EFFECTS OF ORGANIC AND CONVENTIONAL MANAGEMENT ON PLANT HEALTH AND SOIL BIOLOGY IN BLUEBERRIES

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ABSTRACT

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Three studies were conducted to determine the effects of organic management on plant health and soil biology in blueberry production fields in Michigan. The first study evaluated the effects of mulches, cover crops, and organic fertilizer on microbial populations in soil. Significant treatment effects were found, which indicates that microbial populations are responsive to cultural practices and soil inputs. This information may be used to enhance populations of naturally occurring beneficial microbes in soil. Cultivable soil fungi, bacteria, and beneficial microorganisms were positively correlated with soil pH and appeared to be suppressed at the low pH range of blueberry soils. In a second study, we assessed mycorrhizal colonization and soil biology in organic and conventional production fields. Sharp contrasts were observed in N:P enzyme ratios, labile C and N pool sizes, and mycorrhizal colonization, and organic fields had higher values for all of these parameters. Lastly, we evaluated organic options for control of fruit rot and cane diseases in three separate on-farm trials. Promising organic disease control methods were identified, but none matched the control provided by standard synthetic fungicide treatments. These studies will provide a background for further research into improved organic blueberry production methods in Michigan.

Dedicated to my Grandpa Fritz and the blueberry growers of Michigan (and Northern Indiana).

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Northern highbush blueberries and organic production: review and synthesis

Abstract

Northern highbush blueberry (*Vaccinium corymbosum* L.) is a crop well adapted to edaphic and climatic conditions in Michigan, where production area has grown to nearly 20,000 acres in recent years. Blueberries have some traits in common with other perennial fruits, but diverge in their specific requirements for wet, acidic, and high-organic matter soil, high crop value, and susceptibility to numerous pests and diseases that necessitates intensive management and frequent interventions to produce a marketable crop. Organic blueberries represent a small fraction of the total blueberry production area in Michigan, but growers stand to profit if premiums for organic fruit can be matched with yields comparable to conventional production. This review provides a science-based inquiry into the current knowledge and challenges of organic blueberry production systems. Some promising alternatives to conventional (synthetic) fertilizer and pesticide inputs have been identified, but an incomplete understanding of plant and soil responses to crop inputs and the role of ericoid mycorrhizae (ERM) in blueberry production indicates that further research into organic management specific to blueberries is needed. General nutrient and pest management practices, promising organic management tactics, and aspects where research-based knowledge is lacking are discussed. Scientific inquiry into the roles of soil health and ERM in cultivated blueberries may be valuable where an agroecosystem approach is based on the concept of building healthy soils to sustain the health of plants. Although factors controlling colonization by ERM have not yet been fully described, lower colonization levels sometimes observed in managed versus natural ecosystems suggests there is

potential for optimization of cultural practices to harness the benefits of ERM in organic blueberry production.

The Northern Highbush Blueberry

The northern highbush blueberry (*Vaccinium corymbosum* L.) is a deciduous shrub bearing fruit in clusters. The species belongs to the Ericaceae, or heath, family of plants, which also includes cranberry (*Vaccinium macrocarpon* Ait.), rhododendron (*Rhododendron* spp.), mountain (or Western) huckleberry (*Vaccinium membranaceum* Douglas ex Torr.), and scotch heather (*Calluna vulgaris* L.) (Trehane, 2004; USDA, 2008). The native range of highbush blueberry spans from Illinois, Michigan, Nova Scotia, New Jersey, and Texas to Florida (Vander Kloet, 1980). Frederick Vernon Coville, a researcher with the United States Department of Agriculture, initiated domestication of wild highbush blueberry in 1909 using germplasm collected from New Jersey and New Hampshire (Eck, 1988; Trehane, 2004).

The plant forms a crown habit and reaches a height of 2 to 3 meters (6.5 to 10 feet) at maturity (Vander Kloet, 1980). The average amount of fruit produced per bush is 1.5 to 1.8 kg (3 to 4 lbs), while yields of 8 to 10 kg (17 to 21 lb) per bush can be achieved under ideal conditions (Vander Kloet, 1980). Vegetative growth begins two weeks before flowering (Eck, 1988), peaks around fruit set, and continues at a decreasing rate until leaf senescence (Abbott and Gough, 1987a). The rate of root growth is moderate rate at fruit set, tapers off mid-season, and then attains its maximum between fruit maturation and leaf senescence (Abbott and Gough, 1987a). Nearly all roots are located below the canopy of bushes (Gough, 1980) and extend into the upper 25 to 30 cm (10 to 12 inches) of soil (Trehane, 2004), developing almost parallel to the soil surface (Gough, 1994). In blueberries and other Ericaceae, root hairs are not formed, but instead ‘hair roots’ (< 100 μm in diameter) are produced and can account for 80% of root biomass in

some species (Read, 1983, 1996). In a highbush blueberry planting in Pennsylvania, Valenzuela-Estrada et al. (2008) reported that hair roots comprised 90% of the total root length, 40% of the root biomass, and had a median lifespan of 115 to 155 days. Most hair roots are located in the upper 15 to 25 cm (6 to 10 inches) of soil (Gough, 1996; Scagel and Yang, 2005).

Blueberry Production in Michigan

Blueberries are planted on 7800 ha (19,300 acres) on 575 farms in Michigan (Kleweno and Matthews, 2007). About 50% of the plantings in the state are more than 30 years old (Kleweno and Matthews, 2007). Fruit yields average 1,800 kg/ha (4,000 lb/acre) (Longstroth, 2006). Production in 2006 was 37.6 million kg (83 million lb) or 30% of the U.S. total (Kleweno and Matthews, 2007), with a value of US$140 million (Kleweno, 2007). Berries grown for the fresh market comprise about 40% of the harvested crop (Hancock and Hanson, 2002). Often the first harvest is picked by hand with later harvests done by machine (Longstroth, 2006). Over 90% of blueberry acreage in Michigan is machine-harvested at least once (Strik, 2006). Blueberry plantings in Michigan are most concentrated along the Lake Michigan shoreline (Kleweno and Matthews, 2007). Climate extremes in this region are tempered by the prevailing westerly winds that pass over Lake Michigan. The climatic influence of Lake Michigan delays the onset of growth in spring, prolongs growth later into fall, and effectively doubles the number of frost-free days in the growing season compared to areas further inland (Kender and Brightwell, 1966). In southwest Michigan, the annual precipitation averages 82 to 91 cm (32 to 36 inches) and is distributed more or less uniformly throughout the year (Anonymous, 2006). The interval between the last and first killing frost averages 160 to 180 days (Kender and Brightwell, 1966), and the historical mean annual minimum temperature ranges from -18°C to -23°C (0 to -10°F) (USDA, 2006).

Soil and nutrient requirements

In Michigan, blueberries are grown predominantly on acidic, high-water-table spodosols [soils with a distinct upper layer of darkened humic material overlaying a coarse-textured B horizon with accumulated oxides of aluminum, iron, and other metals (USDA-NRCS, 1999)] or organic mucks [20-50% organic matter soils (USDA, 1968)] (Bronick et al., 2004; Longstroth, 2006). Marginal soils can be improved by incorporation of organic materials such as peat moss or compost (Kuepper and Diver, 2004). Highbush blueberry yield and growth were found to be inversely related to soil silt and clay content (Korcak et al., 1982) but positively related to soil organic matter content (Johnson, 1948, 1951, 1959).

Blueberries are acidophilic plants, growing best in soils with a pH around 4.5 to 5.0 (Coville, 1910; Ballinger, 1966). Native *Vaccinium* spp. can be found on soils with pH values as low as 2.7 (Vander Kloet, 1980). In a survey of cultivated blueberries in Oregon, root length and root biomass was highest in fields at lower range of soil pH (Scagel and Yang, 2005). However, productive bushes may inhabit soils with pH values as high as 6.5 (Goulart et al., 1993). Before planting, the soil pH may be reduced by additions of sulfur and/or acidic organic materials such as peat and sawdust (Karp et al., 2004), or increased with lime (Hanson and Hancock, 1998). After planting, soil pH levels can be maintained by appropriate fertilization practices, such as applying nitrogen (N) in ammonium form (which tends to lower soil pH), side dressings of sulfur or lime, or injection of acids into irrigation water (Hanson and Hancock, 1998; Karp et al, 2004; Wolfram, 2007). On organic farms, acetic acid, citric acid, or lignosulfonate may be used to acidify irrigation water, but are more costly than slower-acting elemental S (unpublished data).

Unlike many arable crops, blueberries prefer to absorb nitrogen as ammonium rather than nitrate (Korcak, 1988). High-nitrate fertilizers are usually not recommended for highbush

blueberries because nitrate is more prone to rapid vertical leaching through the soil profile and is taken up and assimilated by plants at a higher metabolic cost than ammonium (Hanson, 2006; Hart et al., 2006). Urea is the most economical synthetic source of N and is recommended on sites with a soil pH < 5, while ammonium sulfate is recommended on sites with soil pH > 5 (Hanson and Hancock, 1998). Split fertilizer applications of one half at bud break and one half at bloom increase plant N-use efficiency (Hanson, 2006). Nitrification (oxidation of ammonia to nitrate) is generally inhibited in strongly acidic soils, but occurs at a high rate relative to adjacent forest soils in many older Michigan blueberry fields, irrespective of pH (Hanson et al., 2002). In Oregon blueberry fields, the soil NH_4^+:NO_3^- ratio is 243:1 (Scagel and Yang, 2005), while the NH_4^+:NO_3^- ratio in Michigan blueberry fields is near 1:1 (Chapter 3). While it is not known whether lower nitrate accumulation in Oregon blueberries is related to more rapid plant uptake of fertilizer N, mid- and late- season plant uptake of fertilizer N appears to be greater under Oregon conditions than those in Michigan (Bañados et al., 2006). However, because the blueberry industry in Michigan was established well before that in Oregon, a higher percentage of inorganic soil nitrogen present as nitrate indicates that selection for acid-tolerant nitrifiers may occur in cultivated blueberry soils over time.

Plant nutrient status is monitored by leaf tissue samples that are collected around August 1 (Hanson, 2005). Leaf tissue analysis is more informative than soil tests for monitoring blueberry nutrition (Hanson and Hancock, 1996).

Irrigation

Blueberry roots lack root hairs and have only one-tenth the absorptive surface area of wheat roots per unit length (Childers and Eck, 1966). Blueberries therefore perform best on sites

where moist (but not flooded) soil is maintained by high water tables (Abbott and Gough, 1987b; Korcak, 1989). An early to mid-season drought limits current-season yield and growth while late-season water deficits reduce flower bud set; a single drought event can negatively impact bush productivity for several years (Hanson and Hancock, 1998; Hanson and Mead, 2006). About three-quarters of blueberry acreage in Michigan is irrigated (Kleweno and Matthews, 2007). Overhead irrigation systems are useful on sites prone to late spring frosts (Longstroth, 2005) and are used on more than 60% of the irrigated area (Kleweno and Matthews, 2007). Irrigation during the growing season is recommended when soil moisture drops to below 50% of field capacity, and frequent, shallow irrigation is preferable to infrequent soaking (Longstroth, 2005). Blueberries will not tolerate extended periods of waterlogged soil during times of active root growth (Pritts and Hancock, 1992). Excessive irrigation may contribute to low fertilizer-N use efficiency by causing mobile forms of soil N (NO_3^-, NH_4^+, and soluble organic nitrogen) to leach below the root zone (Ballinger, 1966; Retamales and Hanson, 1989; Neff et al., 2003).

Diseases

Mummy berry is a disease caused by the ascomycete fungus *Monilinia vaccinii-corymbosi* (J.M. Reade) Honey. Shoot strikes (blighted young shoots) result from infection by ascospores forcibly ejected from asci contained within apothecia that emerge from sclerotia ("mummies"). Ascospore release coincides with shoot emergence in the spring and may last for up to 30 days. There is a latency period of two weeks between infection and the appearance of shoot strikes. Asexual spores (conidia) are produced on infected vegetative tissues and transferred by insects or splashing water to flower pistils. Fruit infection occurs after conidia germinate and grow through the style into the ovary. Prior to ripening, sclerotia (mummy berries), which serve as resting structures, drop to the ground or are harvested along with the

crop. A combination of leaching and accumulation of chilling hours below 7°C are needed for germination of sclerotia and apothecium formation in spring, and spring growth of the host often corresponds with mummy berry germination in spring (Caruso and Ramsdell, 1995).

Cultural methods for mummy berry control include planting of uninfested stock and physical isolation from diseased fields to prevent pathogen establishment, removal of infected berries at harvest, and raking and destroying fallen mummies. Since apothecia require light for development (Caruso and Ramsdell, 1995), the disease may be suppressed by covering them with at least 2.5 cm (1 inch) of soil through disking or hoeing (Cline and Schilder, 2006; Scherm 2006) or covering with at least 3 cm (1.2 inches) of mulch (Drummond et al., 2007; McGovern, 2007) prior to apothecium emergence in spring. Dormant sprays of mined fungicides (lime sulfur, sulfur, and copper) are allowed in organic production and have some efficacy against shoot strikes (Schilder et al., 2006). The bacterium *Bacillus subtilis* (Ehrenberg) Cohn, QST 713, marketed as Serenade, has shown moderate to good control of mummy berry in trials conducted in Michigan (Schilder et al., 2006), Maine (McGovern, 2007), and Georgia (Scherm, 2006). Nita and Ellis (2004) noted that organic fungicides have poor efficacy against mummy berry in fields where disease pressure is high. In sites with high disease pressure, resistant cultivars may present a management option. For instance, 'Bluejay' is resistant, while 'Elliott' and 'Spartan' have moderate resistance (Eck, 1988).

Anthracnose, caused by *Colletotrichum acutatum* Simmonds, is a significant postharvest fruit disease of blueberry in Michigan and other areas (Cline and Schilder, 2006). In addition to causing rot of fruit grown for the fresh market, the pathogen contributes to excessive mold counts on berries harvested for processing, which can lead to rejection of entire fruit lots (Sabaratnam et al., 2004). Symptoms of anthracnose include soft, sunken spots on fruit and

orange, wet conidial masses that develop under humid conditions (Caruso and Ramsdell, 1995). *C. acutatum* also infects twigs (Schilder, 2005) and causes cane dieback of up to 50 cm (20 inches) on highly susceptible cultivars such as 'Jersey' (Caruso and Ramsdell, 1995). The overwintering sites of *C. acutatum* are infected twigs, bud scales, and remaining fruit (Schilder et al., 1997; Schilder, 2005). Spores are released from infected plant tissues from bloom through harvest. Fruit infection most often occurs on immature berries and then remains latent until fruit ripening (Caruso and Ramsdell, 1995).

Recommended cultural measures for reduction of anthracnose include timing overhead irrigation in the morning rather than at night to speed drying and avoid lengthy periods of wetness (Schilder et al., 2006), pruning to reduce inoculum and promote air circulation, timely harvests as fruit ripens, rapid cooling of fruit after harvest, and avoidance of handling fruit while surface moisture is present (Caruso and Ramsdell, 1995). Pruning was shown to reduce anthracnose in lowbush blueberry in Maine by 80% (Yarborough, 2006), and annual removal of 20% of shoot growth was found to have no detrimental effect on yields of highbush blueberry (Pritts and Hancock, 1992). Fungicide sprays are recommended from early bloom up until harvest, especially during warm and wet conditions conducive to disease; application after bloom and immediately prior to harvest may have the most efficacy against anthracnose (Schilder, et al. 2006). Nita and Ellis (2004) reported that sulfur and copper-based products are not effective against anthracnose when high levels of inoculum are present. Duke is an early- ripening cultivar with moderate field resistance to anthracnose relative to other early and mid-season cultivars, most of which are susceptible to anthracnose (Miles and Schilder, 2008). Late-season cultivars Elliott, Liberty, Draper, and Aurora are relatively unaffected by anthracnose (Schilder, 2006).

