences in fertility studies conducted on mature trees is difficult due to ample nutrient reserves and inherent soil fertility (Sanchez et al., 1995). Therefore, our study was conducted in a newly established orchard, where young trees are heavily impacted by available soil N and would more likely reflect impacts of orchard floor management practices.

2. Materials and Methods

2.1 Study site and orchard floor treatments

The trial was established in spring 2005 in an organically managed block previously planted to cherry at the Wenatchee Valley College-Auvil Teaching and Demonstration (WVC) orchard in East Wenatchee, Washington. Soil at the site is Pogue sandy loam (aridic haploxerol), averaging 1-2% OM and 6.1-7.3 pH; annual rainfall

averages 21.6 cm. Following stump removal and disking, apple trees (cv. Piñata on M7 rootstock) were planted with 1.5 m by 4 m spacing (1,541 trees/ha). Individual plots were arranged in a completely randomized block design with five replicates. Each plot consisted of six study trees, flanked by two guard trees.

Orchard floor management treatments included: 1) control (CON), 2) clove oil herbicide (CHE), 3) brassicaceae seed meal herbicide (BHE), 4) wood chip mulch (WC), 5) living mulch legume (LML), 6) living mulch non-legume (LMNL), 7) sandwich legume (SWL), 8) sandwich non-legume (SWNL), and 9) mechanical cultivation (CLT). The LMNL and CLT treatments were evaluated under low, medium and high N rate, LML under low and medium N rate, and WC under medium and high N rate (Table 1). In living mulch treatments, the entire 150-cm wide tree rows were planted to a mix of broadleaf and grass species; in sandwich treatments, this was limited to the central 45-cm of the tree row. LML and SWL treatments included a mix of Mt. Barker Subclover (*Trifolium subulata*), black medic (*Medicago lupulina*), burr medic (*Medicago polymorpha*), birdsfoot trefoil (*Lotus corniculatus*), and bentgrass (*Argostis tenuis*). LMNL treatment contained a mix of sweet alyssum (*Lobularia maritime*), five spot (*Nemophila maculata*), mother of thyme (*Thymus serpyllum*) and bentgrass, while SWNL was transplanted with an alternating sweet woodruff (*Galium odoratum*) and Corsican mint (*Mentha requiennii*). Drive rows were planted to a mix of sheep fescue (*Festuca ovina*) (50%), chewings fescue (*Festuca rubra*) (30%) and perennial ryegrass (*Lolium perenne*) (20%).

Drive rows and living mulch and sandwich treatments were routinely mowed throughout the growing season. Mature weeds in CHE, BHE and WC plots were mowed

as needed, and CLT and sandwich plots were managed using a combination of hand weeding and a tractor mounted Wonder Weeder (Harris Mfg., Burbank, WA). All orchard trees were cut back to whips in summer 2005. In spring 2006, orchard trees were given a liberal pruning, and small plastic mats (0.6 X 0.6 m) were placed around the base of each tree in CON, CHE, LML, and LMNL treatments to reduce competition. The entire research area was irrigated with a semi-micro system (solid set rotators) throughout the growing season as needed.

2.2 Amendments

In spring 2005, pelleted chicken manure (4% N) (NutriRich, Stutzman Farm, Canby, OR) was broadcast in the tree row at a rate of 0.9 (0.5X), 1.8 (1X) or 2.7 (1.5X) kg tree^{-1} and mechanically incorporated prior to tree establishment. The 1X rate equaled 105 kg total N ha^{-1}. In mid-July a soluble N fertilizer (Biolink, Westbridge Ag Products, Vista, CA) (14% N) was injected under each tree at a rate of 18 (0.5X), 36 (1X), or 54 (1.5X) kg total N ha^{-1}. Supplemental foliar applications of fish emulsion (Mermaids, I.F.M., Wenatchee, WA) and kelp (Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada) were applied to all trees at a rate of 2.75 kg total N ha^{-1}. In 2006, N availability from compost amendments was pre-determined and the 1X rate was adjusted upward accordingly. As a result, equal amounts of Nielsen's chicken manure compost (Mossyrock, WA) (3.5%N), and Stutzman's chicken manure compost (4% N) with available N estimated to be 51 and 28% respectively, were spread around the base of each tree in four equal, split-applications (April, early May, mid-May, June) for a total of 2.7

(0.5X), 5.4 (1X), and 8.2 (1.5X) kg tree^{-1}, resulting in approximately 101 kg available N ha $^{-1}$ (1X).

A clove oil based herbicide (Matran, Ecosmart technologies Inc., Franklin, TN) was diluted (60 ml L^{-1}) and the diluted formula was applied to CHE plots using a backpack sprayer at 230-306 L ha^{-1}. Brassicaceae seed meal (*Sinapis alba)* cv. Ida Gold seed meal (J. Brown, University of Idaho) (6.84% N) was broadcast over BHE plots at a rate of 1136 kg ha^{-1} using one application in 2005, and a rate of 3,408 kg ha^{-1} was applied in three equal applications in 2006. All SM amendments were incorporated into soil except the final application in 2006 that was left on the soil surface. A mix of conifer and deciduous wood chips (1.3-2.5 cm) was surface applied each spring. Wood chips were applied to the tree row (1.2 – 1.5 m) to a depth of 0.15 m, and stirred in autumn using the Wonder Weeder.

2.3 Soil sampling and analyses

Following orchard establishment, baseline soil samples were randomly collected from each plot using a 2-cm diameter probe to a depth of 30 cm, air-dried and stored for analysis. Subsequent soil sampling was conducted in mid-summer and autumn of 2005, and again in spring, mid-summer and autumn 2006. Composite samples were collected using a 2-cm diameter probe to a depth of 0-10 cm at a location approximately 15-30 cm from the base of each sample tree in a given plot, pooled and stored at 4 C until analysis.

Soil samples were air-dried and ground to pass through a 2 mm sieve. Total carbon (C) and nitrogen (N) were determined for the initial soil baseline and autumn collected samples. Particulate organic matter fraction (POM) was determined using an

automated dry combustion analyzer (LECO, St. Joseph, MI). Separation of the POM fraction followed established methods (Cambardella and Elliott, 1993). Soil was shaken overnight with a solution of hexametaphosphate and passed over a 53 um sieve. Material remaining on top of the sieve was oven dried at 50 C overnight, weighed, roller-ground and passed through the combustion analyzer. All soil samples were used to determine soluble N and potentially mineralizable N (PMN) following procedures outlined by (Drinkwater et al., 1996). Soil samples were suspended in deionized (DI) water and anaerobically incubated at 40C for 7 d. Nitrate and ammonium concentrations were determined following extraction with 1M KCl using a Lachet automated colorimetric analyzer (Lachet Instruments Inc., Milwaukee, WI) on both initial and incubated samples. PMN was calculated by subtracting the initial amount of available N from that released during incubation.

Autumn soil samples were analyzed for dehydrogenase enzyme activity and C-mineralization. Percent moisture was determined and soil samples were adjusted to 55% water filled pore space using methods outlined by Jarrell et al. (1999). Subsequently, dehydrogenase activity was determined following methods outlined by Tabatabai (1994). Carbon-mineralization and determination of different C pools and their corresponding decay rates were determined following the methods of Robertson et al. (1999) and Collins et al. (2000). Briefly, soil samples were incubated at ambient temperatures in the dark and CO_2 was extracted using a needle and concentration was determined by gas chromatographic analysis (GC-17A, Shimadzu, Kyoto, Japan) at several time intervals. After each CO_2 extraction interval, soils were uncapped and flushed with humidified air, recapped and returned for incubation.

2.4 Nematode abundance and community diversity

Nematodes were extracted from 50 g subsamples of field-moist soil, using a Baermann pan technique (7-day incubation) (Ingham, 1994), and counted under 40X magnification. Estimation of nematode community diversity was determined using a terminal restriction fragment length polymorphism (T-RFLP) assay. Soil community DNA was extracted from 10 g of soil using an Ultraclean Mega Soil DNA isolation kit (Carlsbad, CA), and stored at –20C until analysis. Small subunit ribosomal DNA (18S rDNA) was selectively amplified from soil community DNA using a forward primer (Waite et al., 2003) that targeted variable V3 and V5 regions of the 18S rDNA, and a fluorescently labeled 'universal' reverse primer (5'- AGT CAA ATT AAG CCG CAG-3') that hybridized to highly conserved regions of the eucarya 18S rDNA. Amplification was carried out in 20 µl reactions using 0.6 µl DNA, 1.6 µl dNTP mix, 1.2 µl $MgCl_2$, 2 µl 10X buffer, 0.2 µl Amplitaq Gold DNA polymerase, 0.2 µl of each primer (25 um), and 14.6 µl water. Amplification reactions were conducted in a GeneAmp 9700 thermal cycle (Applied Biosystems, Foster City, CA) using reaction conditions of initial denaturation at 94 C, 5 min, followed by 40 cycles of 94 C, 1 min, 50C, 1 min, 72 C, 3 min, with a final extension for 7 min at 72 C. PCR product was confirmed by visual comparison to a 100 bp ladder following electrophoresis on a 1.5% agarose gel stained with ethidium bromide.

Resulting amplicons were restriction digested using 3 µl DNA, 0.3 µl *Rsa*1 enzyme, 1 µl of 10x digestion buffer, and 5.7 µl sterile DI water, and incubated at 37 C for 3 hours. Restriction product was resuspended in 1 µl of 3M CH3COONa, 1 µl

EDTA, 0.5 µl glycogen, and 30 µl ETOH (95%), and DNA precipitated by centrifugation. The DNA pellet was washed twice with 150 µl ETOH (70%) and dried for 10 min at 65 C. Products were then individually resuspended in 40 µl sample loading solution (Beckman-Coulter, Fullerton, CA) with 0.25 µl 600 bp size standard, and incubated at 37 C for 10 minutes. Samples were then analyzed using a CEQ 8000 Genetic Analysis System (Beckman Coulter) with an injection time of 10 seconds and separation of DNA fragments conducted over 90 minutes. Data sets were constructed using peaks possessing a minimum height threshold of 0.002 fluorescence units. In addition, peaks not occurring in both replicates of each sample were eliminated.

2.5 Tree circumference and leaf sampling

In July 2005, each tree was permanently marked 20 cm above the graft union. Tree circumference was measured at this point in July 2005 and again in October 2005 and 2006. Increase in tree cross sectional area (TCSA) was determined after transforming tree circumference into area, and calculating the difference between the original area and the area in autumn. Composite tree leaf samples were collected in late July 2006 from the middle third of each sample tree in a given plot. From each tree, four leaves of average vigor were randomly selected from the middle of the current season's terminal branches. Leaves were oven-dried at 50 C for 48 hours, ground and sent to an independent laboratory (U of Wisconsin) for analysis of N, P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, and Na.

2.6 Release and partitioning of compost N

In 2006, compost was enriched with ^{15}N fertilizer and applied to the 1X LML, WC and CLT treatments to track N release from compost and its partitioning among orchard components. Nielsen's chicken compost, pre-weighed for each sample tree was spread on a plastic tarp and sprayed with 5.39 g of $(NH_4)_2SO_4$ (~70A% ^{15}N, 0.8 g ^{15}N / tree) dissolved in 50 ml DI water (Monsanto, St. Louis, MO), 48 hours prior to application and hand mixed. Within each plot, ^{15}N labeled compost was applied to a separate individual tree during each of the three compost application periods (April 7, May 9, and June 7). Soil, wood chip and plant residue samples were taken from around each sample tree at monthly intervals following amendment (May 9, June 7, July 13, and Sep 29). In all treatments, three soil samples were taken at approximately 15 cm from the base of each tree with a 2-cm diameter probe to a depth of 10 cm, pooled and dried at 50 C for 24 hours. In WC treatments, three samples of wood chip residue, totaling approximately 10 g were taken from the duff layer just above the soil surface prior to soil sampling, pooled and dried at 50 C for 24 hours. In LML treatments, three plant residue samples (7.6 X 10.2 cm/each) located just outside mulch mats were cut at the soil surface, pooled and dried at 50 C for 24 hours. All dried samples were subsequently roller-ground and analyzed for ^{15}N using an isotope ratio mass spectrometer (Thermo Finnigan, Germany).

2.7 Statistical analyses

All statistical analyses were conducted with SAS 9.1 software (SAS Institute Inc., Cary, North Carolina). Data generated from analyses of trunk cross sectional area

(TCSA), leaf nutrients, soil mineral analyses and dehydrogenase activity were subjected to analysis of variance and mean separation was based upon Fisher Protected LSD. In 2005, active soil carbon (C1 and C2) pools and their associated decay constants (K1 and K2) were estimated using NLIN METHOD-MARQUARDT. Differences among these pools were determined using a multivariate analysis of variance. In 2006, the NLIN model failed to converge so individual sampling dates were analyzed using analysis of variance. Nematode community diversity profiles were evaluated using an analysis of molecular variance (Johnson et al., in press). All analyses were considered significant at $p \leq 0.05$.

3. Results

3.1 Biological soil quality

At the end of the 2005 growing-season total C and N in soil from the 1X LML treatment was significantly greater than the 1X CLT treatment (Table 2). In addition, the 1X LML was significantly greater in mineralizable C (Table 3), its associated decay constant, and dehydrogenase activity (Table 4) than 1X CLT, WC and CHE treatments. By autumn 2006, 1X treatments LMNL and LML had greater total C and N (Table 2), POM-C and N (Table 5), mineralizable C (Table 6), dehydrogenase activity (Figure 1) and nematode abundance (Figure 2) compared to other treatments; CLT and WC treatments were the lowest. The BHE treatment also had significantly greater total C and N (Table 2), nematode abundance (Figure 2), and rapidly mineralizable C (Table 6) than did the WC and CLT treatments. Indices of nematode T-RFLP peaks varied among

treatments (Table 7), but were not significantly different. The 1X LMNL, LML and BHE treatments often had larger pools of available and potentially mineralizable N, while the WC treatment was among the lowest (Tables 8 and 9). Analyses of ^{15}N abundance indicated that initially there existed greater soil N in 1X CLT treatment versus LML and WC treatments, but over time differences among treatments were no longer apparent (Table 10).

3.2 N cycling and tree nutrition and growth

Compost derived ^{15}N was immobilized in living mulch and wood chip residue (Figures 3 and 4), particularly at the first sampling after each compost application. This immobilization temporarily reduced soil N available for tree uptake in LML and WC compared to CLT (Table 10), but this effect was no longer significant by September 29. Tree leaf N reached desirable levels for young non-bearing trees (2.4-2.6%; Stiles, 1994) in LMNL and LML receiving a 1X rate of fertilizer, and CLT treatment regardless of fertility rate, whereas it was deficient in all other treatments (Table 11). Isotopic analyses confirmed that similar levels of compost N accumulated in tree leaves in CLT and LML treatments, while it was significantly less in the WC treatment (Figure 5). In contrast, leaf P levels were inversely related to leaf N, and Ca and Zn levels were deficient in all treatments (Table 11). Each of the other 9 essential nutrients were within the range deemed adequate for young apple trees (Stiles, 1994) in all treatments (data not shown).

Percent increase in tree cross sectional area (TCSA) was low irrespective of orchard floor treatment in 2005 (Table 12) however, treatment differences were apparent; tree growth in soils receiving the CLT and WC treatments were significantly greater than

all living mulch treatments, while BHE, both sandwich treatments, and CHE resulted in tree growth that was intermediate in this range. In 2006, trees in all treatments exhibited greater overall growth (Figure 6), yet differences among treatments followed similar trends. The living mulch and control treatments produced the smallest increase in TCSA, while the WC, CLT, and BHE treatments resulted in significantly increased, though similar levels of tree growth.

4. Discussion

4.1 Impact of orchard floor management on soil quality

Maintaining adequate supplies of available soil N for plant uptake in organic systems is dependent upon supply of soil and amendment-derived organic matter and nutrient release by biological decomposition and mineralization. Both phases of this goal can be enhanced by greater soil quality. These benefits, along with the requirement that certified organic production "maintain or improve soil health" (USDA-NOP, 2001), make soil quality a desired objective for organic systems. In our study, overall tree health was greatest within cultivated treatments; however, this treatment ranked among the lowest in terms of all soil quality indicators. Given rapid nutrient cycling within this treatment, in the short-term soil fertility can be maintained with annual amendment inputs. However, if soil quality continues to decline, there could be negative consequences to soil nutrient cycling and tree health. In contrast, living mulch treatments ranked highest among all soil quality indicators, which implies long-term improvement that may lead to increased nutrient-use efficiency and potentially healthier trees over time. However, consistent

with other studies (Marsh et al., 1996; and Sanchez et al., 2003), living mulch results in severe competition and stunting of orchard trees, which were not compensated for by additional compost amendment. Therefore, during establishment, a more desirable strategy may be application of low C:N ratio plant biomass that could improve soil quality without acting as a competitor to tree development.

Despite substantial inputs of total C from wood chip mulch, this treatment ranked among the lowest in all soil quality indicators. In addition, our results suggest that soluble N compounds are being lost from this system through either gaseous or leaching mechanisms, potentially exasperating air and/or water quality issues. Despite reduced disturbance, the CHE treatment did not have any significant positive impact on soil quality parameters. In contrast, amending soil with Brassicaceae seed meal (*S. alba*) resulted in improved overall soil quality relative to the cultivated standard.

Preliminary analyses of nematode community diversity was not significantly different, yet profiles of different treatments appear to be changing and may become significantly different over time. Living mulch and BHE treatments did result in an increase in nematode abundance relative to that recovered from cultivated soil. Given past research, we expect systems with reduced disturbance and greater inputs of organic matter to become enriched by bacterivore, fungivore, and predatory nematode species. These genera tend to be more common in mature communities and are often correlated with increased N mineralization (Neher, 2001). In contrast, soil cultivation tends to result in enrichment of plant parasitic species responsible for inducing disease and lower overall tree health (Neher, 2001).

4.2 Impact of orchard floor management on nutrient supply and tree uptake

By the end of the second growing season, the standard cultivation practice for weed control successfully eliminated weed competition (Granatstein et al., 2007) and resulted in sufficient available soil N, desirable tree growth, and acceptable levels of leaf N and most other nutrients regardless of the amendment rate. As such, we conclude that in the short-term, tree N needs in cultivated systems can be met and nutrient-use efficiency maximized at a low N compost amendment rate of 1200-2500 kg ha $^{-1}$, depending on N availability of the compost amendment. In contrast, none of the reduced-disturbance alternative treatments provided a combination of good growth and acceptable leaf N.

Although the 1X legume and non-legume treatments had the greatest leaf N, all living mulch treatments produced less tree growth. Sandwich treatments with reduced living cover gave greater tree growth, but leaf N was below desirable levels. Although isotope analysis confirmed immobilization of compost N in living cover crops, periodic mowing allowed mineralization of this N and resulted in sufficient available soil N. Living cover understories also successfully retained available N that was lost from other treatments during winter leaching, and resulted in greater available N throughout the growing season. Given that available soil N was high under living covers, and given the insignificant growth response to higher rates of amendment, reduced tree growth may have resulted from moisture competition. Irrigation was applied uniformly in our study, and may need to be increased in systems with living mulch understories.

An additional factor that potentially contributed to poor tree growth in living cover plots may be the late-season injection of soluble N fertilizer applied mid-season

2005 to all trees. It was visually apparent that living mulch crops recovered much of this additional N, whereas in non-living cover treatments this N was likely taken up by the trees and contributed to their rapid growth in spring 2006. As it results in severe competition to the tree, a living cover crop system is not advisable during orchard establishment or with dwarfing rootstocks that have shallow root systems. However, given the potential benefits to soil quality, this treatment may hold promise in mature orchards or in those systems employing more aggressive non-dwarfing rootstocks with more extensive root systems. These findings support the premise of Stork and Jerie (2003), that future research using living cover understory crops should focus on species with substantial fall growth and winter cover to help retain available soil N, but go dormant during the hot summer season when trees put on maximal vegetative growth.

Application of wood chip mulch resulted in good tree growth, but tree leaf N was well below desirable levels. Increasing compost amendment led to higher leaf nutrient levels, but not enough to meet levels suggested by previous research as desirable for young non-bearing trees (Stiles, 1994). While isotopic analyses indicate initial immobilization of N in the wood chip residue, available N slowly cycles into the soil and is subsequently lost from the system as confirmed by analyses of plant available, labile and total N in the active soil fraction. The wood chip mulch treatment resulted in abundant soil moisture, which may have contributed to the observed adequate tree growth; yet, this may also have contributed to N loss through denitrification or leaching mechanisms, as evidenced by the low available N detected in our study.

Application of clove oil herbicide resulted in lower leaf N and tree growth in comparison to cultivated treatments, likely due to increased competition for water and

nutrients from uncontrolled weeds (Granatstein et al., 2007). In contrast, *S. alba* seed meal amendment resulted in ample pools of available soil N and relatively good tree growth, confirming its potential as an alternative fertility amendment. However, leaf N did not reach acceptable levels and many of the other essential nutrients were lowest in this treatment. Tree leaf chlorosis was observed following early season amendment in 2006 and may be the result of reduced soil iron availability. Ionic isothiocyanate, resulting from hydrolysis of *S. alba* glucosinlates, is thought to complex the pool of plant available soil iron (M. Morra, personal communication). Delayed production of leaf chlorophyll may have reduced the tree's ability to uptake and utilize available soil N. Additional research using different Brassicaceae species is needed to identify application rates and their timing to prevent tree damage and enhance its use as an organic fertility amendment.

Tree leaf Ca and Zn were below acceptable levels in all treatments, but were among the lowest in the cultivated treatments along with leaf P. Interestingly, research has shown that root colonization by mycorrhizal fungi, a process negatively impacted by soil tillage, can increase tree uptake of these elements (Paul and Clark, 1996). In our study, leaf concentrations of these elements were greatest among un-disturbed treatments, suggesting that enhanced mycorrhizal-plant relationships in these systems may facilitate greater P, Ca and Zn uptake.

4.3 Conclusions

Intensive cultivation of orchard understory results in healthy trees with good growth, but a highly disturbed production system remains dependent on annual

amendments to meet N fertility demands. Results of this study indicate that intensive cultivation reduced many soil quality parameters, which may negatively impact long-term nutrient cycling dynamics, tree health, and fruit quality. In contrast, the non-disturbed living cover understory resulted in increased competition with orchard trees and a corresponding early reduction in tree growth. Nevertheless, trees in this system appeared healthy in year two and possessed ideal leaf N values. Our indicators suggest that the living mulch system is improving soil quality and retaining available N pools, which may have positive impacts on long-term tree health and result in a system that is more compatible with organic nutrient cycling dynamics. However, given severe tree competition, the living mulch system is not advisable during orchard establishment.