Alternaria rot, caused by *Alternaria tenuissima* (Nees & T. Nees : Fr.) Wiltshire is a common post-harvest disease of blueberry, second in importance to anthracnose (Schilder et al., 2002). The disease manifests itself as depressed, slightly wrinkled areas accompanied by dark green sporulation, often on the calyx end of fruit, while post-harvest infection occurs on stem-end scars (Cline and Schilder, 2006). A leaf spot caused by the pathogen is also common in the southeastern US and is most prominent in lower portions of the canopy (Caruso and Ramsdell, 1995). Infection is favored by lengthy periods of cool, wet weather during the growing season. Mycelium and spores overwinter on any fruit and peduncles left hanging as well as in plant debris under the bush. Spores are dispersed by wind and rain. Inoculum can be managed through pruning of infected tissues and infection can be reduced by well-timed applications of protectant fungicides (Cline and Schilder, 2006). Minimizing canopy inoculum buildup, handling fruit only when dry, harvesting often and completely to reduce the accumulation of overripe fruit, and lowering fruit temperature rapidly after harvest are recommended to mitigate losses from the disease (Cline and Schilder, 2006).

Phomopsis twig blight and cane canker, caused by *Phomopsis vaccinii* Shear (teleomorph *Diaporthe vaccinii* Shear), are significant diseases of blueberries in Michigan (Schilder et al., 2006). Cane canker is distinct from twig blight in that cankers occur on canes, eventually killing all tissues above the infection site by girdling cambium tissue, while twig blight infection occurs mostly on fruiting twigs and spreads down the stem 15 to 25 cm (6 to 10 inches) until the advance of the fungus is limited by the host (Cline and Schilder, 2006). In Michigan, occurrence of cane canker is more widespread than twig blight (Caruso and Ramsdell, 1995; Schilder et al., 2006). A post-harvest fruit rot caused by *P. vaccinii* has also been reported (Cline and Schilder, 2006). *P. vaccinii* overwinters in infected plant tissues, including dormant buds (Caruso and

Ramsdell, 1995; Schilder et al., 2006). Spores are released from pycnidia and dispersed by rain and wind; sporulation is most abundant between budbreak and bloom (Cline and Schilder, 2006). Infection occurs on buds and extends into the vascular system, which girdles stems and results in symptomatic "flagging" of canes or blighting of shoot tips (Caruso and Ramsdell, 1995). Infection can also extend into below-ground crown tissues (Parker and Ramsdell, 1977). Phomopsis canker symptoms are most severe after harsh winters (Longstroth, 2006) and in production areas with long, cold winters (Lyrene, 2006). Wounding caused by cold injury, frost and mechanical harvest predisposes plant tissue to infection by creating an entry point for the pathogen (Caruso and Ramsdell, 1995).

Effective cultural controls for Phomopsis dieback include eradication of primary inoculum by pruning out and destroying diseased plant tissue, avoidance of field sites prone to winter injury or late spring frosts, and use of cultural practices that encourage plant tissue hardening off in the fall. Removal of infested debris is critical as diseased stems beneath bushes constitute substantial reservoirs of inoculum that contribute to infection of emerging stems (Caruso and Ramsdell, 1995). Several synthetic fungicides provide good control of Phomopsis, while organic-approved materials with good efficacy against Phomopsis have yet to be identified (Schilder et al., 2006). 'Elliott' and 'Bluetta' are relatively resistant to Phomopsis (Baker et al., 1995).

Fusicoccum (Godronia) canker is found on bushes grown in northern regions of Michigan, i.e. in the upper peninsula and northern lower peninsula. The causal organism is *Fusicoccum putrefaciens* Shear, teleomorph *Gondronia cassandrae* Peck. Stems become infected as conidia are released from pycnidia on cankers throughout the growing season, with peak spore release occurring at bloom. The optimal temperature for infection is 10 to 22°C (50 to

72°F. Monthly applications of protectant fungicides and removal of symptomatic stems are recommended for control. 'Jersey' and 'Bluecrop' are among the cultivars most susceptible to the disease (Caruso and Ramsdell, 1995).

Insect pests

Blueberry maggot, *Rhagoletis mendax* Curran, is a serious insect pest of blueberries in areas east of the Rocky Mountains. There is zero tolerance for this pest by the fruit industry and entire shipments are rejected if maggots are found in fruit (Pritts and Hancock, 1992). A single generation appears in June and lays eggs in berries 7 to 10 days after emergence. Each adult may lay up to 100 eggs, each being oviposited singly in berries (Gough, 1994). Eggs hatch 3 to 4 days after oviposition; 18 to 20 days later, maggots drop to the ground and pupate 3 to 4 cm (1.2 to 1.8 inches) below the soil surface where they overwinter.

Yellow sticky boards and spheres may be placed along field margins at two traps per acre for monitoring emerging adults (Gough, 1994). Insecticides such as Imidan (phosmet) are applied every 7 to 10 days after the threshold of two flies per trap in one week is exceeded, which usually coincides with harvest of early-ripening cultivars (Gough, 1994). Pelz et al. (2005) reported organic-approved GF-120 NF baits with the active ingredient spinosad, an aerobic fermentation product of the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao, were highly effective for control of blueberry maggot. Barry et al. (2005) found that GF-120 NF provided control similar to the conventional standard Imidan, while neem and pyrethrum-based products – insecticides allowed in organic production – were less effective. Blueberry maggot larvae are parasitized by a parasitic wasp, *Diachasma alloeum* Muesebeck, at rates of 30-50% in unmanaged blueberry fields (Stelinski et al., 2004), but parasitism is disrupted by chemical

controls (Stelinski et al., 2006). The levels of blueberry maggot parasitism in organic blueberry fields have not been investigated.

Japanese beetle, *Popillia japonica* Newman, is the most important insect pest of blueberries in Michigan (Isaacs et al., 2004). Adult beetles congregate near tops of plants and cause damage to leaves and ripening fruit; damage is apparent by skeletonized leaves (Fleming, 1972). If not controlled, beetles are collected along with berries in mechanically harvested fruit. A zero-tolerance policy for beetle contamination in fruit is enforced by most wholesale buyers (Isaacs et al., 2004). Overwintering larvae feed on roots of grasses and other plants in spring, and then move deeper into the soil to pupate. Adult emergence spans from early July up to the first frost in autumn (Isaacs et al., 2005). Usually only one generation is produced per year (Fleming, 1972).

Over 60% of Michigan blueberry growers use foliar-applied insecticides as the primary means of Japanese beetle control in Michigan (Szendrei and Isaacs, 2006a). Larval populations during spring can be used to predict levels of adult infestation in summer (Szendrei et al., 2005). Timing of foliar insecticide applications is determined by monitoring pheromone-baited traps placed within fields. Soil cultivation in late spring and early fall and the elimination of grassy areas within and around fields, preferred overwintering sites of beetles, are cultural methods useful for reducing populations of Japanese beetles (Szendrei et al., 2005). On sites where clean cultivation is not an option (e.g., sites with frequently waterlogged soils), insecticides may be applied to soil for control of larvae (Isaacs et al., 2003). Planting dicotyledonous cover crops in row middles has the potential for increasing beetle grub predation (O'Neal et al., 2005) and reducing adult egg-laying (Mutch et al., 2005; Szendrei and Isaacs, 2006) compared to grass sod row middles. A commercial product composed of spores of the biocontrol agent *Bacillus*

popilliae Dutky, which caused milky spore disease in Japanese beetle grubs, is available for use in turf grasses (Federici, 2005), but may not be effective in areas with cold winters (Isaacs et al., 2003). A strain of the soil bacterium *Bacillus thuringiensis* Berliner that produces metabolites toxic to Japanese beetle grubs has potential for commercial release (Suzuki et al., 1992), but product development and release have been held up by formulation issues (Potter and Held, 2002). Other biological control organisms endemic to soil, such as entomopathogenic nematodes, do not significantly reduce Japanese beetle populations in Michigan fields (Cappaert and Smitley, 2002). Adult Japanese beetles are competent fliers and can move long distances, which effectively limits the efficacy of control tactics aimed at immature life stages. Biological insecticides such as pyrethrum and spinosad may be applied just prior to harvest in organic production fields to clear bushes of beetles that would be machine-harvested along with fruit (Szendrei et al., 2003), but these do not always provide effective control (Kuepper and Diver, 2004).

The cranberry fruitworm, *Acrobasis vaccinii* Riley, is a nocturnally active lepidopteran (moth) pest that emerges from a cocoon on soil surface debris in the spring and deposits eggs inconspicuously on the inner portion of the berry calyx (Pritts and Hancock, 1992). Larvae hatch and bore into fruit, feed on pulp, and web berries together with silk (Pritts and Hancock, 1992). Infested berries are often filled with frass (Gough, 1994). Larvae feed for about three weeks (Gough, 1994) and then migrate to the ground pupate beneath the plant canopy (Pritts and Hancock, 1992). This pest is most problematic in fields surrounded by native forest stands, as egg laying is highest on the margins of fields adjacent to wooded field edges (Mallampalli and Isaacs, 2002). Soil cultivation reduces overwintering of larvae, while seasonally applied

insecticides may be necessary under heavy infestation. Pheromone-baited traps can be used to optimize timing of insecticide application (Liburd and Arevalo, 2006).

Cherry fruitworm (*Grapholita packardi* Zeller) is another lepidopteran pest. Adults lay eggs on the undersides of leaves in spring (Gough, 1994). The larvae hatch in about one week, bore into the berry calyx, and consume the flesh. The pest overwinters in a cocoon affixed to the plant or debris on the soil surface. Two to three fruit webbed together indicate the presence of cherry fruitworm. Protective chemical sprays are applied in sites with a history of infestation. Parasitoids also attack the larvae. *Bacillus thuringiensis* (Bt) is effective against this pest if applied frequently beginning early in the growing season. Entrust (spinosad) is an effective option for use in organic blueberries (Wise and Isaacs, 2001).

The Michigan blueberry aphid, *Illinoia pepperi* MacGillivray, is generally not a direct threat to blueberry crops, but is economically important as a vector of blueberry shoestring virus (Caruso and Ramsdell, 1995). Aphids overwinter as eggs in bushes, hatch in spring, and colonize tender shoot tips. In late summer, winged aphids appear and spread to new plants, and characteristically move within rather than across rows (Pritts and Hancock, 1992). Insecticides are the primary means of aphid control, though excessive use of chemical sprays may lead to insecticide-resistant populations and reduced numbers of beneficial aphid parasites. Isaacs et al. (2006) reported that substituting reduced-risk, targeted insecticides for broad-spectrum insecticides increased aphid egg parasitism by 30%. Insecticidal soaps and high-pressure water sprays are effective alternatives if good coverage can be achieved (Drees, 1994; Karagounis et al., 2006). Use of insecticides with specificity towards sap-feeding insects could mitigate impact on beneficial species, as naturally occurring enemies of insect pests are the most economical aphid control.

Plum curculio, *Conotrachelus nenuphar* Herbst, is especially problematic for early ripening cultivars and in fields near woodlots. The coleopteran pest is active during bloom after emergence from pupae below the bush. A “C” or “D”-shaped fruit puncture indicates the presence of plum curculio. Grubs feed within fruit for three weeks and then fall to the ground floor with infested fruit, where the grub emerges and pupates in soil surface litter. Monitoring for plum curculio can be done by placing a large white sheet or board under bushes, and collecting adults that fall from the canopy while shaking the branches. In addition to insecticide sprays, frequent cultivation and removal of surface debris can be useful for reducing populations of plum curculio (Gough, 1994).

Vertebrate pests

Losses to birds average 10% in commercial fields (Pritts, 2006) and tend to be highest in small plantings near urban areas (Gough, 1994). Installation of netting as a physical barrier can result in yield savings of up to 20%, but is costly ($1,000 to 3,000/acre). Methyl anthranilate is a foliar-applied bird repellant used by some growers. Table sugar (sucrose) is also sometimes applied to ripening berries to disrupt feeding as birds lack the ability to digest reduced sugars. Combination of noise devices such as bird distress calls and propane or electric cannons in combination with visual scary eye balloons approach the level control attained by netting (Pritts, 2006), while others suggest that visual repellants are completely ineffective (Gough, 1994). A recent study in British Columbia found that a most effective bird repellant was a hawk kite (Steensma, 2009). Deer consume young shoots and may decimate young plantings if left unchecked. Slanted wire fences are often effective for deterrence as deer have poor depth perception. Electric fences are also effective but more costly. Rotten egg-based repellants and dogs are additional options to discourage deer (Pritts, 2006). Mice gnaw on bark, causing

girdling of canes at the ground level, while voles consume roots. Mulches and perennial sods may contribute to rodent problems (Kuepper and Diver, 2004). Recommended controls for rodents include mowing tall grass around bushes and adding a mowed strip between wild and cultivated areas to increase exposure of rodents to predatory birds and deter rodent migration into managed fields (Gough, 1994).

Weed management

Weeds compete with blueberry bushes for light, nutrients, moisture, and space. They can also serve as hosts for insect pests and diseases, impede harvest, and may contribute to late-spring frost damage by trapping heat radiated from soil (Majek, 2006). In addition, tall weeds can increase humidity and thereby increase disease pressure. Managing weeds is especially important during the plant establishment phase, as young blueberry plants are poor competitors for moisture, nutrients, and light (Ballinger, 1966). In conventional blueberries, synthetic herbicides are the most economical method of weed control, while shallow cultivation and mulches are relied upon to a lesser extent (Majek, 2006). Flaming is impractical in most situations due to the potential for fire and damage to low-growing canes. Acetic acid and fatty acid-based organic herbicides have shown potential as "burn-down" herbicides in other cropping systems, but due to high cost and risk of damage to lower portions of canes, these are likely to be most useful for spot spraying in problem areas. Shallow cultivation is sometimes used within the planting row, but specialized equipment and well-trained operators are needed to prevent extensive plant damage because blueberry roots grow close to the soil surface (Ballinger, 1966).

Weed control is a major problem in organic blueberry production (Sciarappa, 2006). Weed management options for organic growers include herbicides made of plant extracts, acids, flaming, weeder geese, and cultivation (Kuepper and Diver, 2004), although mulch within

blueberry rows appears to be the best weed management tactic in organic production (Majek, 2006). Landscape fabric covered with a layer of organic mulch provided almost 100% control of crabgrass, foxtail, and broadleaf weeds (Sciarappa, 2006), which demonstrates that good weed control can be achieved without synthetic herbicides. In addition to suppressing weeds, mulches conserve moisture, insulate soil from daily and seasonal temperature extremes (Cox and Keen, 2008), contribute organic matter (St. Laurent et al., 2008), and enhance soil physical properties (Korcak, 1988). Mulches may influence soil pH (Yao et al., 2005), nutrient availability (Hanson, 2006; White, 2006), and soil biological activity (St. Laurent et al., 2008). Blueberry root penetration into lower soil depths is reduced under mulch culture (Spiers, 1986; Goulart et al., 1998; Krzewinska, 2004). Research has shown that growth and yield of blueberries grown with mulch are superior to bare-ground culture (Savage and Darrow, 1942; Spiers, 1986; Karp et al., 2004; Kozinski, 2004; White and Strik, 2007). Mulch may be an essential input for blueberries grown on drier, upland sites for conservation of soil moisture (Childers and Eck, 1966; Goulart et al., 1995), but mulch adds considerably to the cost of production (Eleveld et al., 2005)

Mulching materials include pine bark, sawdust, straw, polyethylene sheeting, and woven landscape fabric. Each material has potential trade-offs, and cost and local availability are primary factors in the type of mulch utilized by growers. Woven plastic landscape fabric is water-permeable, lasts about 10 years, and provides excellent weed control (Sciarappa, 2006). Its drawbacks include high initial cost and economic and environmental sustainability concerns (Cox and Keen, 2008). Black plastic is generally not recommended for use in mature blueberries, as it requires frequent replacement – about every two years (Eck, 1988) – and due to impermeability, requires use of drip irrigation, adding costs where irrigation is applied through overhead systems that provide spring frost protection (Kuepper and Diver, 2004). Synthetic

mulches were found to increase soil temperature and reduce fruit yield in low-chill growing regions; extremes in temperature can be moderated by overlaying black plastic with white plastic or organic materials (Magee and Spiers, 1995; Cox and Keen, 2008).