Although application of wood chip mulch had positive soil and tree effects in previous studies, it did not perform well in the current study, resulting in low leaf N and poor performance on all soil quality indices. Organically approved herbicides hold promise to reduce soil disturbance and improve soil quality over time, but they will need to be more effective at controlling weeds that compete for N resources. Brassicaceae seed meal amendments have potential to simultaneously provide N, improve soil quality, and control weeds and soil-borne diseases, yet they require additional study to determine optimal rates and conditions for application in organic orchard management systems. None of the treatments applied in this study produced and ideal combination of weed control, maximum tree growth, adequate leaf nutrients, and improved soil quality in newly established organic apple orchards. Changes in soil quality accumulate over time and may have a more significant impact on nutrient cycling processes and tree health and productivity in mature orchards.

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Table 1. Summary of Orchard Floor Management Treatments.

	Treatment	Disturbance	Fertility
CON	Control	Low	0X
CHE	Clove Oil Herbicide	Low	1X
BHE	Brassica Herbicide	Medium	0.5X
LML	Living Mulch Legume	Low	0.5X, 1X
LMNL	Living Mulch Non-Legume	Low	0.5X, 1X, 1.5X
WC	Wood Chip Mulch	Low	1X, 1.5X
CLT	Cultivated	High	0.5X, 1X, 1.5X
SWL	Sandwich Legume	Medium	1X
SWNL	Sandwich Non-Legume	Medium	1X

Table 2. Total Carbon and Nitrogen in autumn collected soil samples (ppm).

	2005		2006	
Treatment	Carbon	Nitrogen	Carbon	Nitrogen
CON	11172 ab	911 ab	11900 fgh	920 d
CHE	9608 ab	732 ab	12500 defg	1100 bc
BHE	11015 ab	878 ab	13400 abcd	1200 ab
LML0.5	8738 b	656 b	10900 hij	890 de
LML1	12255 a	939 a	14300 a	1240 a
LMNL0.5	10690 ab	782 ab	13100 bcde	1050 c
LMNL1	11038 ab	822 ab	14600 a	1210 ab
LMNL1.5	10338 ab	788 ab	13700 abc	1210 ab
WC1	10959 ab	844 ab	11600 ghi	920 d
WC1.5	11462 ab	872 ab	12800 cdef	1120 abc
CLT0.5	9184 ab	711 ab	9800 j	770 f
CLT1	8841 b	683 b	10500 ij	800 ef
CLT1.5	10896 ab	844 ab	12100 efg	1070 c

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

Table 3. 2005 Active and intermediate carbon pools (c) and their associated decay constants (k) in autumn collected soil samples.

Treatment	c1	k1	c2	k2
CHE	28.7 a	0.445 a	378.1 b	0.019 b
LML	30.6 a	0.711 a	547.5 a	0.023 a
WC	26.4 a	0.587 a	418.6 b	0.017 b
CLT	22.1 a	0.499 a	346.0 b	0.018 b

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

Table 4. Dehydrogenase activity (ug TPF g^{-1} soil h^{-1}) in autumn collected soils samples.

Treatment	2005
CON	
CHE	1.53 b
BHE	
LML1	3.26 a
LMNL1	
WC1	1.53 b
CLT1	1.35 b

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

Table 5. 2006 Particulate Organic Matter (POM) Carbon and Nitrogen (mg/kg soil) in autumn collected soil samples.

Treatment	POM-C	POM-N
CON	41.10 b	2.34 bc
CHE	48.18 ab	2.94 abc
BHE	45.50 ab	2.92 abc
LML	50.06 ab	3.0 ab
LMNL	55.26 a	3.28 a
WC	39.21 b	2.11 c
CLT	41.27 b	2.64 abc

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

Table 6. 2006 Carbon mineralization (ug CO2-C g^{-1} soil d^{-1}) in autumn collected soil samples.

	Day 1	Day 3	Day 10	Day 17	Day 24	Day 38	Day 54
CON	17.3 cd	6.7 b	2.8 b	0.3 b	0.3 b	0.2 b	0.1 b
CHE	18.5 bcd	7.4 b	4 ab	1.9 a	1 ab	0.3 ab	0.2 ab
BHE	27.7 abc	9.6 ab	3.5 b	0.8 ab	0.7 ab	0.4 ab	0.3 ab
LML1	30.3 a	13.4 a	6.6 ab	1.8 ab	1.4 a	0.9 a	0.4 a
LMNL1	29.8 ab	13.4 a	7.8 a	1.4 ab	0.7 ab	0.5 ab	0.4 a
WC1	21.8 abcd	7.9 b	3.5 b	2.0 a	1.24 a	0.54 ab	0.4 a
CLT1	16.1 d	6.3 b	3.3 b	1.6 ab	1 ab	0.4 ab	0.2 ab

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

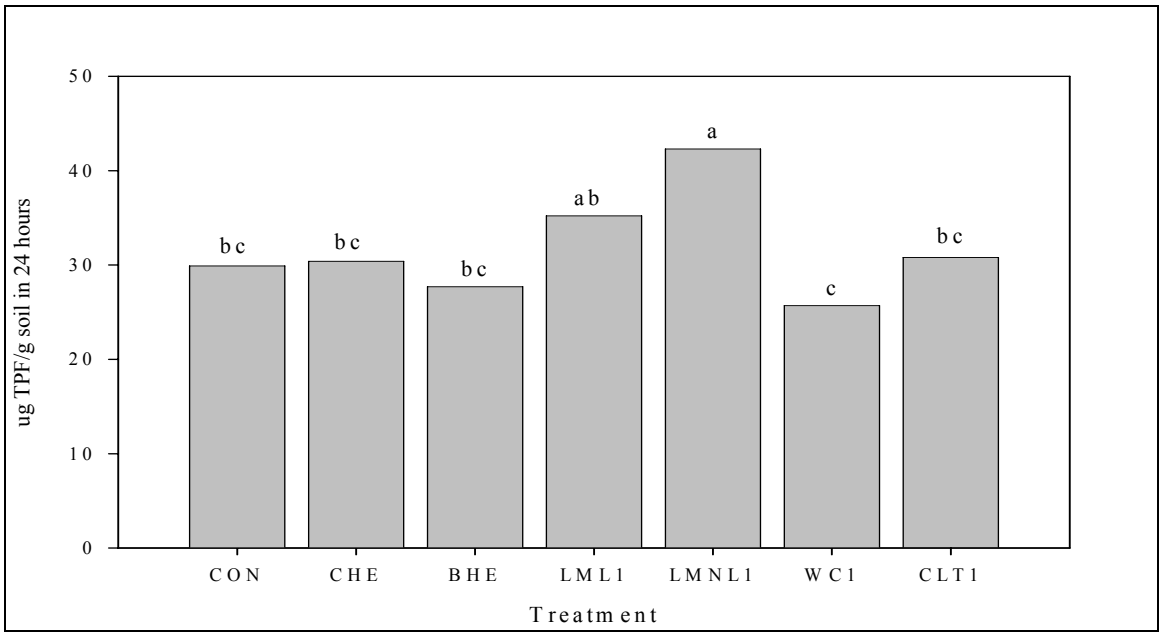

Figure 1. 2006 Dehydrogenase activity in autumn collected soil sample (ug TPF g^{-1} soil h^{-1}).
* Bars with the same letter are not significantly different (P> 0.05; n=5).

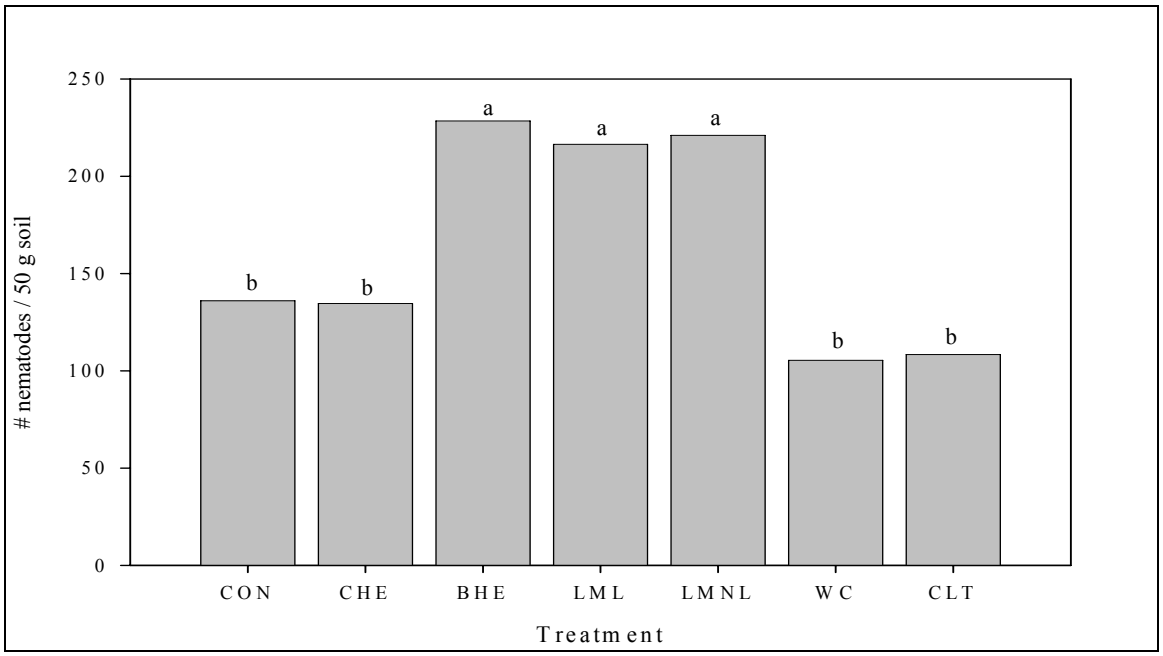

Figure 2. 2006 Nematode abundance in summer collected soil samples.
* Bars with the same letter are not significantly different (P> 0.05; n=5).

Table 7. 2006 Treatment comparisons of nematode diversity using T-RFLP and analysis of molecular variance (n=5).

Treatments		P-Value
CHE	LMNL	0.1005
CHE	CLT	0.1293
BHE	WC	0.1631
CON	CLT	0.1741
LML	CHE	0.25
WC	CON	0.2591
LML	LMNL	0.2901
LMNL	WC	0.2907
BHE	CON	0.2979
BHE	CLT	0.3625
CHE	CON	0.4089
LML	CLT	0.4148
LML	BHE	0.5
LMNL	CLT	0.6196
CHE	BHE	0.6365
BHE	LMNL	0.667
LMNL	CON	0.7141
CHE	WC	0.8242
LML	WC	0.8366
LML	CON	0.9557
WC	CLT	0.9812

Table 8. Total soil inorganic N (ppm) as determined by KCl extraction.

Treatment	Sp '05	1X	Su '05	1X	Fa '05	1X	Sp '06	1X	Su '06	1X	Fa ' 06	1X
CON	4.5 a	a	9.8 cd	c	5.8 c	b	2.2 c	b	9.2 f	d	8.6 d	c
CHE	3.9 a	a	14.1 bcd	bc	35.5 ab	ab	3.0 bc	b	31.8 bc	b	17.9 abc	ab
BHE	5.2 a	a	24.3 a	a	36.4 ab	ab	3.2 bc	b	31.9 bc	b	22.7 a	a
LML0.5	4.5 a		10.8 cd		29.3 abc		4.1 bc		22.4 cdef		15.8 abc	
LML1	4.4 a	a	19.2 ab	ab	33.7 ab	ab	5.8 bc	a	46.1 a	a	21.0 a	a
LMNL0.5	4.9 a		8.1 d		11.9 bc		2.5 bc		32.1 bc		12.2 bcd	
LMNL1	3.9 a	a	12.0 bcd	bc	25.7 abc	ab	3.0 bc	b	28.0 bcd	bc	12.7 bcd	bc
LMNL1.5	4.0 a		15.0 bcd		18.7 abc		16.7 a		37.5 ab		21.8 a	
WC1	5.4 a	a	8.4 d	c	38.1 ab	a	2.5 bc	b	17.9 def	cd	12.8 bcd	bc
WC1.5	5.6 a		16.5 bcd		41.4 a		9.4 b		23.4 cde		12.0 bcd	
CLT0.5	4.2 a		12.0 bcd		19.9 abc		2.5 bc		12.4 ef		11.3 cd	
CLT1	4.7 a	a	14.3 bcd	bc	42.9 a	a	2.5 bc	b	22.9 cde	bc	15.8 abc	abc
CLT1.5	4.4 a		17.2 abc		30.3 abc		3.9 bc		32.2 cde		18.5 ab	

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

Table 9. Potentially mineralizable nitrogen (ppm) in soil as determined by anaerobic incubation and KCl extraction.