Organic mulches are usually applied in a 1- to 1.3-meter- (3- to 4-feet) -wide strip underneath blueberry bushes (Gough, 1994) at a depth of 10 to 20 cm (4 to 8 inches) (Eck, 1988; Kuepper and Diver, 2004; Trehane, 2004). This translates to about 1000 cubic meters of mulch per hectare (526 cubic yards per acre). Doubling the recommended rate of N or adding 1.5 lb N per 500 lb of mulch is recommended to offset N immobilization that occurs during microbial decomposition of mulch (Johnson, 1959; Hanson and Hancock, 1996). Heavy initial application of mulch can reduce the frequency of additional applications needed in subsequent years (Trehane, 2004). Straw decomposes more rapidly than wood chips or synthetic mulches and invites rodents that damage stems and roots (Gough, 1994) and may be a fire hazard (Johnson, 1959). Wood and bark materials decompose more slowly than straw and have been used with success in many regions (Spiers, 1986; Cox and Keen, 2008). Michigan growers using wood chips should beware of wood infected with *Armillaria* sp., a root rot pathogen of blueberries (Trehane, 2004). A recent study of juneberry (*Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem.) establishment in North Dakota, USA showed that mulching suppressed weeds but reduced plant width, stem length and stem number compared to hand weeding or herbicides (Willard and Hatterman Valenti, 2008). Low soil temperature rather than weed pressure may be a limiting factor for growth of perennial crops in cooler climates.

Cover crops in row middles

Many Michigan blueberry growers manage perennial grass sods between rows of bushes for soil conservation, weed suppression, and ease of picker/harvester access to fields during wet

periods (Mutch et al, 2005). Perennial fine hard fescue is often recommended because it tolerates a wide range of soil moisture and fertility conditions and is slow growing, requiring less-frequent mowing than other grasses, while tall fescue tolerates wetter soils (Carroll et al., 2010).

Legumes adaptable to blueberry soils may offer additional benefits to growers besides those provided by grass sods. Legumes fix significant amounts of nitrogen that may partially replace grower inputs (Clark, 1998). When grown under optimal conditions, crimson clover (*Trifolium incarnatum* L.) fixes in the range of 56 to 67 kg N per hectare (50 to 60 lb N per acre), while alsike clover (*T. hybridum* L.) normally fixes 67 to 78 kg N per hectare (60 to 70 lb N per acre) (Clark, 1998). However, the soil nitrogen relations of legumes grown in row middles in blueberry fields have not been determined. Furthermore, optimal management strategies (e.g., mow and blow) to allow growers to most efficiently and practically utilize N fixed by these crops need further investigation (Yao et al., 2005; Moore-Kucera et al., 2008). Legume cover crops can reduce insect pests in blueberry plantings compared to grass sods (Isaacs et al., 2004) and provide refugia for beneficial insects. Certain legumes may attract native pollinators to blueberry fields through synchronous flowering with blueberries in spring (Mutch et al., 2005), although competition between blueberries and cover crop species for pollinators is a concern. Cover crops that flower later in the growing season (e.g., alsike clover, buckwheat) can provide food and shelter to predators, parasitoids, and pollinators.

A concern for legume cover crops is that higher soil N availability in fall could delay the onset of plant dormancy and increase risk of winter injury to plants, as N-scavenging by annual and perennial grasses in fall is beneficial for preventing late-season flushes of shoot growth that are susceptible to winter injury (Mark Longstroth, MSU Extension, pers. comm.). Dense cover crop stands between rows may alter microclimates and provide conditions that are conducive for

disease development (Agrios, 2005). Szendrei and Isaacs (2006) reported that clovers were not useful for reducing Japanese beetle populations. In addition, soil cultivation, which predisposes Japanese beetle larvae to predation and desiccation (Szendrei et al., 2005), is lost as a management option if perennial stands are maintained. Legumes may also attract deer (Kuepper and Diver, 2004), which may necessitate fencing of fields. Further studies are needed to better understand the effects of cover crops on insect and disease cycles, patterns of soil N availability, and winter-hardiness of blueberries in Michigan.

Challenges to establishment of cover crops in commercial blueberry plantings include shading by taller blueberry plants, acidic soils, farm machinery traffic, and periods of cool, waterlogged soils (Mutch et al., 2005). Some cover crops thrive in blueberry fields despite these stress factors. Patten et al. (1990) found that crimson clover, cereal rye (*Secale cereale* L.), and rye grass (*Lolium perenne* L.) can be grown successfully in rabbiteye blueberry fields. Trials done at the Trevor Nichols Research Complex, Fennville, MI, and on grower cooperator farms within Michigan have found that crimson and alsike clovers can be grown successfully in blueberries, and that cereal rye is the most suitable non-legume cover crop (Mutch et al., 2005). Legume cover crops may require additional management (more frequent soil incorporation and reseeding) compared to perennial sods. Perennial mixed legume-grass cover crops planted in tart cherries showed that legumes were overtaken by grasses within four years (Sanchez et al., 2003).

Annual cover crops, such as rye and oats, have long been planted in blueberry fields to curtail shoot growth and hasten hardening off in the fall (Ballinger, 1966). Instead of contributing nitrogen to soils, rye is often used as a catch crop to sequester excess nitrogen in soil. Rye is wind-pollinated (Mauseth, 2003), lacks prominent flowers, and thus is not especially attractive to beneficial insects. However, rye establishes relatively quickly and is adaptable to a

wider range of soils than most cover crops (Clark, 1998). It crowds out weeds through vigorous growth and inhibits weed seed germination through allelopathic compounds released as residues decompose (Creamer et al., 1996). Conventional growers may terminate rye stands with synthetic herbicides, while organic growers rely on mowing and/or incorporation into the soil. Timely management is necessary to prevent rye and other annual covers from setting viable seed and becoming a nuisance in subsequent years (Clark, 1998). No-till crimpers are a novel approach to stand termination and work especially well for killing rye (Ashford and Reeves, 2003).

Compost teas and foliar disease management

Many organic growers use compost teas and other foliar products as a means of applying supplemental nutrients, suppressing diseases, and stimulating plant growth and defenses (Kuepper and Diver, 2004). These authors assert that microorganisms contained in or stimulated by compost teas applied to plant surfaces may outcompete pathogens and reduce levels of plant disease. Few field-based research reports have thus far supported this hypothesis. Schilder, Gillett and Sysak (unpublished data) reported that compost tea sprays resulted in significantly less downy mildew on grape and foliar and cane diseases on raspberry than in untreated control plants, indicating that compost teas may have fungicidal properties. Edwards et al. (2006) demonstrated suppression of Verticillium wilt on strawberries and significant reduction of Phomopsis and powdery mildew on grapes and bacterial rot on cucumber with foliar-applied compost tea.

According to the USDA Compost Tea Task Force Final Report (USDA, 2004), compost tea may be applied to edible crops without restriction, provided that no additives, such as molasses or sugar, have been used during preparation. If additives are used during preparation,

the formulation is required to be tested to ensure that detectible human pathogens are below those of EPA-recommended recreational water quality guidelines for a bacterial indicator of fecal contamination. The requirement for microbial testing of compost tea prepared with additives does not apply if the tea is to be applied at an interval greater than 90 days prior to harvest for below-ground crops or 120 days prior to harvest for crops harvested above-ground. The restriction applies because sugars added during preparation of compost tea increase the potential for rapid growth of human pathogens (USDA, 2004).

Soil and nutrient management

Research conducted to better understand soil nitrogen dynamics where synthetic fertilizers are used to meet crop nitrogen demands have improved fertilizer use efficiency in highbush blueberry production systems (Hanson and Hancock, 1998; Hanson, 2006; Hart et al., 2006). However, less is known about management of nitrogen and other fertilizers derived from organic sources. Nutrient management is a prominent concern of current and prospective organic blueberry growers (2007 Great Lakes Expo survey, Appendix A), and efforts of extension and outreach personnel may be hindered by lack of research in this area. Nutrient deficiency symptoms, suboptimal growth, and below-average yields were observed in organically-managed blueberries in Michigan (Carlos Garcia-Salazar, pers. comm.) and other regions (Muller et al., 2006; Drummond et al., 2007).

No specific recommendations exist regarding the application rate or suitability of organic nutrient sources for blueberry production; this is an ongoing area of research in many organic crop production systems (Moore-Kucera et al., 2008). Organic blueberry growers often apply higher amounts of total N per acre than are recommended for synthetic fertilizers to account for N-mineralization characteristics of organic fertilizer. High-C:N-ratio, plant-based composts

applied to soil may temporarily immobilize soil nitrogen as soil microbes utilize available nitrogen to decompose carbon-rich organic matter, while highly soluble, concentrated sources of organic nitrogen such as bat guano and blood meal are rapidly converted to inorganic nitrogen and in this sense have similar properties to synthetic nitrogen fertilizers (Cowley et al., 1999; Gaskell and Smith, 2007; Kuepper and Diver, 2004). Leaching, runoff, and low N-use efficiency can result from mismanagement of organic fertilizer (Gaskell, 2004). The necessity for understanding patterns of N mineralization and plant uptake is important in organic production systems, since the release of plant nutrients from soil or fertilizers into plant-available forms is dependent on the activities of bacteria, fungi, and other soil organisms. Asynchrony in N-release and plant uptake increases the likelihood of nitrogen losses (Pang and Latey, 2000). Animal manures, compost, meal (soybean, feather, alfalfa)-based protein sources, commercial blended organic fertilizers, and legume cover crops are sources of nutrients acceptable for use in organic production (USDA NOP, 2008). Costs of these materials range from about $1.00 (composted dairy manure) to more than $6.00 (blood meal) per pound of N (pers. comm., Morgan's Composting, Evart, MI). Anticipated within-season mineralization of these materials varies from 10% for compost to 65% for soybean meal (Smith and Hadley, 1989; Sanchez and Demchak, 2004; Agehara and Warncke, 2005; Heckman, 2007). Therefore, the N-mineralization rate should be considered in estimations of fertilizer cost and rate of application. In addition to temperature and moisture (Agehara and Warncke, 2005), nitrogen mineralization of organic fertilizers depends on the initial C:N ratio of the material, with lower C:N amendments providing a more readily available source of N, while high C:N amendments may immobilize soil N (Rowell et al. 2001). Materials with similar C:N ratios can differ in temporal N release patterns (Sanchez et al., 2001). Reeve et al. (2005) postulate that in fields under organic management,

plants may access more N in organic form than in conventionally managed fields. Edaphic conditions in blueberry fields (acidic, coarse texture, high organic matter, and sometimes waterlogged) differ sharply from those of arable crops in which N-mineralization rates of organic fertilizers have been studied. A major goal in organic systems is to build nutrient reservoirs to provide a sustained release of nutrients such that large quantities of annual fertilizer inputs are not required to obtain acceptable yields (Moore-Kucera et al., 2008). Optimizing management to attain a "healthy" soil in organic cropping systems is an emerging area of research (Sooby et al., 2007). More studies to address the suitability of organic fertilizers in blueberry fields and their impacts on soil health are warranted.

Organic sources of nutrients are usually more bulky than synthetic fertilizers, and rates of application are determined by expected N mineralization rather than total N. The additional bulk of these amendments likely affects physical, chemical, and biological properties of soil more than synthetic fertilizers. Locally available materials are preferred by growers due to high transport costs. The effects of organic amendments on soil and plant health may not be immediately apparent (Moore-Kucera et al., 2008). There are reports that soils with high organic matter content allow for acceptable growth of blueberries outside of the range of pH considered optimal (Ballinger, 1966; Goulart et al., 1993), but mechanisms underlying this phenomenon are not well understood. Soil organic matter may buffer iron chlorosis-inducing high pH soils through enhanced nutrient turnover resulting from greater biological activity. Research is needed to determine organic nutrient sources suitable for blueberries and which biological, physical, and chemical measures are most associated with soil health in organic blueberry production.

Soil Biology and Blueberries

There is a close relationship between biological activity and nutrient availability in soils in organic crop production systems (Ferris et al., 1996; Alfoldi et al., 2000; Bloem et al., 2006). Organic management practices tend to improve biological soil properties over time (Glover et al., 2000; Mader et al., 2002). Several studies have suggested that in soils managed with organic methods, the "priming" effect, or short-term burst of soil biological activity and nutrient turnover stimulated by adding organic amendments such as manure or compost, can satisfy plant nutritional requirements without the need for supplemental fertilizers (Drinkwater et al., 1995; Yao et al., 2005). Some studies have reported similarity in yields on organic and conventional farms despite lower levels of soluble N, P, and K in soils under organic management (Drinkwater et al., 1998; Poudel et al., 2002). This may be attributed to enhanced nutrient cycling mediated by soil organisms which are often more abundant in organic systems (Mäder, 2002; Pimentel et al., 2005). This closed-loop N cycle may reduce rates of nitrification, a process which contributes to low fertilizer-use efficiency in older blueberry fields (Hanson et al., 2002), and preserve water resources through reduction of leaching and field runoff (Drinkwater et al., 1995).

Standard soil testing for nutrients other than N is used to estimate fertilizer needs in conventionally grown blueberries (Eck, 1988), but soil nutrient levels do not always accurately predict the nutrient status of bushes (Hanson, 1987). The need for solubilization and mineralization of fertilizers used in organic production systems may require novel measures to predict the supply of plant-useable nutrients in soils managed under organic protocol (Sanchez and Demchak, 2004; Heckman, 2007). Indicators of soil biological activity and labile (readily available) nutrient pools have been studied in relation to nutrient management of organically

grown crops. One such measure is light fraction soil organic matter (LF-SOM), which appears to be directly related to soil N supply in soils under organic management (Wander et al., 2005). This active component of SOM has a turnover time of about 1.5 years and was found to have a three-fold higher N concentration than inorganic N in bulk soil, which is the standard measure for soil-test-based N recommendations (Wander et al., 1994). Willson et al. (2001) determined that C in particulate organic matter (POM, a biologically active organic matter fraction isolated by size separation) was a reliable predictor of nitrogen mineralization. In addition to finding that POM carbon was a more sensitive measure of short-term SOM aggradation than total organic carbon, Marriott and Wander (2006) found that POM nitrogen was the best indicator of short-term-available soil N in organically managed soils. Light fraction C and N content is often linked with other measures of active pools of SOM, including microbial biomass, potential nitrogen mineralization, and short-term soil respiration, and may have potential for use as a composite measure of soil health and plant nutrient supplying capacity in annual cropping systems (Wander, 1994). Nitrogen mineralization is influenced by abiotic factors such as soil temperature, moisture, pH, and oxygen content and reflects the intrinsic ability of soil to meet plant N needs (Bloem et al., 2006). Soil respiration is an indirect measure of the activity of soil enzymes and may be assessed under standardized laboratory conditions (Bloem et al., 2006) or *in situ* (Coleman et al., 2004).

Beneficial and plant-pathogenic soil microorganisms

Soilborne diseases are common in blueberries grown in the Southeastern United States (Cline and Schilder, 2006). Recent work showed that *Pythium* spp. were ubiquitous in Oregon blueberry fields, while *Phytophthora* spp. were present in 24% of fields, but the presence of either pathogen was not associated with site or cultural characteristics or plant vigor (Bryla et al.,

2008). Blasing (1989) isolated *Rhizoctonia solani* J.G. Kühn from roots of chlorotic, low-vigor blueberry plants growing on arable soils in Finland. Problems with Phytophthora root rot and other blueberry root diseases can be avoided through proper site selection, drainage, and irrigation practices (Pritts and Hancock, 1992).

Certain beneficial fungi and bacteria in soils have the capacity to ward off plant disease-causing organisms through antibiosis, resource competition, parasitism, and induction of host defenses (Harman et al., 2004, Hass and Defago, 2005). In addition, beneficial microorganisms can enhance crop growth through the release of plant growth-promoting substances (Kloepper et al., 2004, Hass and Defago, 2005). Although soil and rhizosphere-colonizing biological control microbes have been a focus of extensive research on apple (Yao et al., 2005; St. Laurent, 2008), little research has been done on beneficial soil rhizosphere organisms in blueberry soils. Furthermore, it is not known whether these microbes affect blueberry plant vigor, productivity or disease resistance, or are more abundant in organic blueberry production soils.

There is evidence that beneficial microorganisms are stimulated by organic practices. In a comparison of organic and synthetic nutrient sources applied on three organic and three conventional farms, Bulluck et al. (2002) found higher *Trichoderma* spp. and thermophilic microbe (*Bacillus* spp.) populations, a corresponding decrease in *Phytophthora* and *Pythium* spp., and higher yields in tomato plots treated with cotton gin trash, yard waste compost or manure compared to plots amended with synthetic fertilizer. Swine manure or compost increased *Trichoderma* spp. and fluorescent pseudomonad populations and reduced incidence of southern blight relative to synthetically fertilized control plots (Bulluck and Ristaino, 2002). Aini et al. (2005) found higher populations of actinomycetes and lower *Fusarium* spp. populations in soil in plots supplemented with organic fertilizers on a site with a history of organic management, but

no difference at a site that was previously managed with conventional practices, which suggests that resident populations of soil microbes are more responsive to disease-suppressive inputs on organic farms. Yao et al. (2005) reported differences in fungal and bacterial species composition in apple orchard soil in response to compost application determined by DGGE-PCR (density gradient gel electrophoresis-polymerase chain reaction, a method of characterizing soil microbial communities), but did not find a significant relationship between microbial community composition and apple tree performance. They also found that rootstock genotype, rather than soil amendments, was the primary determinant of rhizosphere microbial community and tree growth and yield. Fierer and Jackson (2006) determined that overall bacterial abundance and species diversity is most closely related to soil pH. In addition to plant disease suppression, fungi and bacteria are the primary decomposers of organic materials in soil (Magdoff and Van Es, 2009), which benefits crops by making nutrients in soil organic matter available for plant uptake.

Nematodes play an important role in soil nutrient cycling and their activity may be especially important in soil N turnover and synchrony between N mineralization and uptake by crops (Ingham et al., 1985). Some efforts to utilize various indices of nematode community structure as a measure of soil food web functioning have been made in recent years (Yeates et al., 1997; Ferris et al., 2001; Neher, 2001; Forge et al., 2003). However, a minimum threshold population is required prior to adoption of these measures as soil quality indicators, and nematode community structure is not likely to differ significantly between conventional and recently established organic fields (Dr. George Bird, pers. comm.). Therefore, for purposes of comparing conventional vs. recently transitioned organic fields, i.e. most organic-certified blueberry acreage in Michigan, assessment of total and plant pathogenic nematode populations is

adequate; detailed nematode community indices are likely more appropriate for comparisons between soils under intermediate to long-term organic and conventional blueberry production.

Ericoid mycorrhizae

Ascomycete fungi have been observed to form a mutualistic association, termed ericoid mycorrhizae (ERM), within the epidermal layer of ephemeral hair roots of blueberry plants (Doak, 1928). Rayner (1927) compiled earlier accounts of ericoid mycorrhizae, which dated to the 1840's, and concluded that the relationship benefits both the plant and fungus. The ERM mutualism has since been shown to enhance plant uptake of nutrients under nutrient-limiting conditions (Stribley and Read, 1974; Smith, 1980; Read, 1996). Ericoid mycorrhizae also buffer plants from phytotoxic metal ion concentrations (Bradley et al., 1981) and are hypothesized to protect plant tissues from infection by soilborne pathogens (Goulart et al., 1993; Koron and Gogala, 1998), although evidence for the latter phenomenon is lacking. In turn, the fungal partner benefits through provision of carbohydrates by the plant (Smith and Read, 2008). Fungal isolation, host reinfection, and evidence of nutrient and metabolite transfer or plant growth enhancement relative to non-mycorrhizal plants are considered as prerequisites to classification of a root association as mycorrhizal by some researchers (Dixon et al., 2002). Conversely, Brundrett (2004) suggests that presence of specialized tissues for nutrient and metabolite transfer between the plant and fungus is sufficient evidence for characterizing a fungus-root association as mycorrhizal.

The ERM morphology consists of intracellular hyphal coils within hair root epidermal cells and a limited weft of extraradical mycelium (Read, 1996). ERM lack the tree-like arbuscules and bulbous hyphal swellings of vesicular-arbuscular mycorrhizae (AM or VAM) and extracellular mantle and intercellular Hartig net characteristic of ectomycorrhizae (ECM)

(Ingrouille and Eddie, 2006). Arbuscular, ecto-, and ericoid mycorrhizae diverge in benefits conferred to the host plant. ERM allow plant uptake and assimilation of organic N and P not directly accessible by uncolonized roots (Smith and Read, 2008). AM mycorrhizae enhance host plant scavenging ability for mineral P by increasing surface-area in contact with soil through production of root hair-like, extra-radical hyphae that extend to a length of one to several hundred centimeters into the surrounding soil matrix (Rhodes and Gerdemann, 1980; Smith and Read, 2008). Along with the mantle and Hartig net, ECM fungi form rhizomorphs, lengthy mycelia that extend up to several meters into the surrounding soil, which extend the plant's range to mine the soil for nitrogen, phosphorus, and other nutrients (Tibbett and Sanders, 2002; Smith and Read, 2008). Taxonomically, ERM fungi fall within the phylum Ascomycota, and the most common ERM fungi belong to the orders Leotiales and Eurotiales. AM fungi were previously assigned to the phylum Zygomycota and order Glomales but were recently reclassified into a separate new phylum, the Glomeromycota (Schubler et al., 2002). ECM fungi are grouped within of several orders of the Ascomycota and Basidiomycota (Smith and Read, 2008). The ERM symbiosis is regarded as the most host-specific mycorrhizal association, as ERM occur only in hair roots of ericaceous plants (Smith and Read, 2008). In contrast, AM fungi are found in greater than two-thirds of plant species (Newsham et al., 1995). The host range of ECM fungi is intermediate to that of ERM and AM fungi (Smith and Read, 2008). Arbutoid or ectendomycorrhizae are formed by basidiomycete fungi on a limited range of plants, *Arbutus* and *Arctostaphylos* spp., in the Ericaceae, along with a few hosts outside the Ericaceae (Smith and Read, 2008). Arbutoid mycorrhizae combine the morphology of ERM and ECM, consisting of a mantle (dense fungal tissue covering root tips) and Hartig net (intercellular hyphae) along with intracellular hyphal coils (Zak, 1976; Harley and Harley, 1987).

ERM fungi are facultative biotrophs; they survive as saprotrophs but are capable of colonizing roots of compatible hosts (Hutton, 1996; Rice and Currah, 2006; Smith and Read, 2008). In the absence of a host, ERM fungi survive as hyphae (e.g., *Rhizoscyphus ericae* Zhuang and Korf, *Oidiodendron maius* Barron) (Hutton et al., 1996), as conidia and sterile gymnothecia (ascocarps lacking an outer covering of parenchymous tissue) (*O. maius*) (Rice and Currah, 2002), or within thickened epidermal cells shed by the host (Smith and Read, 2008). ERM fungi may be recovered via use of bait plants, isolation from colonized roots, or incubation of organic matter from ericaceous soils, e.g., boreal forests, peat bogs, and heathlands (Rice and Currah, 2002, 2006). At infection, a hypha attaches itself to a root, penetrates the epidermis, and forms a filamentous knot or coil of hyphae that occupies individual cells within the epidermal layer but does not disrupt the interfacial cell membrane or advance into the xylem or endodermis (Schenck, 1982, Smith and Read, 2008). Hyphal coils and a plant host-derived membrane serve as the interface for nutrient and carbohydrate exchange between host and fungus (Smith and Read, 2008). Infection may occupy up to 80% of the cellular area within root segments (Schenck, 1982) and is confined mainly to epidermal cells of hair (feeder) roots (Pearson and Read, 1973). External hyphae extend no more than 1 cm (0.4 inch) into surrounding soil (Read, 1984), although Gough (1994) noted that the lengths of extraradical hyphae may reach 2.5 cm (1 inch). Colonization of the root cells is transient (Kemp et al., 2003), lasting about 11 weeks from initial contact through root cell senescence (Mitchell and Gibson, 2006). Temporal fluctuations in mycorrhizal colonization may parallel carbohydrate levels within the host (Korcak, 1988; Scagel and Yang, 2005). The most abundant infection occurs on roots within the upper organic soil horizon on native, undisturbed sites (Rachel, 2004), but highly infected roots also occur deep in the soil profile (Scagel and Yang, 2005).

The most common ERM associations in the Ericaceae, including *Vaccinium* spp., are formed by the ascomycetes *Rhizoscyphus ericae* (Read) Zhuang & Korf and *Oidiodendron maius* Barron (Smith and Read, 2008; Mclean et al., 1999; Monreal et al., 1999; Berch et al., 2002). *R. ericae* (anamorph *Scytalidium vaccinii* Dalpé, Sigler & Litten) is part of the broader *Hymenoscyphus ericae* aggregate in the order Leotiales (Egger and Sigler, 1993; Zhang and Zhuang, 2004; Read and Smith, 2008). This ERM-forming species was first designated as *Pezizella ericae* Read (Read, 1974), and is also referred to as *Hymenoscyphus ericae* (Read) Korf & Kernan in the older literature. The updated classification of *R. ericae* is based on DNA sequence-based taxonomy (Zhang and Zhuang, 2004; Sigler and Egger, 2005). The *H. ericae* complex includes non-ERM saprophytes and species that are ectomycorrhizal on *Pinus* spp. (Vrålstad et al., 2002). Villarreal-Ruiz et al. (2004) demonstrated that an isolate of *R. ericae* was capable of simultaneously forming mycorrhizae on *Pinus sylvestris* L. and *Vaccinium myrtillus* L. under controlled conditions. Vrålstad (2004) suggested that fungi capable of forming both ecto- and ericoid mycorrhizae may allow for transfer of nutrients and carbohydrates between coniferous and ericaceous plants in forest ecosystems. *Oidiodendron maius* var. *maius* is a hyphomycete that performs dual roles as a saprophyte and ERM-fungus (Rice and Currah, 2006). In previous reports of *O. griseum* Robak isolated from ericoid roots, the fungus was likely misidentified, since *O. maius* is the only species within the genus that forms ERM *in situ* (Rice and Currah, 2005).

In addition to ERM fungi, Stevens et al. (1997) isolated three fungal species not typically reported as associated with blueberry roots from various sites in Pennsylvania: *Cenococcum* sp. (typically an ectomycorrhizal fungus), *Scytalidium vaccinii*, later identified as an *R. ericae* anamorph (Egger and Sigler, 1993), and an additional unidentified isolate. Koske et al. (1990)

reported a *Glomus* sp. AM fungus alongside ERM fungi colonizing roots of several native Hawaiian *Vaccinium* species, apparently members of an ancestral clade of ericaceous plants that have not yet lost the capacity for the AM symbiosis. Interestingly AM fungi are more ancient than plant roots and predate the ERM symbiosis by 300 million years (Brundrett, 2002). Basidiocarps of *Clavaria argillacea* Fr., known to exchange phosphorus and carbohydrates with plant hosts (Englander and Hull, 1981) were observed at the base of lowbush blueberry (*Vaccinium angustifolium* Ait.) plants in Maine (Peterson and Litten, 1989). The importance of the association between basidiomycetous *Clavaria* sp. and ericaceous roots is not known. *Clavaria argillacea* is commonly regarded as a mycorrhizal symbiont of the Ericaceae (Legon and Henrici, 2005) but at this time there is no clear evidence for this (Smith and Read, 2008). Overall, these findings indicate high potential diversity of ERM fungi and a less clearly defined boundary between ERM, AM, and ECM fungi than was previously known.

Use of culture-independent methods has allowed identification of previously unclassified root endophytes of the Ericaceae. An unculturable *Sebacina*-like basidiomycete was found in roots of salal, *Gaultheria shallon* Pursh (Ericaceae), in British Columbia using DNA sequence analysis. The discovery was made after endophytes were recovered from 10% of visibly mycorrhizal root segments via culture-based isolation techniques, but DNA isolation and amplification revealed that 65% of colonized root segments were inhabited by an unknown basidiomycete, while less than half of the roots contained DNA of ascomycetous ericoid mycorrhizal fungi (Berch et al., 2002). The authors concluded that molecular methods are superior to traditional methods for identification of mycorrhizal fungi in roots. Several researchers have investigated basidiomycetes suspected as ERM fungi, but the functional significance has not yet been determined due to the difficulty of isolation and resynthesis *in vitro*

(Allen et al., 2003); Bougoure and Cairney, 2005; Smith and Read, 2008). Culture-based and molecular techniques have also helped identify additional ascomycete orders (Pleosporales, Chaetothyriales, Rhytismatales, and Xylariales) capable of colonizing ericaceous roots and transferring nutrients to the host (Berch et al., 2002; Allen et al., 2003).

The preferred method for isolating culturable ericoid fungi from roots is a sterile water rinse followed by tissue maceration and serial dilution, although surface sterilization with bleach, hydrogen peroxide, or ethanol is also common (Read, 1996; Smith and Read, 2008). Assessment of colonization is carried out using a microscope with a grid-line technique modified to estimate the percentage of root cortex cells containing hyphal coils (Pearson and Read, 1973; Giovannetti and Mosse, 1980; Scagel, 2005). Other methods of ERM quantification, such as ergosterol content (Olsrud et al., 2007), maximum density of colonization (Yesmin et al., 1996), or number of extra-radical hyphae (Starast et al., 2006) have been proposed, but the percentage of total root cells colonized seems to best correlate with plant benefits derived from the ERM symbiosis (Diaz et al., 2006). In future studies, quantitative PCR may allow investigators to forgo time-consuming clearing of roots and manual counting using microscopy, while DNA sequencing may prove useful for identification, regardless of endophyte amenability to isolation on laboratory media.

The ERM association allows increased uptake of soil nutrients by plants, which occurs by hyphal secretion of extracellular enzymes that depolymerize organic residues in soil and subsequent uptake of mobilized soil nutrients (Smith and Read, 2008). Yang et al. (2002) found lower concentrations of ^{15}N in mycorrhizal plants compared to non-mycorrhizal plants, but similar levels of total N, indicating that mycorrhizal plants had greater access to organic soil N because dilution of the heavy isotope is assumed to be due to uptake of non-labeled organic N

from soil. ERM increase plant uptake of organic nitrogen, including amino acids (Bajwa et al., 1985), peptides (Bajwa and Read, 1985), proteins (Bajwa et al., 1985; Spinner and Haselwandter, 1985), and chitin (Kerley and Read, 1997, 1998). ERM also increase plant uptake of ammonium (Read, 1983) and nitrate (Kosola et al., 2007), although Read et al. (2004) reported that plant uptake of nitrate was unaffected by ERM. There are inter- and intraspecific differences among ERM fungi in terms of their ability to absorb organic N and transfer it to the plant host (Cairney et al., 2000), indicating that natural processes may select for isolates suited to a particular ecological niche. Yang (1999) reported that ericoid fungal collected from native blueberry habitats had higher protease activity than those collected from commercially managed fields. ERM fungi enhance host utilization of inorganic P (Myers and Leake, 1996) and synthesize phosphatases (Read, 1983), allowing access to organic P (phosphomonoesters such as Al and Fe phytates and phosphodiesters such as nucleic acid) (Leake and Miles, 1996), which may be important to the fitness of the host plant because most phosphate is present in esterified rather than ionic form in acid-organic soils inhabited by the ericaceous plants (Mitchell and Gibson, 2006). Host Fe uptake from soil is increased by ferricrocin and siderophores, chelating agents with high affinity for Fe that are synthesized by ERM fungi, while iron toxicity that may occur in wet, acidic soils is also ameliorated by ERM colonization (Shaw et al., 1990; Haselwandter, 2008).