Treatment	Sp '05	1X	Su '05	1X	Fa '05	1X	Sp '06	1X	Su '06	1X	Fa ' 06	1X
CON	16.7 a	a	8.5 abcd	ab	26.0 b	a	27.2 ab	a	27.7 abc	ab	32.8 abc	ab
CHE	17.0 a	a	1.9 d	c	8.9 cd	bc	22.7 ab	ab	12.9 c	b	28.0 abcde	ab
BHE	22.1 a	a	2.5 cd	bc	19.6 ab	ab	22.4 ab	ab	33.0 ab	ab	35.4 a	ab
LML0.5	23.4 a		6.5 abcd		13.2 cd		19.0 ab		36.7 a		29.1 abcde	
LML1	24.5 a	a	8.0 abcd	abc	10.6 bcd	bc	15.4 b	b	20.3 abc	ab	34.5 ab	ab
LMNL0.5	21.7 a		11.0 ab		24.3 bc		31.5 a		22.1 abc		32.5 abcd	
LMNL1	17.2 a	a	5.9 abcd	abc	22.6 bc	ab	24.6 ab	ab	25.9 abc	ab	38.8 a	a
LMNL1.5	15.9 a		3.9 bcd		51.7 a		20.8 ab		18.0 bc		27.7 abcde	
WC1	23.9 a	a	10.2 abc	a	8.2 cd	bc	21.3 ab	ab	26.6 abc	ab	21.3 bcde	b
WC1.5	19.4 a		13.3 a		9.6 bcd		19.5 ab		14.0 c		16.2 e	
CLT0.5	22.1 a		7.9 abcd		18.7 bc		17.9 b		24.7 abc		21.0 bcde	b
CLT1	19.0 a	a	5.2 bcd	abc	1.5 d	c	15.9 b	b	32.5 ab	a	19.7 cde	
CLT1.5	19.3 a		8.8 abcd		9.1 cd		16.1 b		25.1 abc		18.8 de	

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

Table 10. 2006 Abundance of ^{15}N in soil in plots amended with ^{15}N-enriched compost over time (atom%^{15}N).

Treatment	Compost application date	Collection Date			
		5/9	6/7	7/13	9/29
CLT1	**4/7**	0.6135 a	0.5279 b	0.5134 a	0.4033 ab
CLT1	**5/9**		0.5129 b	0.4991 a	0.4004 abc
CLT1	**6/7**			0.4727 abc	0.3958 abc
LML1	**4/7**	0.5233 ab	0.5130 b	0.4929 ab	0.3984 abc
LML1	**5/9**		0.5568 ab	0.5146 a	0.4106 a
LML1	**6/7**			0.4728 abc	0.3866 bc
WC1	**4/7**	0.4872 a	0.6281 a	0.4325 bc	0.4040 ab
WC1	**5/9**		0.5789 ab	0.4241 c	0.3819 bc
WC1	**6/7**			0.4338 bc	0.3788 c

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=3).

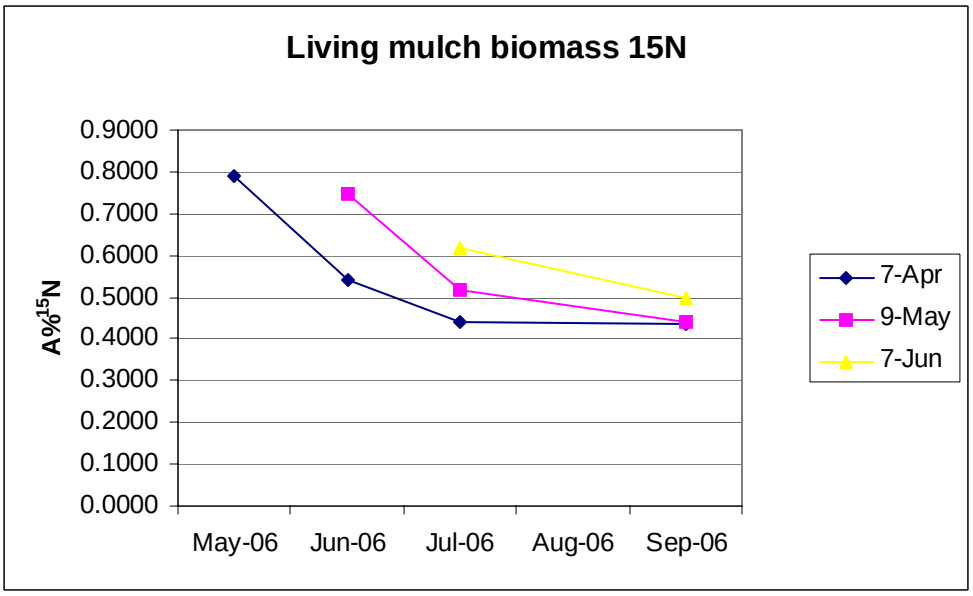

Figure 3. 2006 Abundance of ^{15}N in living mulch biomass in plots amended with ^{15}N - enriched compost over time (n=3).

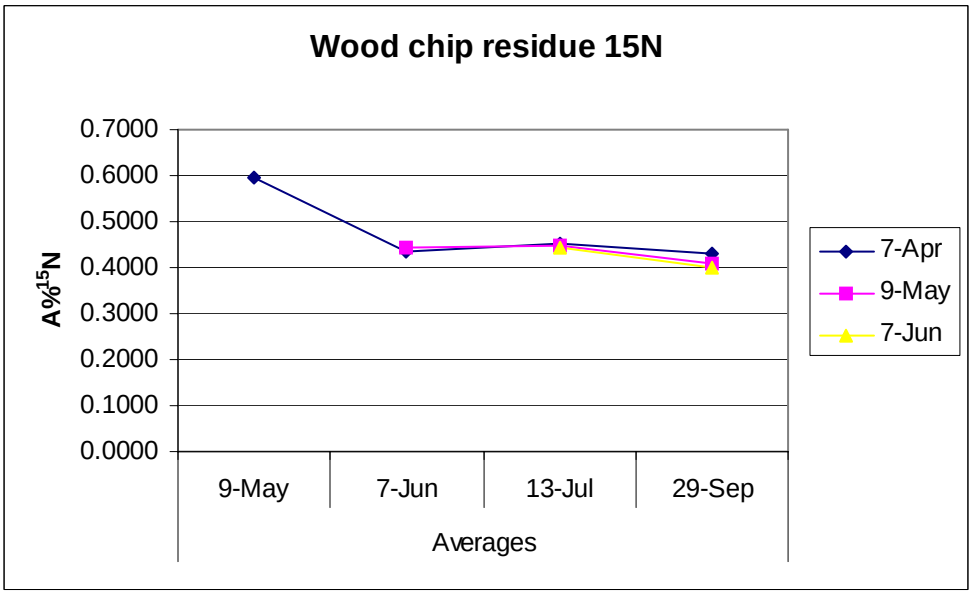

Figure 4. 2006 Abundance of ^{15}N in wood chip residue in plots amended with ^{15}N - enriched compost over time (n=3).

Table 11. 2006 Tree Leaf Nutrients.

Treatment	% N	%P	% Ca	Zn ppm
CON	2.08 de	0.40 a	1.26 cd	9.47 abcd
CHE	2.35 abc	0.26 cd	1.31 abcd	10.49 ab
BHE	2.25 cde	0.21 d	1.17 d	10.33 abc
LML0.5	2.24 cde	0.32 abc	1.38 abc	10.34 ab
LML1	**2.55 a**	0.24 d	1.43 ab	10.37 ab
LMNL0.5	2.33 abc	0.32 bc	1.28 bcd	9.78 abcd
LMNL1	**2.50 ab**	0.24 d	1.37 abc	10.68 ab
LMNL1.5	2.34 abc	0.22 d	1.34 abc	11.11 a
WC1	2.05 e	0.38 ab	1.30 abcd	9.28 bcd
WC1.5	2.26 cde	0.38 ab	1.36 abc	9.91 abcd
CLT0.5	**2.48 abc**	0.22 d	1.27 bcd	8.32 d
CLT1	**2.45 ab**	0.21 d	1.27 cd	9.36 bcd
CLT1.5	**2.41 abc**	0.22 d	1.27 bcd	8.61 cd
SWL	2.33 abc	0.23 d	1.36 abc	10.18 abc
SWNL	2.29 bcd	0.23 d	1.44 a	9.3 abcd

- **Desired N level for young non-bearing apples 2.4-2.6% (Stiles, 1994).**
- **Desired P level in apple 0.11-.3%.**
- **Desired Ca level in apple 1.5-2.0%.**
- **Desired Zn level in apple 15-200 ppm.**

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

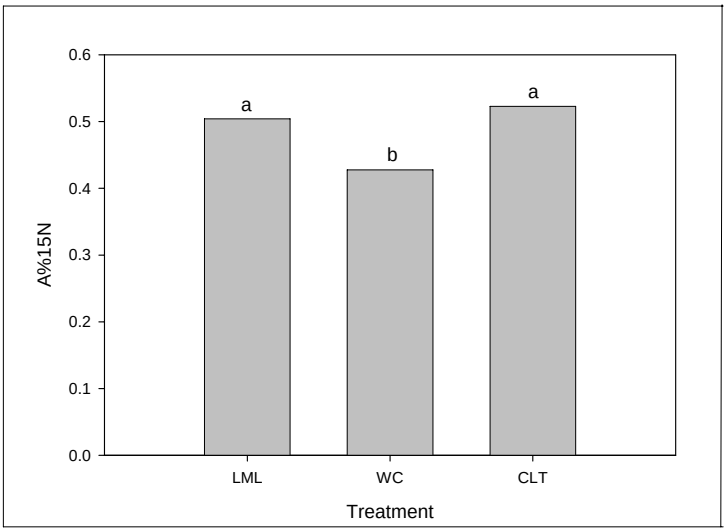

Figure 5. 2006 Tree Leaf ^{15}N abundance (averaged across application date).
* Bars with the same letter are not significantly different (P> 0.05; n=3).

Table 12. 2005 % Increase in Tree Cross Sectional Area (TCSA).

Treatment	Autumn 2005
CON	13.7 def
CHE	20.8 cd
BHE	24.8 bc
LML0.5	11.7 def
LML1	10.6 ef
LMNL0.5	5.6 f
LMNL1	8 f
LMNL1.5	11.6 def
WC1	28 abc
WC1.5	27.6 abc
CLT0.5	31.5 ab
CLT1	28.5 abc
CLT1.5	35.9 a
SWL	11.1 ef
SWNL	19 cde

* Means in the same column followed by the same letter are not significantly different (P> 0.05; n=5).