The ecological importance of the ericoid mycorrhizal symbiosis to ericaceous plants becomes apparent if one considers the nitrogen-limited, lignin- and phenol-rich soil types where these plant species have evolved (Read, 1996). For example, in the case of ERM and non-ERM plants growing side by side in a nitrogen-poor environment, the ERM plant bypasses the need for N mineralization and is able to monopolize soil organic N, giving a competitive advantage to

ERM-colonized plants where soil N supplies are limited. Read (1984) reported that plants grown with alanine as an organic N source had 25% greater biomass when inoculated with ERM fungi. For ericaceous plants in natural ecosystems, it is typical to find that >90% cells of fine roots are colonized by ERM fungi (Read, 1996; Scagel, 2003b). On N-limited sites, up to 86% of plant N uptake occurs through ERM hyphae in exchange for up to 20% of C fixed by the host (Hobbie and Hobbie, 2006). Conversely, loss of the competitive ability of Ericaceae under conditions of elevated inorganic soil N is demonstrated by declining native heath populations and resulting transition of the dominant flora to more mineral N-competitive grasses on sites where significant atmospheric deposits of nitrogen occur (Bobbink and Lamers, 2002).

Ericoid mycorrhizae in Vaccinium *spp.*

All *Vaccinium* spp. are colonized by mycorrhizal fungi (Caruso and Ramsdell, 1995). Although *Vaccinium* spp. are not obligate mycorrhizal symbionts (some Orchidaceae require association with mycorrhizal fungi for seed germination, for instance), even early observers of ERM on *V. corymbosum* noted that colonization "seems to benefit the plant in unknown ways" (Coville, 1910). Rice and Currah (2006) suggest that the plant is the major beneficiary in the ERM association, describing ERM as "controlled parasitism of a fungal partner by the host plant" after finding no evidence of C transfer to the fungus. In contrast, Hobbie and Hobbie (2006) reported that ERM received up to 20% of C fixed by the host (Hobbie and Hobbie, 2006). Since blueberries lack root hairs (Gough, 1994), mycorrhizal infection serves to increase cell surface area in contact with soil. ERM colonization often increases uptake of both mineral and organic forms of nutrients, particularly N (Smith, 1980; Yang and Goulart, 2000; Kosola et al., 2007). Direct access to nutrients in organic polymers via association with ERM bypasses mineralization as a prerequisite to nutrient assimilation by plant roots, which explains the

positive effect on plant growth often reported in studies where ericaceous plants were inoculated with ERM fungi. Although some have hypothesized that mycorrhizal colonization may increase the range of pH where blueberries can be cultivated (Reich et al., 1982), maximum protease synthesis occurs at the optimal soil pH for blueberries (pH < 5.5), which provides evidence of the blueberry plant's dependency on ERM and requirement for acidic soil (Read, 1984b; Yang et al., 2004; Scagel and Yang, 2005).

Mycorrhizal colonization of highbush blueberries and other cultivated *Vaccinium* spp. has been associated with varying cultural practices (mulch, irrigation, fertilization rate, soil disturbance), edaphic factors (pH, available nitrogen, texture) and cultivar specificity (Stevens et al., 1997; Scagel, 2003a, 2005; Scagel and Yang, 2005; Scagel et al., 2005). Mycorrhizal colonization of rabbiteye blueberry, *V. ashei* Reade, in the southeastern United States was less than 5% in younger fields and up to 50% in established fields and native sites (Jacobs et al., 1982). Native blueberry populations surveyed in North Carolina had high levels of infection (>85%), while commercially grown blueberries in the same region had mycorrhizal colonization levels of only 1 to 3% (Boyer et al., 1982). Considerable variation in mycorrhizal colonization in northern highbush blueberry fields has also been reported. Goulart et al. (1993) surveyed native and commercial sites in Michigan, New Jersey, and Pennsylvania and found that ERM colonization ranged from 2% to 100%. Mycorrhizal colonization averaged of 22% in cultivated blueberry fields in Oregon (Scagel and Yang, 2005). Kasurinen et al. (2001) reported lower levels of colonization (4 to 27%) in commercial plantings of *V. corymbosum* than in native stands of *Vaccinium* spp. (21-48%), which parallels the findings reported in Stevens et al. (1997), where 20% mycorrhizal colonization was reported for native stands and 5% for cultivated fields. Schroeder et al. (1993) surveyed mycorrhizal colonization of nursery-grown plants from

Michigan, Pennsylvania, and North Carolina and found that all plants were mycorrhizal. Overall, there appears to be a trend of higher ERM colonization in native compared to commercial blueberries, with significant variation in ERM levels among commercially managed sites.

One possible explanation for lower rates of mycorrhizal infection in commercial plants relative to those in undisturbed areas is suppression of mycorrhizal colonization by synthetic N inputs. With few exceptions (Goulart et al., 1993), high inorganic soil N levels appear to suppress mycorrhizal colonization in cultivated blueberries (Goulart et al., 1993, 1995, 1998; Schroeder et al., 1993; Percival and Burnham, 2006; Scagel, 2005; Scagel and Yang, 2005). Similarly, a reduction in ERM colonization of *Calluna vulgaris* L. corresponded to an increasing gradient of added mineral N (Yesmin et al., 1996). Frequent soil cultivation practiced by some growers could also be detrimental to ERM. Soil disturbance was associated with a decrease in mycorrhizal colonization in native *Vaccinium* spp. (Hutton et al., 1997). Fungicides may reduce, increase, or have no effect on ERM colonization (Percival and Burnham, 2006; Kosola and Workmaster, 2007; Walker et al., 2010). The lack of fungal inoculum due to no prior site history of ERM hosts may contribute to low ERM colonization levels of cultivated Ericaceae (Scagel, 2003b). In addition, genetic traits of hosts and root symbionts interact to affect colonization levels (Eynard and Czesnik, 1989; Noe et al., 2000; Starast et al., 2006; Scagel and Yang, 2005). Ongoing studies of mycorrhizae in wheat (*Triticum* aestivum L.) suggest that breeding efforts may select for plant responses to synthetic fertilizers and eliminate host dependence on mycorrhizal symbioses in modern cultivars (Zhu et al., 2001).

The contribution of ericoid mycorrhizae to the vigor and productivity of managed *Vaccinium* spp. has not been fully determined. Mycorrhizal colonization has benefited plant growth or yield in some studies (Yang et al., 1996, 1998a, 1998b; Yang and Goulart, 1997;

Starast et al., 2006), but had no effect in others (Goulart et al., 1995). Scagel (2005) reported that nursery plants rated as grade 1 or grade 2 had higher mycorrhizal infection levels than inferior grades. Goulart et al. (1995) suggested, albeit based on preliminary data, that "practices that increase blueberry plant growth and vigor do not increase the concentration of mycorrhizas in the roots". As concluded in recent reports (Yang et al. 2002; Percival and Burnham, 2006), research is needed to more conclusively determine benefits of the ERM symbiosis to blueberries grown under different production regimes.

Ryan (1999) hypothesized that mycorrhizae may be of great importance on organic farms. This logic seems reasonable since: 1) plant nutrients are supplied mostly as insoluble forms on organic farms, and thus availability to crops is not as easily manipulated as with the use of synthetic fertilizers; 2) nutrients, especially N, have been a limiting factor to crop yields on organic farms (Edwards, 1990; Berry et al., 2006); and 3) it is well documented that mycorrhizal colonization can enhance plant uptake of nutrients under limiting conditions.

ERM may be important interface for nutrient uptake and transfer in organically grown blueberries. Read (1984) found no difference in plant biomass between ERM-inoculated and uninoculated *V. corymbosum* plants when provided with ammonium as a nitrogen source, but inoculated plants accumulated 25% greater plant biomass when alanine, an amino acid, was provided as the N source, which demonstrates a significant advantage of ERM-colonized plants in organic N assimilation. In a greenhouse experiment, Scagel (2003a) found that inoculation of blueberry roots with three species of ericoid fungi had little effect on above-ground or root biomass of plants grown with mineral-N fertilizers, but noted that inoculation increased biomass of plants grown with a commercially available blended organic fertilizer. In a later study, Scagel (2005) found higher levels of ERM colonization in blueberries grown with organic fertilizer than

those given synthetic fertilizer. Under field conditions, mycorrhizal colonization of agronomic crops increases linearly with duration of site history of organic management (Gollner et al., 2005). Strawberry roots had higher levels of AM root colonization in organic fields than conventional fields as well as higher yields in later years of organic transition, which correlated with mycorrhizal infection level (Werner et al., 1990). Li et al. (2004) reported that ERM colonization in blueberries was increased by addition of organic matter to soil. Litten et al. (1985) reported that field concentrations of the pre-emergent herbicide hexazinone were not inhibitory to an isolate of *H. ericae* (=*R. ericae*) in culture, but speculated that herbicide use may have far-reaching consequences on ERM fungi. Muller et al. (2006) did not quantify mycorrhizal infection, but reported a three-fold difference in foliar N levels of young blueberry plants grown with conventional vs. organic fertilizer, suggesting difficulty in providing a constant supply of nutrients to blueberries with organic fertilizers. Scagel (2003a) concluded that colonization by mycorrhizae may not fully compensate for soil nutrient deficiencies in organically grown blueberries.

Mycorrhizal colonization has been reported to provide protection from infection by plant pathogens (Smith and Read, 2008). Martins et al. (2003) reported that an isolate of the ectomycorrhizal basidiomycete *Pisolithus tinctorus* (Pers.) Coker & Couch inhibited *Phytophthora cinnamomi* Rands *in vitro*. Inoculation of tomato, *Solanum lycopersicum* L., with arbuscular mycorrhizal fungi induced systemic resistance to early blight, caused by *Alternaria solani* (Ellis and Martin) Jones & Grout, but did not induce plant resistance to late blight, caused by *Phytophthora infestans* (Mont.) de Bary (Noval et al., 2007). Some have suggested that ERM colonization may provide a barrier against invasion by root pathogens (Koron and Gogala, 1998; Grunewaldt-Stoecker, 2003), but this has not been observed empirically. Grunewaldt-Stoecker

(2003) found that ERM fungi were antagonistic to a *Phytophthora* sp. in culture. *Oidiodendron maius* serves as a biocontrol of root diseases when associated with non-ericaceous plant species (Rice and Currah, 2006), but a similar effect has not been reported in ericaceous roots. Although impacts of ERM on plant disease suppressiveness may be indirectly related to improved overall health status of the colonized plant via improved nutrient scavenging and heavy metal tolerance (Smith and Read, 2008), the long history and ubiquity of the mycorrhizal association in the Ericaceae supports further investigation into the relationship between plant pathogens and ERM.

Chapter 2.

Effects of fertilizer, cover crop and mulch treatments on soil microbes during blueberry field establishment

Abstract

Monitoring soil biological characteristics is of interest to organic growers under the prevailing notion that healthy soils support healthy plants. Few studies have examined soil microbial communities in blueberry production systems. Furthermore, the relationship between cultural practices and soil microbial communities during the organic transition phase has not been well documented. Total and beneficial cultivable bacteria and fungi were monitored in a newly established organic blueberry planting. Management practices included fertilizers (compost, protein meal, and ammonium sulfate), mulches (various locally available organic and plastic materials), and between-row cover crops (alsike clover, crimson clover, cereal rye). Compost application generally resulted in higher populations of streptomycetes. The protein meal fertilizer treatment had higher fungal populations than the synthetic fertilizer treatment by the second year of the study. Organic mulches increased populations of total and beneficial bacteria and *Trichoderma* spp. fungi but did not affect total fungal populations. The cereal rye cover crop enhanced bacterial populations compared to alsike clover in the first but not second year of the study. Significant positive correlations in population sizes were observed among many of the microbial groups assessed. However, fluorescent pseudomonads and streptomycetes were negatively correlated in the cover crop and fertilizer trial but not in the mulch trial. Overall, we observed measureable effects of soil amendments on populations of cultivable soil microbial over short (months) and intermediate (2−3 years) time intervals. Populations of cultivable microbes were often positively correlated with soil pH, and appeared to be limited at the lower

range of soil pH tolerated by blueberries. This finding suggests use of cultivable microorganism populations as a criterion of soil health may be at odds with nutritional requirements of blueberries where sulfur is continuously applied to maintain an acidic soil pH.

Introduction

Monitoring soil biology is of interest to organic growers, for whom a major goal in crop management is to facilitate an active and resilient community of soil organisms, under the assumption that healthy soils support healthy plants (Sooby et al., 2007). In addition to standard laboratory analysis (e.g., NH_4^+-N, NO_3^--N, P, total C), an increasing number of growers are interested in monitoring changes of biological characteristics of soils to better understand how soil management contributes to soil function and crop productivity (USDA-NRCS, 2004). Biological assays of soil are an informative means for assessing management-induced changes in soil health in farming systems (van Diepeningen at al., 2006, Birkhofer et al., 2008; Bonanomi et al., 2009). The terms soil quality and soil health are often used interchangeably, but soil quality most broadly refers to "the fitness of a specific kind of soil, to function within its capacity and within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain water and air quality, and support human health and habitation" (Karlen et al., 1997), while soil health explicitly emphasizes biological characteristics of soils (Van Bruggen and Semenov, 2000). Various indices, such as relative and total microbial populations, suppressiveness to plant disease, and carbon (C) and nitrogen (N) cycling in relation to plant nutrient availability, are used to assess soil health and quality, and some of these are becoming standardized for routine use in state-run (e.g., Cornell University, Ithaca, NY) and private (e.g., Soil Food Web Inc., Corvallis, OR) soil testing laboratories. Other methods are under development and have not been employed by farmers, but have potential for tracing management

impacts on soil properties over short to intermediate time scales (Moore-Kucera et al., 2008). The understanding of biologically mediated functions of soil is still in its infancy (Sugden et al., 2004). Identification of measures of soil health that are responsive to management practices and related to crop productivity are needed to improve sustainability of crop production (Weinhold et al., 2004; USDA-NRCS, 2004).

Soils are highly complex and interactive ecosystems. Communities of soil organisms and their responses to various management practices are virtually impossible to characterize in a single study (Sturz and Christie, 2003). This is due to a myriad of feedbacks and interdependencies among various orders of the soil food web and abiotic (pH, moisture, and temperature) factors that shape microbial community structure and activities (Coleman et al., 2004; Bardgett, 2005). Soil health indicators are now being used to quantitatively assess the impacts of management (Gugino et al., 2009). Applied, grower-driven research into soil health has its origin in studies of annual cropping systems such as corn-soybean rotations (Wander et al, 1994) and tomatoes in California (Drinkwater et al., 1995) and North Carolina (Bullock and Ristaino, 2002; Tu et al., 2006), and more recent studies in perennial crops such as cherries (Sanchez et al. 2003; Moore-Kucera et al., 2008) and apples (Rumberger et al., 2007). Measures of soil health in these studies include microbial diversity and abundance, carbon (C) and nitrogen (N) turnover as a proxy for microbial activity and soil N-supplying capacity, respectively, and plant yield response to assess the connection between plant and soil health.

One criterion often used to predict gross responses of soil microbial communities to inputs is the carbon to nitrogen (C:N) ratio of various inputs. C inputs to soils include plant litter (leaves, sloughed root cells), root exudates, and the byproducts and cell remnants of heterotrophic (non-CO_2-assimilating) organisms such as decomposer fungi and bacteria

(Bardgett, 2005). The most biologically active C pools in soil appear to be plant-derived (Grandy and Neff 2008), while more recalcitrant C pools are protected from decomposition via physical inaccessibility, such as organic matter complexed within soil aggregates (Grandy et al., 2002), or biochemical recalcitrance found in highly lignified, microbially processed, and chemically resistant compounds in humic soil fractions (Grandy and Neff, 2008). Inputs of labile C to soil on organic farms may be higher than on conventional (non-organic) farms, where weeds are controlled by herbicides and soil fertility is maintained by fertilizers supplied in mineral form. C inputs to soil promote and sustain functioning of the soil food web (USDA-NRCS, 2004).