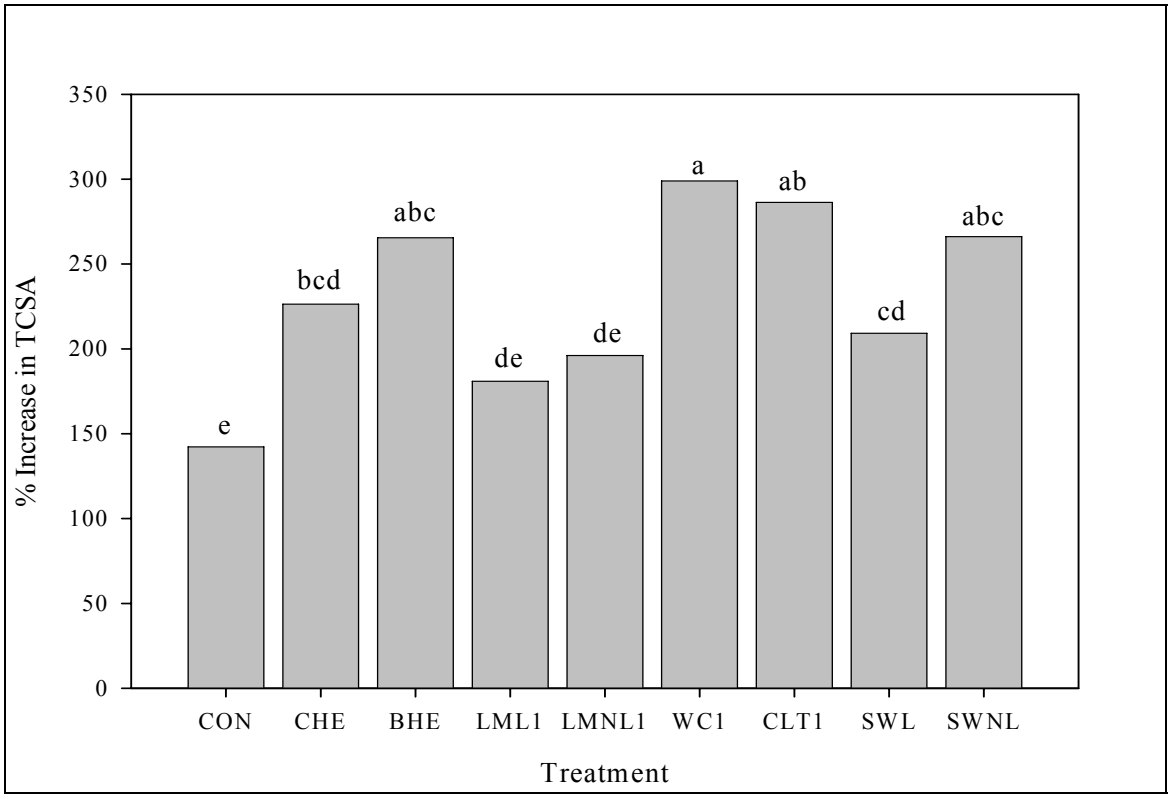

Figure 6. 2006 Percent increase in Tree Cross Sectional Area (1X treatments).
* Bars with the same letter are not significantly different (P> 0.05; n=3).

Appendix I

Plot map

Table A1. Plot map

Replicate 5		Replicate 4		Replicate 3		Replicate 2		Replicate 1	
75 CLT 0.5		60 BHE		45 CLT1		30 LMNL1		15 CLT1	
74 SWNL1	67 CLT1.5	59 CHE	52 LML1	44 LML0.5	37 LMNL1.5	29 CLT1	22 LMNL0.5	14 CLT1.5	7 LML0.5
73 BHE	66 SWL1	58 CON	51 WC1.5	43 LML1	36 LMNL1	28 CLT1.5	21 LML1	13 SWL1	6 LMNL1
72 WC1	65 LML0.5	57 CLT1	50 LML0.5	42 SWNL1	35 CHE	27 CON	20 WC1.5	12 LMNL0.5	5 WC1.5
71 CHE	64 LMNL1.5	56 CLT 0.5	49 SWL1	41 LMNL0.5	34 CLT 0.5	26 WC1	19 BHE	11 CON	4 BHE
70 CON	63 WC1.5	55 SWNL1	48 WC1	40 WC1	33 CLT1.5	25 SWNL1	18 LMNL1.5	10 SWNL1	3 LMNL1.5
69 LMNL0.5	62 LMNL1	54 LMNL1	47 CLT1.5	39 CON	32 SWL1	24 SWL1	17 CHE	9 CLT 0.5	2 CHE
68 LML1	61 CLT1	53 LMNL1.5	46 LMNL0.5	38 BHE	31 WC1.5	23 CLT 0.5	16 LML0.5	8 WC1	1 LML1

MANAGING ORCHARD UNDERSTORY AND ORGANIC AMMENDMENTS FOR NITROGEN FERTILITY

(*to be submitted for publication as an extension bulletin*)

Introduction

Organic orchards represent a significant and growing component of agriculture in the Pacific Northwest. Yet, meeting the nitrogen (N) needs of orchard trees with organic fertility amendments can be a significant challenge. Extensive information is available to help determine ideal timing and application rates for N fertilizer in conventional systems. However, most of this information relates only to synthetic fertilizers that contain N in readily available, plant accessible forms. In most organic fertilizers, these plant available forms make up only a fraction of the total N. The remaining N in organic amendments is released slowly as a result of decomposition by soil organisms. The speed of nutrient release from different organic amendments is dependent not only on the make-up of the amendment, but also site-specific soil, management, and environmental conditions. As a result, development of an organic N fertility management plan can be challenging and requires consideration of multiple factors.

The purpose of this bulletin is to help orchard managers confront the challenge of developing a site-specific organic N fertility management plan. The importance of soil organic matter content, organic amendments, and biological processes responsible for decomposition and release of nutrients are outlined. Strategies for the use of living cover crops, along with the nitrogen content and average availability of a range of commonly

used organic fertility amendments are included. Examples of fertility plans employed by several organic orchardists with extensive experience have been summarized. Yet, because an optimal organic N fertility management is site-specific, orchard managers are encouraged to experiment with on-farm research trials, and actively track the effects of different practices.

Healthy Soil as a Foundation

A healthy soil is the foundation of any sustainable agricultural production system, but this is particularly true for maintenance of adequate plant nutritional supply in organic orchards. In most conventional synthetic fertilizers the total N is supplied in forms that are immediately soluble and available to plants. Therefore nutrients can be applied in exact amounts during high nutrient uptake periods. In contrast, most organic fertilizers and amendments supply N slowly over a matter of months and years. Therefore meeting tree N needs in an organic system requires greater planning, and relies on soil stewardship. Plant available nutrients are released from organic amendments as a result of decomposition, which requires biological activity in the soil. There are few quick fixes to correct nutrient deficiencies in an organic system. Instead, producers must shift thinking from curative to preventative. The focus on an organic N fertility management plan will be long-term, and geared toward feeding the soil, which will in turn, feed the plant. While this strategy requires additional time and fertility amendments during establishment, over the long-term N fertilizer costs are reduced and fertility supply becomes more reliable.

Soil Organic Matter

Soil organic matter is fundamental to the concept of soil quality and nutrient supply in organic production systems. Soil organic matter is often compared to the soil's financial capital, because it contains a large pool of N much like money put away in the bank. Soil organic matter is made up of both living and non-living soil organisms, as well as plant and animal residues in various stages of decay. Scientists often divide soil organic matter into different fractions based on soil activity. The active fraction refers to material that is easily decomposed by soil organisms, releasing plant available nutrients. Materials more resistant to decay are called humus, and are responsible for maintenance of soil structure, and nutrient and water-holding capacity.

In the fruit-growing region of the Pacific Northwest, soil organic matter levels in native soils are generally quite low. It is common to find soil organic matter levels near 1%. Amending soil with plant residues, animal manures and/or compost can significantly increase levels of soil organic matter over time. Building soil organic matter enhances N availability and reduces N fertility costs over time. Each year, 2 to 5% of organic matter decomposes to release plant available nutrients. At a rate of just 2%, each one percent of organic matter could release as much as 20 pounds of plant available N per acre (Edwards, 1998).

Soil Nitrogen Cycle

Because N release from organic amendments and soil organic matter is dependent on biological cycling in the soil, understanding the N cycle will help to better predict availability for orchard trees. The majority of N in soil and organic amendments is bound

with carbon (C) and is unavailable for plant uptake. Through decomposition, a fraction of this N is converted into forms available for plant uptake. Soil organisms are responsible for this transformation. Large soil organisms work to shred the organic debris. Soil dwelling microorganisms then convert organic N into ammonium ions (NH_{4+}). This N conversion process is termed mineralization. A different set of microorganisms then transform ammonium ions into nitrate ions (NO_{3-}) through a process called nitrification. Both ammonium and nitrate ions are soluble in water and readily available to plant roots for uptake. Ammonium and nitrate released from organic matter and organic fertility amendments are the same forms of N contained in most commercially available synthetic fertilizers.

The rate of decomposition and N release from organic amendments is dependent on site-specific soil and environmental conditions. Decomposition will occur fastest under conditions of neutral soil pH, temperature of 95 F, and moisture near field capacity. In addition, the ratio between C and N in the residue will affect the rate of decomposition. Residue with a C:N ratio of less than 20-25:1 will usually decompose relatively quickly. In contrast, if the C:N ratio is greater than 25-30:1, N compounds can become temporarily immobilized, as soil microbes tie-up N in their biomass during decomposition.

In addition to the challenge of releasing ample plant available N to meet tree needs, all producers whether conventional or organic must understand and reduce sources of N pollution. If soil pH is alkaline, especially above 8.3, ammonium ions can be converted to ammonia (NH_3) gas and lost to the atmosphere through a process called volatilization. Alternatively, if soil is waterlogged, available N ions can be reduced to

gaseous forms of N and lost to the atmosphere via denitrification. Some of these gaseous N compounds are potent greenhouse gases associated with global warming. Excess nitrate ions not taken up by plants or microorganisms are subject to leaching, which can pollute local water systems and wells. Therefore, it is important to synchronize nutrient release with crop uptake patterns in order to maintain optimal productivity while protecting air and water quality.

Cover crops

Cover crops can be an economical means to build soil organic matter and supply N in agricultural production systems. Prior to the advent of synthetic fertilizers, cover crops were used extensively to supply N. Leguminous plants like clover, peas and alfalfa, living in symbiosis with specialized bacteria, convert atmospheric N into ammonium in the roots. Growth of a legume cover crop can contribute between 50 – 200 lbs N per acre. The rate of N-fixation is dependent on the plant species, its vigor, density and duration of growth, and the effectiveness of its bacterial partner. Plant residues from legume crops generally have a low C:N ratio and thus will decompose and release N relatively quickly. When plant residues from legumes are soil incorporated, up to 10 – 50% of N in their residue is released in just four to six weeks (Horwath, 2005).

Grass species also benefit N dynamics when planted as a cover crop. Because of their dense, fibrous root systems grasses are often planted as a 'catch crop' to capture excess soil nitrate that may otherwise be lost to leaching. Residues from grass species have a greater C:N ratio than legumes, and thus can greatly enhance soil organic matter content. However, some grass residues have C:N ratios as high as 40-80:1, which will

result in significant immobilization of plant available N as soil organisms work to decompose this residue. To avoid starving trees during this immobilization, soil incorporation of high C:N plant residues should occur at least 4 to 6 weeks prior to planting trees, or periods of high tree uptake. Shallow tillage should be practiced to prevent root damage and minimize erosion potential.

Deep-rooted cover crops like alfalfa provide additional N benefit through their ability to recover N and other nutrients from deep within the soil profile. In addition, cover crops benefit production systems by aerating soil, breaking up hard pans, reducing weed and disease pressure, and providing habitat for beneficial insects and soil organisms. However, while cover crops are ideally suited to improving N dynamics in annual production systems, their use in perennial orchards systems can be more challenging.

Cover crops can be used as a 'green manure' or as a 'living cover'. A green manure is killed and incorporated into soil, as an amendment while the crop is green and rich in nutrients. These nutrients are released to the main crop. Incorporation of large amounts of fresh plant matter also affects the soil biological community, and can cause temporary increases in pathogen populations.

In perennial orchard systems, cover crops are generally planted as a perennial companion, or 'living cover'. Living covers provide multiple benefits to orchard agroecosystems. These include N provision and retention, organic matter enrichment, better soil structure, more soil life, weed suppression, less tillage and fossil fuel use, less phthophthora, and provision of habitat for a wide variety of beneficial organisms.

Living covers also have potential drawbacks. Living covers growing in close association with tree roots can compete with the trees for nutrients, water and space. Competition can severely reduce growth in young trees that have shallow, limited root systems. Leguminous living covers may increase soil N supply both when N is needed and when it is not wanted. In fruit tree systems, high soil N late in the growing season can delay crop maturity and result in immature fruit that does not store well. In addition, many living cover crops harbor rodents that can damage tree bark or roots. Applying mouse guards or repellents at the base of each tree can help to reduce rodent damage.

In spite of these issues, living cover crops can be successfully used in orchard systems to improve soil quality and reduce N fertility costs. Yet, their use requires careful management consideration, and because of the difficulties they are not currently advisable during orchard establishment. To assist orchard managers, a number of production strategies have been designed to help achieve the benefits living covers provide while minimizing competition with fruit trees.

Soil building prior to tree establishment

If economically feasible, orchard managers can use cover crops to prepare a future site for orchard establishment. Growth of cover crops for 2 to 3 years can result in substantial soil quality improvement. When preparing to replant trees in an existing orchard, cover crops can be used to build soil quality in the drive row, where new trees will be planted. Inclusion of cover crops prior to new tree establishment can help disrupt soil pathogens responsible for tree disease. For example, growth of certain wheat

cultivars can reduce the impact of soil organisms known to incite replant disease (Gu and Mazzola, 2003).

Plant residue mulch

Living cover crops can be planted into the drive row of an existing orchard. Living cover residues are routinely cut and physically transferred to the tree row in a strategy often called 'mow and blow'. In a Michigan study that compared mow and blow, herbicide and cultivated soil treatments, the mow and blow system was the most cost-efficient and resulted in the highest fruit yield (Edson et al., 2003). Researchers in the Netherlands used this strategy to contribute up to 2 tons of dry matter and 50 lbs N per acre to young fruit trees with a mixture of grass and 10% clover (Bloksma, 2000). However, over time clover tended to out-compete grass species, and high clover density led to trafficability problems. As a result, the researchers recommended removal of the cover crop when clover compromises 50% of the total plant clover.

The use of plant residue as mulch will provide numerous benefits to orchard systems. Plant residue mulch has been found to increase soil organic matter, improve water infiltration and retention, and reduce weed pressure in tree fruit systems. In addition, using the mow and blow approach with living cover can help eliminate the issue of excess N late in the growing season. Residues with high N and a low C:N ratio can be physically removed from the system during critical fruit development periods. In addition, residues with a high C:N ratio could be applied during critical fruit development periods to help tie-up and immobilize plant available N compounds. Although, bringing in plant residues from off of the farm can be expensive.

Living cover in tree row

Establishment of living cover crops in the tree row is an efficient way to produce and sequester N to help meet fruit tree needs. One strategy is to establish living cover late in the summer when soil N is in excess for proper fruit development. Desirable living cover species can be planted, or producers can simply let weeds establish. However, weed seed heads should be cut before they mature. With this approach, living cover is maintained throughout the winter and early spring, and removed at the start of the next growing season. Living cover species can be removed using soil cultivation, severe mowing or flaming. Alternatively, plant species like Subterranean clover will reseed itself in early summer, die back during intense summer heat, and reestablish in the autumn. This strategy will help reduce excess soil N during critical fruit development periods and reduce leaching of excess soil nitrate during winter when plant uptake and microbial activity are minimal.

Another desirable strategy involves establishment of limited cover in the tree row. Leguminous clover islands can be established directly around the base of each tree to help supply N and limit establishment of grass species that are more competitive with fruit trees. Alternatively, scientists at the international organic research institute (FiBL) in Switzerland have had success with a sandwich system. With the sandwich approach, cover is limited to the interior 30 cm of the tree row. Soil area in the tree row not planted to cover is kept free of vegetation to help limit competition with orchard trees.

In mature irrigated orchard systems where trees have deeper more extensive root systems, living cover can be established over the entire tree row. Researchers in

Michigan found living cover in the tree row to contribute multiple soil quality benefits, yet the living cover did not compete with orchard trees or lead to any reduction in fruit yield or quality (Edson, 2003). Beginning with a legume-dominated mixture is desirable, as grass and weeds will slowly take over. Living cover crops should be routinely mowed to no less than 4 inches. The greatest contribution of N will occur when the legume is cut at 25% bloom, or prior to seed set in grass species.

Organic Fertility Amendments

A number of biological based amendments are commercially available to help supply N and build soil organic matter. These amendments are made from a variety of different animal waste products or byproducts of processing industries. The nutrient composition and concentration of these amendments vary considerably, from less than 1% to greater than 12% N. However, unlike synthetic fertilizers, only a fraction of the N in these amendments will mineralize and become available for plant uptake during the current growing season. In addition, release of plant available N from different organic amendments can vary by orders of magnitude. Variability in total and available N in organic amendments results from the nature of the materials used, and how they were processed. Like organic residues, the rate of decomposition and release is dependent upon the C:N ratio, the complexity of C and N bonds, and inherent soil and environmental conditions. Additionally, the method used to process the material will affect release rates. Table 1 lists the average nutrient concentration and availability of many commonly used organic amendments, along with urea, a commonly used chemical fertilizer.

Table 1. Average concentration and % availability of common organic fertility amendments (McLaurin and Reeves, 2000).

Amendment	N	P_2O_5	K_2O	Relative availability
Alfalfa Meal	3	1	2	med-slow
Blood Meal	12-15	1.5	0.6	med-rapid
Bonemeal (steamed)	0.7-4.0	11-34	0	slow-med
Compost	2	1	1.5	slow
Feather Meal	10-15	0	0	slow
Fish Emulsion	3-5	2	2	med-rapid
Guano (peru)	12.5	1.2	2.4	medium
Kelp	0.9	0.5	1-4	slow
Poultry Manure (fresh - 30% water)	3	2.5	1.8	med-rapid
Cattle Manure (fresh)	0.25	0.15	0.25	med-rapid
Urea	42-46	0	0	rapid

Estimating the amount of plant available N that will be released from an organic amendment during the current growing season can be difficult. Laboratories that routinely analyze soil samples can perform tests to develop a rough estimate. However, actual availability in the field will vary considerably based on soil and environmental conditions. Therefore, it is helpful to track release indirectly by monitoring soil N, plant tissue N, and yield. For more information on how to track N movement, see the section below on developing an organic fertility plan.

Many manure and compost amendments can contain a significant amount of moisture. To determine the real cost of the product, the moisture level needs to be accounted for. Laboratory analyses can be used to determine the percent moisture of the product, but this can also be estimated at home. To determine percent dry weight, spread out 10 pounds of the product, allow it to air dry, and then reweigh it when dry. Divide the end weight by the beginning weight, and multiply by 100. The real cost is then

calculated as the cost per unit of nutrient (with moisture correction) multiplied by the number of units needed for the crop.

The majority of organic fertility amendments on the market are suitable for use in certified organic production systems. However, some amendments can contain unknown ingredients or contaminants, or they may have been processed in a way that prevents their use in a certified organic system. Therefore, it is advisable to check with your certifier or the OMRI materials list before use to ensure approval for use in certified organic systems. In addition, some amendments may contain appreciable levels of salts, heavy metals or boron that can harm crop plants. Consequently, careful evaluation of potential fertility amendments is encouraged to help maintain a sustainable, healthy production system.

Compost

Compost amendments can be an economical way to supply N in an organic production system. Compost may be made from a variety of animal and plant materials. The composting process stabilizes nutrients, resulting in slow release of plant available compounds, much like soil organic matter. Compost amendment can contribute significantly to soil organic matter content and beneficial soil microbial communities responsible for nutrient cycling.

The total N content of compost can range from between 0.8 to 4.2%. For example, compost made from cattle manures may contain less than 1% N, while those made from chicken manure can have N concentrations greater than 4%. Variability in the concentration and availability of N can also result from the processing method, moisture

content and maturity of the compost. Mature compost will be relatively stable when most biological activity has ceased. Compost that is mature will have a dark color, be at or near air temp, and have an earthy smell with no detectable ammonia. The mineralization rate for release of plant available N will vary from 10 to 50% during the initial growing season. The remaining N in compost amendments will continue to be released over a period of 2 to 3 years. Relative N availability of different compost amendment can also be estimated based on C:N ratio.

While compost is an effective amendment to meet tree N needs, prolonged use can lead to problems. In addition to N, compost amendments contain appreciable amounts of phosphorous (P), potassium (K), and salt. Yet, because the concentration and availability of N in compost is so low, orchard managers often apply between 2 to 4 tons per acre to meet tree N needs. Because plant P needs are relatively low, excessive levels of soil P can build-up. Phosphorous enrichment can reduce the availability of other plant nutrients like zinc. In addition, erosion of soil high in P can pollute local water systems. Soil K seldom builds to levels that can injure plants, yet it can contribute to soil salinity. High soil salinity can damage plants and disrupt soil aggregates responsible for soil structure. A good soil structure is necessary to anchor trees and facilitate water and air movement near tree roots. Salt build-up is of particular concern in arid land with high evaporation rates. Flushing soil with high quality irrigation water can help to move salts through the soil system.

Manure

Animal waste products are an important source of plant available N and soil organic matter. The total N concentration in manure ranges from between 1 and 5%. Variability in N concentration is dependent on the animal, and whether the material is fresh, or aged. Because manures generally have a relatively low C:N ratio, their N compounds tend to mineralize and become plant available relatively quickly. In addition, fresh manure can have appreciable levels of ammonium compounds that are immediately available for plant uptake.

While animal manures are an excellent N fertility resource, there are issues regarding their use in agricultural production systems. Unless manure is immediately incorporated, ammonium compounds are subject to volatilization. In addition, like composts, fresh manure can also contain high levels of P and total salts. Direct application of ammonium and other salts to plant roots can harm orchard trees. Further, because manures are high in moisture, the cost of transportation can become excessive. Finally, there are strict guidelines regarding the use of manure in certified organic production systems. Under the national organic program standards, fresh manure must be soil incorporated within 90 days of harvest if the fruit is not in contact with the soil. As a result, orchard managers must plan ahead and be aware of these issues when including manure in their fertility plan.

Poultry litter

Poultry litter consists of a mix of bedding materials, feathers, and waste products leftover from broiler operations. The product tends to have a relatively high amount of

both total N (4-5%) and ammonium compounds. Poultry litter is usually marketed as having been composted, but is often times found to be less than mature. As a result, this product tends to decompose and release plant available N relatively quickly. The material will often have an ammonia smell, resulting from the high amount of N present in the form of uric acid in the poultry manure, or because it is not fully composted. While poultry litter is an exceptional source of readily available N, orchard managers should be careful to avoid direct contact with plant roots and incorporate the material to reduce loss through volatilization.