Although C inputs in cropping systems generally improve soil properties and stimulate microbial activity, less is known about how inputs of varying chemical composition differ in their effects on soil microbial communities. Because various forms of C are decomposed by distinct categories of soil microbes (Cleveland et al., 2006; Hanson et al., 2008), each is likely to differ in its influence on specific microbial taxa. For example, sugars released in the rhizosphere of plants are readily degraded and utilized by rhizosphere microbes such as fluorescent *Pseudomonas* spp. (Vančura, 1980). Cellulose added with plant biomass inputs is hydrolyzed to glucose and utilized as a source of energy by a variety of saprotrophic microbes, including ascomycete fungi, such as *Trichoderma* spp. (Sinsabaugh and Linkins, 1989), actinomycetes including *Streptomyces* spp. (Srinivasan et al., 1991), and non-filamentous bacteria, such as *Bacillus* spp. (Paul, 2007). Conversely, organic matter containing a high proportion of C as lignin or humic fractions is a relatively poor quality source of C and turns over slowly due to its high-molecular weight, aromaticity, and steric hindrance to enzymatic degradation (Grandy and Neff, 2008). A distinct consortium of microorganisms, known collectively as “miners”, release

non-specific, reactive oxygen species and other oxidative enzymes to access biochemically shielded nutrients in more refractory soil organic matter pools (Sinsabaugh, 2010).

Highbush blueberries (*Vaccinium corymbosum* L.) are an economically important crop in Michigan. Michigan has nearly 20,000 acres of cultivated blueberries and produces more blueberries than any other state in the US (Kleweno and Matthews, 2008). Statewide, organic blueberry production is growing, but comprised less than 0.3% of the total cultivated area in 2007 (pers. comm., Michigan Department of Agriculture accredited organic certifiers, list available at http://www.michigan.gov/mda/0,1607,7-125-1569_25516-55175--,00.html). Recent studies that are particularly relevant to organic blueberry producers have examined the relationship between sawdust amendment and plant fertilizer-N uptake and partitioning (White, 2006), effects of organic inputs on nitrogen acquisition by ericoid mycorrhizae (Yang et al., 2002), and the efficacy of substituting organic for conventional pest management inputs (Sciarappa et al., 2008). Studies of soil microbial communities in blueberry production systems are conspicuously lacking.

Growers in Michigan with experience in both conventional and organic production agree that organic blueberry farming is vastly different from conventional farming (Michigan blueberry growers, pers. comm.). A typical sequence observed upon conversion from conventional to organic practices is a precipitous decline in crop vigor and yield followed by an eventual rebound in productivity that was previously attained with conventional practices (Wander et al., 1994). Although Martini et al. (2004) suggest that a learning curve may be associated with adapting management strategies to the distinct challenges of organic farming, there is evidence that changes in soil microbial communities during the organic transition period are tightly coupled to changes in plant growth and yield (Ryan, 1999; Phelan, 2004; Birkhofer et

al., 2008). Soil microbial communities during the organic transition phase have not been investigated in blueberry production fields.

Organic inputs added to soil by regional blueberry growers vary widely (Michigan blueberry growers, pers. comm.). The relative effects of multiple soil inputs in a field setting have not been widely tested, as recent field studies of soil inputs in organic farming have largely focused on two or three locally available inputs. Furthermore, the wet and acidic nature of blueberry soils foster microbial communities that may respond differently to management practices compared to most other agroecosystems, as was found in a recent study of cranberry (*V. macrocarpon* Aiton), a close relative of blueberry (Stackpoole et al., 2008). Therefore, characterizing the response of soil microbes to varied cultural practices is needed to understand changes in soil microbial communities that may occur during the transition from conventional to organic management. With this aim, my objective was to document changes in soil microbial communities in response to different management practices in a new organic blueberry research planting.

Materials and methods

Site preparation and cultural practices

The trial was conducted in a 1-acre field at the Michigan State University Horticulture and Teaching and Research Center (Figure 2.1). The soil type is a Spinks loamy sand (Mixed, mesic Psammentic Hapludalfs). Pre-plant field preparation began in the summer of 2007 and included soil disking and an herbicide (glyphosate) application to manage perennial weeds. An elemental sulfur (S) application was made to lower soil pH; pelletized S (Tiger Organics 0-0-0-90) was applied to reach a target soil pH of 4.5 to 5.5, which was necessary because blueberries develop iron chlorosis at a soil pH > 5.5 (Johnson, 1959; Korcak, 1989, 1993). In the fall 2007,

prior to planting, soil organic matter (SOM) in the field ranged from 2.0 to 1.1% along an east-to-west gradient while soil pH was 6.2 ± 0.05 (mean ± standard error). SOM content was low compared to the highly productive, 3- to 5%-SOM sites along the Lake Michigan shore where the highest density of commercial blueberry plantings are located in Michigan (NRCS soil survey maps, http://websoilsurvey.nrcs.usda.gov/app/; Kleweno, 2007). Sand content decreased from 88% to 79% along an east-to-west gradient, with a corresponding increase in silt content. In the eastern portion of the field, which was designated for the mulch trial, aged dairy compost was spread at 101 metric ton/ha (45 ton/a) in the fall of 2007. Compost was not added to the west half of the field to avoid confounding the effect of the fertilizer treatments, which included compost as one treatment. In the spring of 2008, the soil pH where compost was applied was 6.5, while soil with no added compost had a pH of 5.6. Two-year-old container-grown blueberries were planted over two weeks in late April and early May of 2008 in 14 rows oriented perpendicular to the SOM and soil texture gradient. 'Bluecrop' was grown in the cover crop and fertilizer study, while 'Elliott' was planted in the mulch-trial portion of the field. After planting, chipped brush mulch was spread uniformly to a depth of 15 cm (6 in.) and width of 0.75 m (2.4 ft) (Gough, 1996) in all rows except rows 10 through 13, which were designated for a mulch comparison. Plots were hand-weeded several times per year and mulch was supplemented in 2009 to maintain a 15-cm (6-in.) depth. Irrigation from a well-water source was applied through drip tubing when the soil water tension measured less than 50 centibars. Total irrigation water applied in 2008 was approximately 3 acre-inches. Increases in soil pH appeared to be related to the high pH of irrigation water and consequently S was broadcast evenly in all rows on several dates to maintain the soil pH below 5.5. Total S applied over three years (including site preparation) is estimated at 5.6 metric ton/ha (2.5 ton/a).

Cover crop treatments

Cover crop treatments – annual winter rye (*Secale cereale* L.), crimson clover (*Trifolium incarnatum* L.), and alsike clover (*T. hybridum* L.) – (Table 2.1), were seeded in fall 2007 and replicated four times in approximately 50-m (164-ft) long plots in alleys [2-m (6.6 ft)-wide strips] between blueberry rows. Rye established and overwintered well, while crimson and alsike clover treatments failed to overwinter in some plots and required reseeding in spring and supplemental irrigation applied with a traveling sprinkler during establishment. The above-ground biomass of crimson clover was 3730 ± 104 kg/ha (3290 ± 229 lb/a) and N content was 2.2% (dry weight). Biomass of alsike clover and rye were not determined. Cover in alsike clover matched that of generally well-established crimson clover stands in some plots but was poor in others. Rye was about 1.7 m tall at anthesis in early June before it was terminated with a tractor-mounted, water-filled roller-crimper (Magdoff and Van Es, 2009). In fall 2008, rye plots were rototilled and reseeded, and alsike and crimson plots were flail-mowed. In spring 2009, the alsike clover and crimson clover treatment plots were rototilled and seeded to different cover crop treatments but these did not establish and plots were managed as untilled fallow for the remainder of the year. Surviving clover and weeds were present to varying degrees in the former clover treatment plots in 2009 but ground cover in these was sparse and only the rye accumulated biomass at a comparable level to that of 2008.

Fertilizer treatments

Three fertilizer inputs were compared: 1) Synthetic fertilizer, 2) McGeary Organics Fertilizer, and 3) a custom blended compost (Morgan's Composting, Evart, MI) (Table 2.2). The synthetic fertilizer consisted of ammonium sulfate. The McGeary Organics Green-Up & Side Dress Fertilizer 8-1-1 (McGeary Organics, Inc., Lancaster, PA) had an analysis of 8% N, 0.9% P

(as P_2O_5), and 1% K (as K_2O) and provided N mostly as of amino acids and proteins. Compost (Morgan Composting, Sears, MI) consisted of a blend of dairy manure compost, poultry litter compost, bone meal, wood ashes, and feather meal and had an analysis of 2.0% N, 0.3% NH_4^+-N, 0.3% NO_3^--N, 0.3% P (as P_2O_5), 1% K (as K_2O), pH of 7.7 and C:N ratio of 11. The rate of the synthetic fertilizer treatment was based on MSU Extension recommendations (Hanson and Hancock, 1996). Rates of McGeary's Fertilizer and Compost were estimated based on an expected seasonal N mineralization rate of 60% for McGeary Fertilizer and 20% in compost (E. J. Hanson, pers. comm.). The fertilizer rates applied are shown in Table 2.2. In year one (2008), the compost was incorporated into soil before bushes were planted. In year two, the compost was left unincorporated but added prior to supplemental mulch. McGeary and synthetic fertilizer treatments were broadcast over the planting row in both years. Compost treatment plots were amended with elemental S (1 lb S per 100 lb compost, wet weight) to mitigate compost-induced iron chlorosis in blueberries that was observed in preliminary trials (E. J. Hanson, unpublished data). The rate of S was determined by titration of a 1:2 (w/v) soil:H_2O with 2N sulfuric acid over 2 days to pH 5.0. Compost was applied in April and applications of synthetic fertilizer and McGeary Organics Fertilizer were split evenly between April and May. In 2009 the rate of all fertilizer treatments was doubled to mitigate observed N deficiency. Fertilizer treatments were arranged in a split-plot design with cover crop treatments blocked by row and fertilizer treatments randomized as complete blocks within cover crop plots. Treatments were applied in even-numbered rows in the west half of the field. Seasonal N availability in this trial was monitored by Hanson (unpublished data).

Mulch treatments

Nine mulch treatments were selected based on cost, availability, prior studies (Majek, 2006), and consultation with growers (Table 2.3). Treatments were arranged in a randomized complete block design in four rows in the eastern portion of the field; each row served as one block. Each mulch treatment was replicated once per row in four-bush plots. Two weeks after planting, mulches were applied to the same depth and width as described in the cover crop and fertilizer study. Woven plastic landscape fabric (black and white, respectively) and burlap coffee sacks were secured with metal stakes. Plant nutrient requirements in the mulch trial were met by the compost applied to the entire trial in fall 2007, additional compost applied in late fall 2008 at 9 metric ton/ha (4 ton/a), and McGeary Organics Fertilizer 8-1-1 applied at 44 kg N/ha (39 lb N/a) in 2008 and 88 kg N/ha (79 lb N/a) in 2009. Soil pH, temperature, nitrogen availability, and weed management costs were monitored by E. J. Hanson (unpublished data).

Soil sampling

Soil samples were collected on 19 Oct 2007, 30 Oct 2008, and 29 Oct 2009. Late fall sampling was chosen for accuracy in measuring the effects of organic amendments, which were applied at different times within the first half of the growing season, e.g., split applications of some (McGeary Organics, ammonium sulfate) but not other (compost) fertilizer treatments and annual supplementation of some (hay, burlap) but not all mulch treatments. We assumed that late-season sampling would measure longer-lasting, more constant effect of organic inputs after the initial "pulse" of biological activity had subsided (Yao et al., 2005). Freeze-thaw, wet-dry cycles, and soil temperature fluctuation would also be avoided to a greater extent by fall sampling. In addition, root growth in highbush blueberry mostly occurs between fruit maturation in late summer and the onset of dormancy in late fall (Abbott and Gough, 1987a). Profiles of soil

microbial communities during periods when plants invest substantial energy to belowground portions may be more meaningful than during periods when plants allocate energy to shoots and fruit. Due to these factors and finite availability of resources, we collected samples over three years (2007 to 2009) in the fall.

Each sample consisted of eight soil cores taken at a depth of 0 to 10 cm (0 to 3.9 in.) with a diameter of 2.5 cm (1 in.) diameter 0.2 to 0.3 m (0.7 to 1 ft) apart within each plot. Samples were collected outside the drip line of bushes to avoid collection of peat added with container-grown blueberries at planting. Soil was placed in 3.78-L (1-gallon) polyethylene zipper bags, transported in chilled coolers and then stored at 4˚C for up to 10 days before use.

Soil dilution plating and colony enumeration on selective media

Soils were diluted to 10% (w/v) in phosphate-buffered saline (Sanbrook et al., 1989) and placed on a shaker table set at 128 oscillations per minute for 30 minutes. The selective media and microbes isolated were TSP for *Trichoderma* spp. (Askew and Laing, 1993), Dichloran Rose Bengal Chlortetracycline (DRBC) (King et al., 1979) with chloramphenicol substituted for chlortetracycline for general fungi, STR for *Streptomyces* spp. (Conn et al. 1998), S1 for fluorescent *Pseudomonas* spp. (Gould et al., 1985), STR for *Streptomyces* spp. (Conn et al. 1998), S1 for fluorescent *Pseudomonas* spp. (Gould et al., 1985), heat treatment at 90°F for 30 minutes and tryptic soy agar (TSA) for *Bacillus* spp. (Bashan et al., 1993), and $1/10^{th}$-strength TSA for general bacteria (Bashan et al., 1993). DRBC was preferred to RBC due to the presence of zygomycetes that filled petri dishes with mycelium within 3 to 5 days after plating and impeded or prevented precise colony counts. A preliminary experiment showed that addition of dichloran had no effect on the number of colonies recovered (unpublished data). The volume and soil dilutions plated were, 800 µl10^{-1} on TSP, 200 µl 10^{-2} on DRBC, STR, S1, and TSA

(*Bacillus* spp.), and 200 µl 10^{-4} on $1/10^{th}$-strength TSA (bacteria). Beneficial groups of microbes were chosen based on pathogen suppression, plant growth promotion, or induced plant disease resistance attributed to the respective taxa in other studies, for example, total cultivable bacteria (Postma et al., 2008; Bonanomi et al, 2010), *Bacillus* spp. (Bulluck et al., 2002; Orhan et al., 2006), fluorescent *Pseudomonas* spp. (Bulluck and Ristaino, 2002; Hass and Defago, 2005; Bonanomi et al., 2010), *Streptomyces* spp.(Crawford et al., 1993; Aini et al., 2005; Postma et al., 2008), and *Trichoderma* spp. (Bulluck et al., 2002; Bulluck and Ristaino, 2002; Vargas Gil et al., 2009; Bonanomi et al. 2010), while general media for fungi and bacteria indicate the population size of cultivable species. Drawbacks of the dilution plating method include preferential selection for fungi with prolific sporulation traits (Van Bruggen and Semanov, 2000), disregard for uncultivable species that comprise a sizable and often overlooked component of microbial communities (Torsvik et al., 1990), underrepresentation of taxa with a slow growth response to glucose that are outcompeted by so-called "sugar fungi", e.g., *Mucor* spp. and *Penicillium* spp. (Hudson, 1968; Rice and Currah, 2002), and lack of correspondence between *in-situ* microbial populations and those assessed on agar media (Thormann, 2006). Sewell (1959) contends that information provided by soil plating methods is not reflective of dominant microflora in acidic and highly organic heath soils, characteristics that also apply to blueberry soils in Michigan. Still, the dilution plating method is useful for monitoring changes in microbial communities (Vargas Gil et al., 2009).

Three petri-dish subsamples per soil sample were included in 2008 and this was reduced to two in 2009. After diluted soil was spread on agar, plates were incubated in the dark at 22°C. Colony numbers were assessed after 3 days for fluorescent pseudomonas, general bacteria, and bacilli and after 7 to 10 days for streptomycetes and fungi. If plate counts could not be completed

within these intervals they were stored at 4˚C in darkness for up to two weeks. Soil moisture content was determined by drying 10 g of soil for 3 days at 80˚C. Colony forming units (CFU) per g dry soil was the unit of measure for microbial populations and calculated from the total number of colonies on petri dishes and soil moisture according to the formula

$$\text{CFU/g dry soil} = \frac{(\text{\# of colonies, mean of plate replicates})*(\text{dilution factor})*100}{(100 - \%\ \text{soil moisture})}$$

Other measures

Soil pH was determined on 5 g soil in a 1:1 (w/v) deionized water:soil mixture after shaking for 1 minute and allowing soil particles to settle for 30 minutes (North Central Region Recommended Soil Testing Procedures, http://extension.missouri.edu/explorepdf/specialb/sb1001.pdf). Soil moisture content was determined by drying field-moist soil in a drying oven at 80°C for 72 hours.

Leaf spot and twig dieback were assessed on 25 September 2009. Pre- and post-field establishment nematode community structure was profiled according to Sanchez et al. (2003).

Statistical analysis

Cover crop and fertilizer main effects and their interactions were analyzed as a split-plot design using the GLIMMIX procedure in SAS (SAS Institute, Cary, NC). Block and the cover crop by block interaction were specified as random effects, and the block by cover crop by fertilizer interaction was specified as a within-subject factor for the effect of year. Inclusion of soil pH as a covariate in the cover crop and fertilizer study is justified as differences in soil pH were expected among fertilizer treatments due to the high pH of compost. The effect of compost was mitigated by adding an acidifying agent (elemental S) to the compost treatment plots at the

time of compost application. Use of soil pH as a covariate thus partitioned error due to field variability from the effects of treatments under investigation. If interactions between main effects were not significant ($P < 0.05$), means were compared with the LINES option of the LSMEANS statement, which is roughly equivalent to Fisher's least significant difference but takes heterogeneous variance among treatments into account (SAS, 2009). If significant interactions between fixed effects were observed, means were compared using the SLICE option of the LSMEANS statement; a Tukey-Kramer adjustment was used for comparison of simple effect means. Analysis of interactions and the pH covariate applies only to the cover crop and fertilizer study. Mulch treatment means were compared with the LINES option of the LSMEANS statement. The relationship between measured variables was assessed with Pearson's r statistic, computed with the CORR procedure in SAS.

Results

Pre-and post-field establishment microbial populations

Pre-field establishment samples were collected to compare microbial populations in the years before and after treatments were applied. In 2007, microbial populations were highest in the portion of the field where dairy compost was applied (for the mulch trial) one month prior to soil sampling and a similar trend was observed in the abundance of bacteria-feeding nematodes (Table 2.4). Populations of fungi, streptomycetes, and fluorescent pseudomonads increased by 10 to 25, 20 to 50, and 2 to 6 fold, respectively, between 2007 and 2008 (Table 2.4). Total nematode populations doubled in size between 2007 and 2008 (Table 2.4).

Cover crops and fertilizers

There was a significant main effect of fertilizer treatments on the populations of fungi, fluorescent pseudomonads, and streptomycetes (Table 2.5) and year on populations of bacteria,

fluorescent pseudomonads, and streptomycetes. Due to interactions between the effects of cover crop, fertilizer, and/or year on most microbial populations, treatment effects were analyzed separately (Table 2.5). For total bacterial populations, there were no significant interactions between cover crop, fertilizer and year (Table 2.5), but main effects of cover crops within years are shown for consistency with subsequent data. In 2008, the population of bacteria was significantly affected by cover crop ($P < 0.003$) and was higher in rye than alsike clover but not significantly different between rye and crimson clover or alsike clover and crimson clover (Figure 2.2). Bacterial populations were not significantly different among cover crop treatments in 2009 (Figure 2.2) or fertilizer treatments in either year (Table 2.5). There was a significant interaction among cover crop, fertilizer, and year effects on streptomycete populations ($P = 0.005$). Streptomycete populations in cover crop and fertilizer treatments were pooled across 2008 and 2009 samples, i.e., only the cover crop by fertilizer interaction was considered. Within the rye cover crop, streptomycete populations were higher in the compost than the McGeary Organics fertilizer treatment and intermediate in the synthetic fertilizer treatment (Figure 2.3). Within the alsike clover cover crop, streptomycete populations were highest in the compost treatment, followed by McGeary Organics fertilizer, and lowest in the synthetic fertilizer treatment (Figure 2.2), while bacterial populations did not differ among fertilizer treatments within the crimson clover cover crop. Populations of fluorescent pseudomonads were affected by fertilizer treatments within alsike clover ($P = 0.03$) and significantly higher in the McGeary Organics fertilizer than compost treatment (Figure 2.4). Populations of *Bacillus* spp. were not affected by treatments in 2008 or 2009 (Table 2.5).

Significant cover crop by year and fertilizer by year interactions were observed for populations of fungi. In 2008, fungal populations were highest in the rye, intermediate in alsike

clover, and lowest in crimson clover, but not significantly affected by cover crops in 2009 (Figure 2.5). Conversely, among fertilizer treatments, fungal populations were not significantly different in 2008. However, in 2009 fungi were more abundant in the McGeary Organics fertilizer than the synthetic fertilizer treatment, but not different between compost and McGeary Organics fertilizer or compost and synthetic fertilizer treatments (Figure 2.6). *Trichoderma* spp. populations were not assessed in 2008 but were not affected by cover crop or fertilizer treatments in 2009 (Table 2.5).

When soil pH was not considered as a covariate, significant main or interactive effects of cover crops and fertilizers were observed only for fungal and streptomycete populations (Table 2.6). Mean responses of microbial populations to cover crop × fertilizer combinations in 2008 and 2009 are shown in Table 2.7.

There were significant positive correlations between populations of bacteria and fungi and streptomycetes and fungi, while significant negative correlations were observed between pseudomonads and fungi and between pseudomonads and streptomycetes (Table 2.8 and Figure 2.7).

In the fertilizer trial, populations of most nematode trophic groups (herbivore, omnivore, and carnivore) tended to be highest in compost-amended plots in 2008 (Table 2.9). Compost supported the greatest number of dorylaims, non-plant-parasitic nematodes, and total nematodes, while compost and synthetic fertilizer treatments had more bacterial feeding nematodes than McGeary Organic fertilizer. The ratio of non-plant parasitic to plant-parasitic nematodes also did not vary significantly among fertilizer treatments but was lowest in the synthetic fertilizer treatment (Table 2.10). Nematode populations were not assessed in 2009.

Soil pH was not affected by main effects of cover crop or fertilizer but was a significant covariate in all analyses (Table 2.5). Over the entire trial, soil pH was lower in 2009 than in 2008 (Table 2.5). Soil pH was positively correlated with populations of fungi, bacteria, and streptomycetes and negatively correlated with populations of fluorescent pseudomonads (Table 2.8 and Figure 2.8).

Leaf spot and twig dieback occurred at low levels in the field in 2009 but were unaffected by the cover crop, fertilization or mulch treatments ($P < 0.05$).

Mulches

Mulches significantly differed in their effects on soil pH and all microbial populations except fungi (Table 2.11). Bacterial populations were significantly higher in chipped brush compared to straw, pine bark, and no mulch (Figure 2.9). Organic mulches, with the exception of straw and pine bark, tended to increase bacterial populations compared to the woven white and black plastic and unmulched treatments (Figure 2.9). Streptomycetes were most abundant in the burlap mulch treatment (Figure 2.10). Fluorescent pseudomonad populations showed high variability in response to different mulch treatments, with approximately eight-fold higher population sizes in the chipped brush and wood chip treatments compared to the unmulched control (Figure 2.11). *Bacillus* spp. populations were increased two to three-fold by the spoiled hay mulch treatment but remained relatively unaffected by the other treatments (Figure 2.12). Although the difference was not statistically significant, fungal populations tended to be highest under burlap and woven white plastic mulches and least under no mulch, black plastic, and wheat straw (Figure 2.13). *Trichoderma* spp. populations were significantly higher under chipped brush and burlap mulches than pine bark, and woven white or black plastic (Figure 2.14).

The soil moisture content at sampling was about 1.5 fold higher under spoiled hay compared to black plastic or no mulch and intermediate in remaining treatments (Figure 2.15). The soil pH ranged from a mean of 5.6 under the chipped brush mulch to 4.1 with no mulch, while the pH remained between 4.7 and 5.0 in the remaining mulch treatments (Figure 2.16).

All significant ($P < 0.05$) correlations between microbial taxa in the mulch trial were positive (Table 2.12). Furthermore, the correlation between bacteria and fluorescent pseudomonad populations was highly significant (Figure 2.17). Soil pH was positively correlated with populations of fluorescent pseudomonads and bacteria (Figure 2.17).

Discussion

As expected, the conversion of the field site from fallow conditions in 2007 to an established blueberry planting in 2008 resulted in substantial increases in microbial populations. This finding reflects microbial C-limitation under fallow conditions and stimulation of microbial growth by C inputs to soil provided by cover crops, fertilizers, and mulches. Some studies have shown that fungi are favored over bacteria as the C:N ratio of soil organic matter inputs increases (Rousk et al., 2010a). Therefore, we expected the high C:N ratio of rye to stimulate fungal growth and the lower C:N ratio of clover to encourage bacterial populations. The observed treatment effects agreed to some extent with this hypothesis, but interpretations of our results are complicated by statistically significant interactions between cover crop and fertilizer treatments and variability in soil pH among treatment plots and between sampling dates.

The highly significant effects of cover crops on soil microbial populations were somewhat unexpected given that the above-ground portions of plants were limited to row middles. One explanation for cover crop effects on soil microbes is root encroachment into blueberry rows, which were irrigated and amended with mulch and fertilizer and thus likely

provided more favorable conditions for roots than row middles which were not fertilized or irrigated. The rhizosphere – or soil within a few millimeters of plant roots – has one to two orders of magnitude higher biological activity than bulk soil (Lugtenberg and Bloemberg, 2004; Kourtev et al. 2002). Therefore, root encroachment of between-row cover crops into blueberry rows at least partially account for the significant effects of cover crops on microbial populations despite the fact that they were not planted in the blueberry rows. Cover crops differed in their effects on the microbial taxa assessed in this study, possibly due to both the quality and quantity of root exudate and biomass inputs. Roots of alsike clover, which was rated only fair in its stand development in 2008, likely encroached less into blueberry rows than plants in the well-established rye or crimson clover stands. Lower root encroachment in the alsike clover stand may explain why the differences in the effect of fertilizers on streptomycetes and fluorescent pseudomonads were most pronounced within in the alsike clover treatment. Also, differences in nutrient availability among the fertilizer treatments (Hanson, unpublished data) may have influenced root growth of both blueberries and cover crops and thereby favored certain microbial groups depending on the fertilizer × cover crop combination.

Rhizosphere effects may also explain differences in fungal populations between the clover and rye treatments. Due to atmospheric N fixation by clover, the soil in clover treatments may be less N limited (i.e., more N-enriched) than that of cereal rye, a non-N-fixing plant that requires a significant amount of soil N during its life cycle. In broad terms, fungi may be better adapted to soil N-limitation than bacteria because mycelia allow for redistribution of N from N-rich to N-poor (high C:N) patches. Bacteria, in contrast to fungi, advance through soil passively, via movement of soil water or other organisms, and lack the ability to translocate nutrients due to unicellular growth. We expected that soil N availability would be higher in the clover cover

crops than rye, and populations of fungi would be favored by lower N availability in the rye treatment. The results appear to support our initial hypothesis. However, the magnitude of the effects of alsike and crimson clover depended on their degree of stand establishment. In 2008, fungal populations were least in the crimson clover cover crop (most N added and no N immobilized), intermediate in the relatively poorly established alsike clover (some N added and no N immobilized) and highest in the rye cover crop (no N added and some N immobilized). Therefore, variation in N availability among cover crop treatments may have been one mechanism for their effects on populations of cultivable soil fungi. The reason fungal populations differed significantly among cover crop treatments in 2008 but not 2009 may be due to the fact that plots that were planted to clovers in 2008 were managed as fallow and only the rye plots were in place in 2009.

Compared to fungi, bacteria have a lower C:N ratio and are less able to scavenge N from the surrounding environment (Paul, 2007). Therefore, lower C:N biomass of clover and higher soil N availability in the clover treatments were expected to create an advantage for bacteria over fungi, while higher C:N ratios in rye were hypothesized to favor fungi. However, increased bacterial populations by low C:N of inputs was not observed in the cover crop study, as total bacterial populations were highest in rye. Thus, cover crop effects on bacteria appeared to reflect the amount of aboveground cover crop biomass, which was estimated as highest in rye, intermediate in crimson clover, and lowest in alsike clover. Cleveland et al. (2006) observed that dissolved organic matter added to soil mesocosms incubated under laboratory conditions stimulated mainly opportunistic bacterial species. This indicates that quantitative differences in the amount of C added by each cover crop species may have contributed to the differences in bacterial populations that we observed. Yao et al. (2005) observed a similar relationship between

bacterial populations and aboveground inputs in a comparison of ground cover treatments in an apple orchard in New York.

Despite the fact that compost fertilizer treatment plots had 20 tons/ha (9 tons/a) greater total organic matter inputs over the course of the study, compost addition showed only modest effects on microbial populations compared to the other fertilizer treatments. Total bacterial populations were not significantly different among fertilizer treatments in either year of the study. Likewise, fungal populations were unaffected by fertilizer treatments. A significant enhancement of streptomycete populations by compost within the alsike clover plots was the only significant increase in microbial populations directly attributable to compost. Lack of a general stimulatory effect of compost on microbial populations may be due to several factors. Additional sulfur added with compost to offset the alkaline pH of the compost may have detrimentally affected soil microbial populations, either by acidifying the soil or having a direct fungicidal effect (Agrios, 2005). However, the soil pH was not significantly affected by fertilizer treatments.

A second explanation for lack of a positive effect of compost on beneficial soil microbes populations is that sampling in fall may have measured microbial populations after most of the biologically labile components of compost had been utilized (Abbasi, 2007). The media used in this study to assess microorganism population sizes select for organisms with certain traits. Bacteria and fungi cultivated on enriched laboratory media may reflect populations of r-strategist bacterial taxa (abundant spore producers that proliferate where carbon is available in the form of sugars), while microbial taxa well-adapted to the oligotrophic (carbon- and nutrient-limited) soil environment are likely to have been severely underrepresented by our methodology. It is noteworthy that streptomycetes were the only microbial group to show a consistent positive

response to compost, and the streptomycete-selective medium was the only isolation medium to contain a carbon source in a more complex form than glucose or sucrose. Thus, compost may be most beneficial to microbes that are able to utilize more complex forms of carbon and that were not selected for on the media utilized here.

A final consideration in the lack of beneficial compost effects is that chipped brush mulch was added to all plots in the compost and fertilization study. Chipped brush stimulated populations of most microbial taxa in the mulch trial (discussed below), and may have overshadowed any positive effects of cover crop and fertilizer treatments if the magnitude of the effect of chipped brush outweighed that of the cover crops or fertilizers.

Competition between microbes may explain the effect of compost on fluorescent pseudomonads and streptomycetes. Within the alsike clover plots, streptomycete populations were significantly increased by compost compared to McGeary and synthetic fertilizer treatments, apparently at the expense of fluorescent pseudomonads. Streptomycetes are able to utilize complex forms of carbon, including starch, lignocellulose, and chitin (Srinivasan et al., 1991). In contrast, fluorescent pseudomonads grow best in the presence of sucrose and other forms of carbon contained in fresh (undecomposed) plant inputs (Rovira and Ridge, 1973; Vančura, 1980). Compost addition may favor microbes able to utilize the complex C that remains after the organic residues have undergone a preliminary round of microbial processing. As plant C inputs pass through microbial decomposition, the most labile constituents are consumed while the more resistant C fractions remain and increase in abundance relative to readily utilizable compounds (Grandy and Neff, 2008).