Blood meal

Blood meal is an animal byproduct with a significant amount of both total and plant available N. These products generally contain approximately 12% total N, and much of this is in the form of ammonium. As such, this amendment is often used under emergency situations when corrective action is needed to correct a deficiency. However, this product is relatively expensive. Because of the high ammonium content, it is subject to volatilization, and may burn plant roots. In addition, there has been some concern over its use because of food safety and disease issues. Blood meal products have been shown to promote fungal growth and may contain harmful pathogens.

Feather meal

Feather meal is a byproduct of the poultry industry with significant total N that ranges between 7 and 10%. Unlike other organic amendments with high total N, the N in feather meal is tightly bound with carbon compounds. These N compounds are not easily

decomposed and so the availability of feather meal N tends to be low. This product will help to build soil organic matter, but it will not contribute as much plant available N as other similarly priced products.

Fish meal and emulsion

Fish meal is an animal byproduct that is rich in N at around 10%. The meal is commonly used as a feed additive, while the emulsion is more often used as a fertilizer amendment. The emulsion has been digested by either phosphoric acid or special enzymes, to yield a product with around 4% total N. The N compounds in fish emulsion tend to have relatively high availability, but this resource can be expensive in comparison to other amendments. It is often applied as a foliar amendment to help supplement nutrient needs.

Kelp and Seaweed

Kelp and seaweed based amendments are a relatively low source of N at only 1%. These products are also thought to contain plant hormones and growth regulators that stimulate plant growth. As a result, while application of kelp and seaweed products are not likely to correct a nutrient deficiency they are often used as a "tonic" in foliar sprays. In addition, they contain small amounts of P, K, Mg, S and trace minerals. These products tend to be an expensive source of N per unit weight.

Brassicaceae seed meal

Brassicaceae seed meal is a byproduct of the extraction process for bio-diesel production. High-oil seed from a variety of canola and mustard plants are crushed to remove oil, and the remaining seed meal is utilized as animal feed or as a soil amendment. Brassicaceae seed meal is high in total N (6%), and, because it has a low C:N ratio, this N will usually mineralize and become plant available relatively quickly. Seed meal from certain Brassicaceae species can also supplement weed and disease suppression. A mixture of *B. napus* (Canola) and *B. juncea* (Indian mustard) is highly effective at controlling soil organisms known to incite replant disease (Mazzola et al., 2006). In addition, soil amendment with *B. napus* and *S. alba* seed meals have been shown to result in weed suppression. *S. alba* seed meal tends to be the most effective seed meal for weed control, yet early season applications can reduce iron availability to trees. Therefore, careful management is recommended when using Brassicaceae seed meals as a soil amendment.

Chilean sodium nitrate

Under organic certification guidelines, the use of Chilean sodium nitrate is currently authorized on a regulated status, but its use is under scrutiny. This natural amendment is derived from Caliche, a nitrogenous rock found mostly in northern Chile. It contains a high amount of plant available N, but its use is generally authorized only under emergency situations to correct a deficiency. Because this amendment contains significant levels of sodium, there is concern over the adverse effects of its long-term use on soil quality and soil aggregate stability.

Biosolids

Biosolids are not currently approved for use under certified organic production systems. However, this byproduct of municipal sewage systems has appreciable amounts of total N (3-6%) which can contribute to N fertility needs in systems that are not certified as organic. However, there are several concerns associated with this material, including heavy metal, chemical and human pathogen contamination. In addition, consumer preference may limit their use in many food based production systems.

Microbial inoculants

A number of commercially available microbial inoculants are available to help supplement plant available N. These inoculants generally contain one or more microorganisms in dry or liquid form. The most well-known and commonly used are Rhizobium-based. These soil dwelling microorganisms form the mutually beneficial, symbiotic relationship with plant legumes to fix atmospheric N. The plant-rhizobial interaction is very species specific. Therefore, to achieve optimal N-fixation, producers will need to apply the appropriate Rhizobial species for the specific legume species. While these microorganisms can survive for many years without a plant host, soil should be inoculated if the given legume species has not been grown within the past 10 years.

Impact of orchard floor weed management

Controlling weeds in the tree row is essential to minimize competition with young orchard trees. In conventional systems, orchard managers effectively control

weeds with synthetic herbicides. In the absence of reliable herbicides approved for use in organic systems, orchard managers often use extensive soil tillage to control weeds. While tillage can be an effective tool for weed control, incorporated organic amendments and aerating soil, too much tillage can damage soil. The disturbance of tillage increases microbial activity, and therefore increases organic matter decomposition. This results in short-term nutrient release, and increases potential for nutrient loss. Extensive soil tillage reduces long-term nutrient storage and other measures of soil quality. Beneficial soil fauna such as earthworms and their burrows are destroyed by tillage. Loss of these organisms can slow nutrient cycling. Finally, tillage breaks soil aggregates and in the long-term degrades soil structure. Deeper and more frequent tillage will intensify these negative effects, while occasional shallow tillage will minimize them. Other weed control measures such as severe mowing, flaming or herbicides approved for use in organic systems have little effect on soil quality.

Application of weed suppressing organically based mulch products (plant residue, paper, fabrics or wool) or growth of a living cover understory will reduce disturbance and potentially improve N fertility dynamics. Mulch and living cover enrich soil with organic matter and beneficial soil nutrient-cycling fauna. This will reduce N fertility needs and result in a system that is more compatible with organic fertility management. In addition, application of residues with high C:N ratios can help to boost soil structure and long-term nutrient and water holding capacity.

However, while these reduced disturbance strategies can benefit N dynamics over time, they may initially increase N fertility amendment needs. For example, without soil incorporation, decomposition and nutrient release from amendments will likely be

reduced. In addition, unincorporated amendments will be more susceptible to nutrient loss via volatilization and surface erosion. Living cover crops and uncontrolled weeds will compete with young orchard trees. Alternatively, plant cover will immobilize N, and reduce amendment needs over time. Orchard managers must evaluate the trade-offs of these alternative strategies and decide on a management plan that will accomplish their multiple orchard management goals.

Developing an Organic N Fertility Plan

Developing an organic N fertility program for a tree fruit system is a challenging process that will take several years of experimentation to establish. Fertility programs will be affected by the local availability of different organic amendments. Decomposition and release of plant available N from organic fertility amendments will be dependent upon site-specific soil, environmental, and management conditions. In addition, fruit trees store excess N in above and below ground woody tissues. As a result, changes in fertility management programs conducted on mature trees may take several years to become apparent. Because of these conditions, there is no one specific formula that can be used to meet orchard tree N needs. Instead, producers much evaluate alterative amendments, rates, and management practices to develop their own site-specific management plan.

Monitoring soil quality

Soil quality improvement is not only desirable for organic N fertility dynamics it is also a requirement of organic certification. However, quantifying soil quality is

difficult. The definition of soil quality depends on the context, and may change given requirements of different agricultural systems. It is also difficult to relate measurable indicators to a specific function or management goal. Despite this ambiguity, there are some general indicators that can be used to monitor the health or quality of soil over time.

A number of laboratory tests can be conducted to determine both total and potentially available pools of N and organic matter. In addition, tests can be done to determine the abundance and activity of soil fauna responsible for nutrient cycling. Yet, these types of test can be expensive. In contrast, there are a number of procedures that can be conducted in the field to help monitor soil quality. The USDA has developed a soil quality test kit that uses many common or easily obtained materials to conduct a variety of tests on the farm. These include water infiltration, water-holding capacity, pH, soil nitrate and aggregate stability. There are also even simpler, more qualitative ways to measure and track changes in soil quality. For example, dig a hole and make general observations. Does soil feel soft and crumble easily, does the soil have a rich earthy smell, or, does it soak up rain and result in little runoff? A number of other helpful evaluation tools are listed on the Soil Quality Institute's webpage listed in the resource section below.

On-farm research trials

Conducting your own research trial is the best way to evaluate alternative practices and develop an optimal site-specific N fertility management plan. Decide what your different treatments will be. For example, you may want to evaluate different fertility amendments, or you may want to determine the optimal rate of a given

amendment to meet your tree's N needs. But remember to keep it simple, and test only one question at a time. The fewer experimental variables you have, the more likely you are to identify reliable differences among treatments. Be sure to include a control or check plot in your experiment. The most logical choice for the control is your standard practice. Make sure to replicate the experiment by repeating each treatment at least three times. In addition, randomize by mixing up individual replicate plots. For example, do not follow the same pattern of Treatment A, followed by B, and then C in a given tree row.

The first step to implementing your experiment will be to locate a uniform experimental area. For example, the area will be characterized by uniform soil conditions, slope, and tree cultivar and age. If a uniform area that will accommodate all treatments and replicates is not available, use several locations each with one plot of each treatment. For example, one set of treatments will be placed at the bottom of a hill, while the other set is up higher in a drier area. Clearly mark off plots that contain at least three to four trees to represent each individual treatment. Make sure to allow at least one to two trees between each treatment plot to act as a buffer. Carefully monitor and record observations of soil and tree health. When you have collected data from the experiment, use the statistical tools in worksheet programs or WSU's on-line program at http://pnwsteep.wsu.edu/onfarmtesting/. See the research section below for more helpful publications on conducting on-farm field trials.

Orchard nitrogen requirements

The typical N needs of orchard agroecosystems are lower than most other agricultural crops. Once an orchard becomes established, N needs are relatively low because little N leaves the system in fruit harvest. Thus, when soil quality is high, N fertility can be maintained with as little as 60 to 100 lbs available N per acre. Alternatively, tree N needs can likely be met with cover crops and mulches alone once established. However, during orchard establishment and when a system is in transition to an organic production strategy, N needs are much greater. Additional amendment is required to help build soil organic matter and soil biological communities responsible for nutrient cycling. Young trees also need greater amounts of N to stimulate root and vegetative growth.

Orchard trees take up the greatest amount of plant available N prior to petal fall in the spring and after leaves fall in autumn. Nitrogen reserves established in autumn will contribute to early season growth the following spring, while N needs of plant organs will result from soil N taken up during the current growing season. High levels of plant available N spring early in the spring will encourage vegetative growth in young trees. Yet, when tree begin to bear fruit, excessive soil N can lower fruit yield and poor fruit quality. A number of textbooks and extension publications are available to obtain more information on tree fruit nutrition. Because nutrient release from organic amendments is so low, they should be applied at least 1 to 3 months prior to periods of high tree uptake. Application of organic amendments post-harvest after trees stop growing and set terminal buds in desirable. This generally occurs from mid-August to mid-September.

Soil tests

Regular soil tests are an essential tool for optimizing an organic N fertility plan, and they are a requirement of organic certification. Soil tests can be used to help determine residual soil N levels so that amendment levels can be adjusted accordingly. However, because they only tell you the amount of nutrient present at the time of sampling and not what will become available, they should be combined with field observation and plant tissue tests. Soil tests are recommended every 3 to 5 years, but if you are conducting an on-farm research trial you many find it helpful to test every year.

A minimum of 7 to 10 samples should be collected from an area with similar characteristics. Remove the organic debris on the soil surface and sample soil to a depth of 10 inches. This is where the majority of feeder roots of fruit trees are located. Mix the soil samples together and allow the composite sample to air dry in a cool space. When dry, collect a sub sample to send in to the lab.

Plant analyses

Simple visual observations of tree health throughout the growing season can tell you a lot about N fertility levels in an orchard system. Lush tree growth and delayed flowering and fruiting can signify an over-availability of soil N. In contrast, poor yields, pale green leaf color, slow branch elongation, and low overall tree growth signify N deficiency. Yet, leaf nutrient analysis is the best way to track changes in nutrient supply and will identify excesses or deficiencies well ahead of visual symptoms. Mid-July through mid-August is the best time to collect tree leaves for nutrient analyses. Collect matures leaves from random trees at the mid-section of current season terminal shoot

growth, approximately 5-7 feet above ground. An average of 2 to 3 leaves should be collected from each tree for a minimum of 50 total tree leaves. Allow the leaf sample to air-dry and then submit it to a laboratory for analyses. Desirable leaf N in pome fruit range from 1.8 to 2.6 %, desirable levels in stone fruit are higher at between 2.6 to 3.5%. Desirable leaf N values in young non-bearing fruit trees will be greater than in mature trees. For example, the ideal range of leaf N in young non-bearing applies trees is 2.4- to 2.6%.

Sample organic N fertility management plans

To assist orchard managers in developing their own site-specific N management plan, interviews were conducted with orchard managers and industry representative that have extensive experience in organic orchard production. These interviews were summarized to include current management practices as well as indicators used to track fertility and soil quality.

Example A.

Previous experience with a mow and blow system worked well. Alfalfa was planted in the drive row during orchard establishment and the residue was routinely mowed and transferred to the tree row. Gypsum was mixed into irrigation water to help alfalfa compete with grasses, and all tree N needs were met with this system. However, the labor and fuel needed to sustain this system became too great. In addition, in some cases N availability became too high and trees had low bloom strength and produced small fruit.

The new approach consists of a fall planting of a 50/50 mix of legume (Dutch white or New Zealand clover) and grass species in the drive row. Every other row is mowed down to 4 inches when the cover reaches approximately 6-8 inches high. The other row is left to harbor beneficial insects. Tree N needs in apple trees are met with a yearly chicken compost amendment rate of 1500-3000 lbs/ac in young trees, and 1500-2000 lbs/ac in mature trees. In cherries, amendment rate is generally 2 tons per acre. Half of the compost amendment is applied in the spring and the other half is applied in autumn. In addition, 5 to 10 gallons of fish emulsion is applied per acre each year to help stimulate microbial activity. When N deficiency becomes apparent additional fish emulsion is infected directly under the tree. Tree leaf color and overall growth is continually monitored and additional fish emulsion is applied as a foliar spray if leaves start to shut down. Soil tests are routinely performed. The type of weeds growing on the orchard floor is used to help judge nutrient levels in the soil. The look and feel of the soil as well as the density of earthworm populations are used to monitor soil quality.

Example B.

Nitrogen needs vary substantially based on location and tree age. Amendment rates are based on tree health and vary from 1 to 10 lbs of chicken compost per tree. Most compost application occurs in the fall, but if trees are young or deficient, extra compost is applied in the spring. In addition, 60 to 200 lbs of Feather meal is applied per acre, again depending on tree needs. Because organic matter levels tend to be naturally high, soil cultivation is used extensively during establishment, but the tree floor is mowed in older blocks. Alfalfa cover has been tried, but it tends to become choked out by

grasses. Tree growth and health are used to monitor N needs. Soil samples are routinely performed to check nutrient and organic matter levels.

Example C.

Living cover crops work well in cherries where N needs are greater and traffic through the orchard is lower. Chickweed is planted in the tree row and mowed regularly. Vetch is fall planted in the drive row and turned under in the spring. The cost of seed for alfalfa is cheaper than vetch, but it does not supply as much N. Trees are supplemented with compost and blood meal under conditions of severe deficiency. In apple orchards, chicken manure compost is applied yearly at a rate of 3 tons per acre in young trees, and 1 to 2 tons per acre in mature trees. Compost amendments are split, with 1 ton applied in the spring, and the other 1 to 2 tons applied in the fall approximately one month prior to harvest. Laboratory analyses of soil, leaves and fruit are conducted regularly, but tree health is monitored continually to assess orchard fertility. The quality of the soil is monitored by evaluation of soil tilth, the smell of the soil and the quality and health of orchard trees.

Example D.

Yearly application of 1 to 2 tons per acre of chicken manure based compost is the most cost-effective way to build soil organic matter and meet tree N needs. Late August to mid-September is the best time to apply compost amendment to ensure N availability in the spring. However, over 5 to 6 years, salt levels can build-up from continued compost amendment. At this point, fertility needs are generally lower, and protein based

sources of N like Feather meal become the next most cost-effective way to supply N. Blood meal is used to correct severe nutrient deficiencies, but this is becoming rare. White Dutch clover can supply a lot of N as a living cover, but the orchard floor becomes slick and makes traffic difficult. Soil and leaf nutrient analyses are performed regularly and leaf color is monitored throughout the season. Soil quality measurement is based on organic matter level, soil tilth, pH, infiltration and intuition.

Example E.

Mint compost has become a readily available source of organic N amendment in the Pacific Northwest. Mint compost tends to have relatively low total N concentration, but it can help to significantly increase soil organic matter levels. To meet tree N needs, yearly application of 4 to 5 tons of mint compost is applied per acre, along with 400 to 500 pounds of feather meal to help supplement N and bolster microbial activity.

Resources

- **Appropriate Technology Transfer for Rural Areas (ATTRA) – National Sustainable Agriculture Information Service** http://www.attra.org/ This site features a multitude of excellent short publications on all aspects of sustainable and organic agricultural management. Specific publications regarding organic fertility management, organic amendments, soil quality and organic tree fruit production are available.
- **Cascade Analytical Inc.** http://www.cascadeanalytical.com/ Laboratory located in Wenatchee, WA, offers a wide range of soil and plant testing procedures.

- **Good Fruit Grower** http://www.goodfruit.com/ This regular publication provides a number of excellent articles focused on fruit production.
- **Organic Materials Review Institute (OMRI)** http://www.omri.org/ Provides up to date information on materials that are approved for use in certified organic production systems.
- **University of Idaho – Analytical Science Laboratory**. http://www.agls.uidaho.edu/asl/index.htm Laboratory located in Moscow, ID offers a wide range of soil and plant testing procedures.
- **University of Illinois – On-farm Research Guidebook** http://web.aces.uiuc.edu/vista/pdf_pubs/GUIDEBK.PDF Guidebook provides extensive information on how to design and carry out on-farm research.
- **USDA National Organic Program** http://www.ams.usda.gov/nop/indexIE.htm This is the official website of the national organic program which sets minimum standards for organic certification standards.
- **USDA-NRCS Soil Quality Institute - Soil Quality Assessment** http://soils.usda.gov/sqi/assessment/test_kit.html Website provides extensive information on how to measure soil quality that include the USDA test kit, scorecards and simple visual observations.
- **Washington State University - On-farm testing resources** http://pnwsteep.wsu.edu/onfarmtesting/ Website provides tips on how to design and carry out on-farm research projects as well as a simple statistical analysis program.

- **Washington State University – Tree Fruit Research and Extension Center** http://www.tfrec.wsu.edu/ Website provides number of links to tree fruit nutrition, soil fertility and organic and integrated tree fruit production.
- **Washington State University – Organic Nutrient Management and Water Quality** http://www.puyallup.wsu.edu/soilmgmt/Default.htm Website has extensive information on availability of different compost amendments.

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Appendix I

Orchard manager/industry consultant questionnaire

Grower/Consultant Questionnaire

General

- How long have you been involved with organic orcharding?
- Reasons for transition to an organic system?
- Difficulties encountered during transition?

Orchard Floor Management and Nitrogen

- What is your general orchard floor management strategy, and does it change with the age of the orchard? (replant issues?)
- Have you tried or plan to try any alternative strategies?
- What is the source and method of application with respect to Nitrogen?
- How much nitrogen is applied per year and when?
- If comfortable, what is the price you pay for your N source?
- How do you evaluate N source alternatives? (tests, economics, etc.)
- What might help you in this decision?
- What indicators do you use throughout the season to judge proper N levels?
- Do you plan to try any alternative N strategies in the future?
- Thoughts on use of legumes for N, pros and cons of integration?
- How do you (particularly if using legumes) avoid late season N problems? (ie. Fruit staying green)
- Will the rising cost of fuel influence your Nitrogen management?

- What are the biggest challenges you face in organic orcharding, and specifically with regard to N management?

- Do you see N management as a barrier to adoption or continuation of organic orchard management?

Soil quality

- How do you define soil quality?

- Do you manage for soil quality? If so, how do you measure it?

Information exchange

- Where did you learn your strategies (Family, Neighbors, School, Internships, Consultants, other)?

- Where do you get your information (extension, consultants, researchers, meetings, field days, other)?

- Are organic orchard field days available? If not, would you find them helpful?

- In general, what are future research areas that you see of value to you, how could WSU researchers help you?

GENERAL CONCLUSIONS

Weed control and nitrogen (N) fertility are substantial management challenges in organically managed, perennial orchard agroecosystems. None of the orchard floor management strategies applied in these experiments were able to meet the multiple objectives of weed control, soil quality improvement, N supply, and production of healthy orchard trees. However, findings from these studies have contributed an extensive amount of information that can be used to offer practical advice to orchard managers and guide future research programs.

An effective organic orchard floor management strategy will likely be site-specific and will likely consist of a combination of different practices that vary given orchard age. During orchard establishment, when weed growth is high and young trees are particularly susceptible to competition, soil cultivation will continue to be the best approach for effective weed control. However, because of the negative impacts of soil cultivation on soil quality, long-term use is not advised, and we must continue to search for alternatives. Application of clove oil herbicide and wood chip mulch is not recommended for use during orchard establishment, but may hold more promise in mature orchards.

Soil amendment of BSM can be used to provide weed and disease suppression, supply N, and build soil quality during any orchard stage, but seed meal type is an important consideration. Our results indicate that a microbial mechanism is likely involved in SM induced weed suppression and that selective enhancement of resident pathogenic *Pythium* spp. can be utilized for the purpose of weed control. *Sinapis alba*

seed meal is very effective at weed control, but also results in a temporary reduction in soil iron availability and can negatively impact orchard trees. However, because *S. alba* is so effective at weed suppression, further research to evaluate application times that do not result in negative tree impact is warranted. *Brassica napus* seed meal amendment is not as effective as *S. alba* at weed suppression, but it has very low GC concentration and will not likely impact soil iron availability. Since weed suppression by BSM is only temporary, periodic applications at a lower rate may be more desirable than a few, heavy applications. Brassicaceae seed meal amendment will not control all weeds and may work better for weed control in mature orchards or in combination with other weed control strategies.

Establishment of living cover crops in the tree row has the potential to provide multiple benefits to orchard agroecosystems. Soil quality improves rapidly in the presence of living cover crops, N availability and retention are enhanced, and weed species are suppressed. However, because living cover can compete with young orchard trees and result in low tree growth, it is not recommended during orchard establishment. Amending soil with low C:N crop residue, blown in from the drive row or brought in from outside the orchard, may help to achieve many of the benefits that living cover provides with less negative impact to orchard trees. Temporary removal of living cover in the spring during critical tree nutrient uptake may allow living covers to become a desirable strategy in young orchards and permanent cover may provide benefits without tree impact in mature orchards.

During orchard establishment, young fruit trees need high amounts of plant available N at specific times during spring and autumn. Because compost amendments

tend to be the cheapest source of N in organic systems, producers often apply substantial amounts to meet tree N needs, but this can cause problems. Application of organic fertilizer amendments with high total N and rapid mineralization may be a more desirable way to meet young tree N needs, despite higher cost. As trees age and N needs become lower, or as soil quality is improved by desirable management practices, modest amounts of slow-release composted amendments can likely be used to meet tree N needs without negative impact.

An optimal orchard floor management strategy will be dependent upon local availability of different organic amendments as well as site-specific soil, environmental, and management conditions. Because of these conditions, there is no one specific formula that can be used to supply N and control weeds. Instead, producers must evaluate alterative amendments, rates, and management practices to develop their own site-specific management plan.