Streptomycete populations increased in the compost treatment but only within the alsike clover plots, which established poorly compared to the rye and crimson clover, suggesting that,

in the absence of confounding factors (e.g., root encroachment), compost directly stimulates streptomycetes. An alternative explanation is that fluorescent pseudomonads were able to inhibit or outcompete streptomycetes in synthetic and McGeary's fertilizer treatments but not in the compost fertilizer treatment. Direct stimulatory effects of compost on the populations of streptomycetes seem more likely because streptomycetes can access recalcitrant C (Srinivasan et al., 1991). While fluorescent pseudomonads are able to degrade cellulose, starch, and lignin, they are found in the greatest abundance in the rhizosphere of plants and preferentially utilize simple forms of carbon (Vančura, 1980; Gould et al., 1985; Paul, 2007)

The sharp population decline in soil fungi between 2008 and 2009 may be related to a decrease in soil pH that occurred over this time frame (Rousk et al., 2010a). No significant difference among fertilizer treatments was observed in 2008, but in 2009, fungal populations were significantly higher in the protein-based McGeary Organics fertilizer compared to synthetic fertilizer treatment. McGeary Organics fertilizer 8-1-1 contains 93% of N as water-insoluble proteins and amino acids (http://mcgearyorganics.com/organic-fertilizer/8-1-1-side-dress-green-up.html). Input of protein-based fertilizer to soil may have stimulated fungal populations as it was broken down into soluble forms of N. Amino acid inputs to soil have been associated with increased fungal abundance compared to inputs of N supplied in mineral form (Lucas et al., 2007). Organic fertilizers that contain amino acids and proteins tend to stimulate secondary decomposition that follows an immediate pulse of biological activity (Hadas, 1994), which means that elevated populations of soil fungi initially stimulated by McGeary Organics fertilizer applied in late spring may have persisted into fall, when soil was collected for assessment of microbial populations. In addition, fungal populations in the compost treatment were intermediate to that of McGeary Organics and synthetic fertilizer treatments. The compost we

applied contained about 25% of N in the form of soluble inorganic N (NO_3^- and NH_4^+) and had been through an initial round of microbial processing (i.e., passage through the animal digestive system and composting), which may have rendered the C remaining less available for utilization as a C source by microbes including fungi (Robertson and Grandy, 2005; Paul, 2007). N contained in the ammonium sulfate fertilizer is completely soluble. Fertilizer effects on fungal populations in soil may be related to the ratio of insoluble organic N relative to the total amount of N applied by each fertilizer treatment. A high population of bacterivorous nematodes in the McGeary treatment in 2008 provides indirect evidence that compost applied in this study preferentially stimulated populations of bacteria rather than fungi. As mentioned previously, compost appeared to stimulate streptomycetes, which were isolated on media with more complex C. These explanations are tentative at best, and continued monitoring of bacterial and fungal populations is needed to determine whether protein-N-based fertilizers such as McGeary Organics fertilizer favor fungal-dominated decomposer webs in soil.

Trichoderma spp. populations were not affected by cover crop or fertilizer treatments. In the current study, populations of *Trichoderma* spp. quantified on TSP agar (Askew and Laing, 1993) might not fully reflect treatment effects on the total *Trichoderma* spp. populations. TSP contains several fungicides that suppress cultivable fungi besides *Trichoderma* spp., but may also selectively inhibit certain *Trichoderma* spp. (A. M. C. Schilder, L. H. Hanson and G. Samuels, pers. comm.) and favor isolation of *Trichoderma* spp. with fungicide resistance (Papavizas, 1985). Additional methods of assessing *Trichoderma* spp. should be sought to determine if the method used in this study produces results comparable to methods less reliant on fungicides for selectivity. Additionally, in a survey of organic and conventional tomato farms in North Carolina, *Trichoderma* populations were highest on conventional farms (Liu et al. 2007),

indicating *Trichoderma* spp. may be favored less by organic management that other microbial taxa.

Populations of total nematodes, in particular bacterial-feeding nematodes, were affected by fertilizer treatment in 2008, with a significant boosts in populations of dorylaims and total nematodes by compost. The McGeary Organics fertilizer treatment had much lower bacterial-feeding nematode populations those of the compost and synthetic fertilizer treatments, indirectly indicating that bacteria were less favored by McGeary Organics than compost or synthetic fertilizers. In a survey of Dutch organic and conventional farms, the populations of total, fungivorous, and bacterivorous nematodes were not affected by management type (van Diepeningen et al., 2006). A trend of higher cyst nematode counts in compost compared to McGeary Organics and synthetic fertilizers in 2008 was unanticipated given that Sanchez et al. (2003) reported significantly higher ratios of non-parasitic to parasitic nematodes after compost amendment compared to synthetic fertilizers. Bongers et al. (1997) reported an increase in populations of plant-parasitic nematodes along a gradient of increasing N-availability. In the current study in 2008, NH_4^+-N plus NO_3^--N levels were highest in the compost treatment throughout the season (Hanson, unpublished data), but non-plant parasitic nematodes were unaffected by fertilizer treatments. This indicates that C inputs may have greater influence on nematodes than N in the fertilizer study in 2008.

The significant effect of mulch treatments on soil pH and moisture levels means that the influence of mulches on microbial populations could have been mediated by effects on soil pH and moisture in addition to the quantity and quality of added C and soil N status (Hanson, unpublished), which appeared to play an important role in shaping microbial communities in the nutrient and cover crop study. Since the quantity of mulch applied was similar among organic

mulch treatments, the quality (C:N ratio) was probably a contributing factor in the effect of mulches on soil microbes.

Total populations of bacteria in the mulch treatments appear to be positively related to the lability (low C:N) among treatments. A significant difference in populations of bacteria but not of fungi among mulch treatments suggests that populations of bacteria may respond more quickly to C inputs than fungi, which were not significantly affected by mulch treatments after they were in place for two years.

Similar to total bacteria, beneficial soil bacteria populations were enhanced by the organic mulches. We observed positive effects of burlap sacks on streptomycetes and spoiled hay on *Bacillus* spp., and a near-absence of fluorescent pseudomonads in the unmulched treatment. The significance of high populations of beneficial soil bacteria in blueberry soils has yet to be determined.

The TSP medium appeared to have some utility for discernment of mulch treatment effects on *Trichoderma* spp. populations which was not apparent in the cover crop and fertilizer study. The reason for relative sensitivity of *Trichoderma* spp. mulch inputs but not cover crops or fertilizers is not known. *Trichoderma* spp. fungi are considered beneficial to plants due to multiple modes of inhibition of soilborne plant pathogens (Papavizas, 1985). Beneficial fungi may be particularly valuable as biocontrol agents in blueberry soils due to their adaptation to acidic soils compared to bacteria assessed in this study, as evidenced by the non-significant correlation between their populations and soil pH in both the mulch and cover crop study. Populations of *Trichoderma* spp. were stimulated by mulches with the most labile C (C:N ratio), in accordance with the abundant cellulase production but limited lignin degradation traits of this taxon (Sinsabaugh and Linkins, 1989). Ratios of populations of *Trichoderma* spp. to total

populations of fungi were significantly affected by mulches as well. This finding , albeit preliminary, may be applicable to blueberry sites where the incidence of root rot caused by *Pythium* and *Phytophthora* spp. is problematic. In non-traditional sites (i.e., upland soils) in other blueberry-growing regions, there have been reports of an association between use of bark and plastic mulches and root rot caused by *Pythium* and *Phytophthora* spp. (Brannen et al., 2006). In comparison, root rot has not been reported on blueberries in Michigan (Caruso and Ramsdell, 1995). Brannen et al. (2006) reported that incidence of blueberry root rot increases as the labile components of high carbon mulch materials are consumed. On non-traditional blueberry soils, maintenance of a pathogen-suppressive soil microbial community, including high populations of *Trichoderma* spp. may be desirable for promotion of plant vigor and staving off root pathogens. Our findings indicate that populations of *Trichoderma* spp. may be increased and enriched relative to total cultivable fungi by the addition of mulches with low C:N ratios.

The populations of the majority of microbial taxa were positively correlated in the cover crop and fertilizer study. This was expected, according to our hypothesis and previous findings (e.g., Yeoung-Seuk et al., 2002) that populations of microbial taxa should increase in tandem.

Populations of fluorescent pseudomonads were negatively correlated with those of both streptomycetes and fungi. This suggests that these organisms occupy opposing niches in blueberry soils, which is not surprising considering their contrasting life strategies. Fungi and streptomycetes have a filamentous growth habit and decompose a variety of C compounds while pseudomonads agglomerate in polysaccharide-dense colonies and proliferate when labile C is readily available (Vančura, 1980; Rovira and Ridge, 1973). Fluorescent pseudomonads and streptomycetes appear to occupy opposing niches, as observed in the increase in populations of streptomycetes by compost at the expense of pseudomonads within the alsike clover treatment.

A significant positive relationship between soil pH and bacterial and fungal populations (or their cultivable representatives) indicates microbes may be limited by the soil acidity required for cultivation of blueberries. Soil pH has been shown to explain divergent patterns of bacterial communities among ecosystems (Fierer and Jackson, 2006). Direct inhibition of bacteria by low soil pH (pH<5.5) may contribute to the relative dominance of fungi over bacteria in acidic soils (Rousk et al, 2010a). Fungal dominance over bacteria at low soil pH was not apparent in the cover crop and fertilizer study, where both fungal and bacterial populations were positively correlated with soil pH. However, it is possible that rapid growth characteristics of "sugar fungi" which are isolated on DRBC agar may overwhelm slower-growing fungal taxa which were likely overlooked in this study. Rice and Currah (2002) demonstrated that fungal taxa common to acidic bog soils in Canada were not represented on typical laboratory media used for studies of population sizes of cultivable soil fungi.

The upper quantile, median, and lower quantile soil pH of samples in the cover crop and fertilizer study in 2009 were 5.9, 5.6, and 5.2, respectively, with a similar range observed in the mulch study. At a soil pH between 4 and 5, values typical for blueberry soils, populations of all microbial groups appeared to taper off sharply in both the cover crop and fertilizer and mulch studies. Interestingly, fluorescent pseudomonad populations did not appear to decrease with decreasing soil pH in the cover crop and fertilizer study. In fact, in contrast to other microbial groups, populations of fluorescent pseudomonads increased between 2008 and 2009 despite the decrease in soil pH which occurred over this time span. It is possible that the chipped brush material applied throughout the cover crop and fertilizer study provided suitable environment or a C substrate for fluorescent pseudomonad populations to thrive. It is possible that fluorescent pseudomonads may be more affected by C availability than soil pH. Nonetheless, a highly

significant positive relationship between fluorescent pseudomonads and soil pH in the mulch trial provides some evidence that fluorescent pseudomonad populations are reduced as soil pH declines from 6 to 4, as was found for populations of other soil microbes.

Soil sulfur was effective for reducing the soil pH to a level appropriate for blueberries. The upper quantile, median, and lower quantile soil pH of samples in the cover crop and fertilizer study in 2009 were 5.9, 5.6, and 5.2, respectively, with a similar range found in the mulch study. However, lower pH was likely a factor in reduced populations of most in microbial taxa in 2009 compared to 2008. The rate of S applied to soil over three years in this study is roughly equivalent to 42 years of elemental S fungicide applications (assuming 5 sprays per year and 12 lbs per acre per application). A recent study demonstrated that soil bacteria are more sensitive than fungi to decreasing soil pH (Rousk et al., 2010b). Because sulfur was applied to the entire experimental site, we not able to partition the direct effects of sulfur from its effect on soil pH. Extra S applied with the compost to neutralize its alkaline pH may have limited the beneficial effects on soil microbial populations that are sometimes attributed to compost application (Guidi et al., 1998; Sivapalan et al; 1993; Bulluck et al., 2002; Liu et al., 2008).

Conclusion

In a three-year study of organic blueberry establishment, cover crops, fertilizers, and organic amendments varied significantly in their effects on populations of cultivable soil microbes. Beneficial bacteria were most affected by mulch applications and were generally enhanced by organic mulches compared to plastic or no mulch treatments. Compost provided lower than anticipated benefits to cultivable microbes other than *Strepomyces* spp., while an organic protein-based fertilizer (McGeary Organics 8-1-1) increased the abundance of fungi compared to ammonium sulfate fertilizer in the second year of the fertilizer trial. Cover crops in

row middles had an unanticipated significant effect on microbial communities within the row, possibly due to root encroachment into blueberry rows and resultant effects on soil C and N availability. A highly significant decline in microbial populations that occurred between 2008 and 2009 (with the exception of fluorescent pseudomonads) seems to be best explained by the marked decrease in soil pH in 2009. Repeated S inputs were required to prevent iron chlorosis that occurs in blueberries when the soil pH rises above 5.5.

The effects of soil amendments such as mulches and organic fertilizers on soil microbes will need to be studied in the coming years to establish the longevity of the effects fertilizers and mulches observed during the first years of field establishment. In addition, it would be informative to characterize the contribution of microbial communities to suppression of blueberry root pathogens in traditional native blueberry field soils; root diseases have not been observed in Michigan despite the long history of blueberry cultivation in the state (Caruso and Ramsdell, 1995). Further investigation into the contribution of soil inputs to the structure and functions of microbial communities may provide additional insights into soil biology in blueberry production fields.

Table 2.1. Cover crop treatments in an organic blueberry trial at the MSU Horticulture Teaching and Research Center, Holt, MI, 2007-2009.

Treatment	Plant type	Mature height	Annual seeding date	Stand cover in 2008	Stand cover in 2009
Cereal rye	Grass	1.7 m (5.5 ft)	Fall	good	good
Alsike clover	Legume	0.4 m (1.3 ft)	Fall/spring	fair	-[z]
Crimson clover	Legume	0.25 m (0.8 ft)	Fall/spring	good	-[z]

[z]Reseeded to different cover crops that did not establish; subsequently managed as fallow.

Table 2.2. Fertilizer treatments in an organic blueberry trial at the MSU Horticulture Teaching and Research Center, Holt, MI, 2008-2009.

Treatment	Description	Supplier	N-P-K (%)	2008 kg ha^{-1} ($lb\ a^{-1}$)	2009 kg ha^{-1} ($lb\ a^{-1}$)
McGeary Organics 8-1-1	Dried blood, soybean meal, cocoa expeller cake, compost (plant or animal source not specified)	McGeary Organics, Lancaster, PA	8-1-1	92 (82)	184 (164)
Compost - custom blend	Dairy manure compost, poultry litter compost, feather meal bone meal, wood ashes	Morgan Composting, Evart, MI	2-0.3-1	1,100 (981)	2200 (1963)
Synthetic	Ammonium sulfate	-	22-0-0	43 (38)	86 (77)

Table 2.3. Mulch treatments in an organic blueberry trial at the MSU Horticulture Teaching and Research Center, Holt, MI, 2008-2009.

Treatment	Supplier/product name	Application timing	Carbon to nitrogen ratio
Burlap sacks	Paramount Coffee, Lansing, MI	2008, 2009	-[z]
Chipped brush	MSU Recycling Center, East Lansing, MI	2008, 2009	93:1
Coarse wood chips	Hammond Farms, Lansing, MI	2008	151:1
Coarse pine bark	Hammond Farms, Lansing, MI	2008	169:1
Spoiled hay	MSU Horse Teaching and Research Center, East Lansing, MI	2008, 2009	35:1
Wheat straw	Riv-Al-Ree Farms, Williamston, MI	2008	161:1
Plastic landscape fabric - white	Dewitt white weed barrier pro grade landscape fabric, Dewitt Company, Sikeston, MO	2008	-[z]
Plastic landscape fabric - black	Dewitt Pro 5 weed barrier, Dewitt Company, Sikeston, MO	2008	-[z]

[z]Not determined.

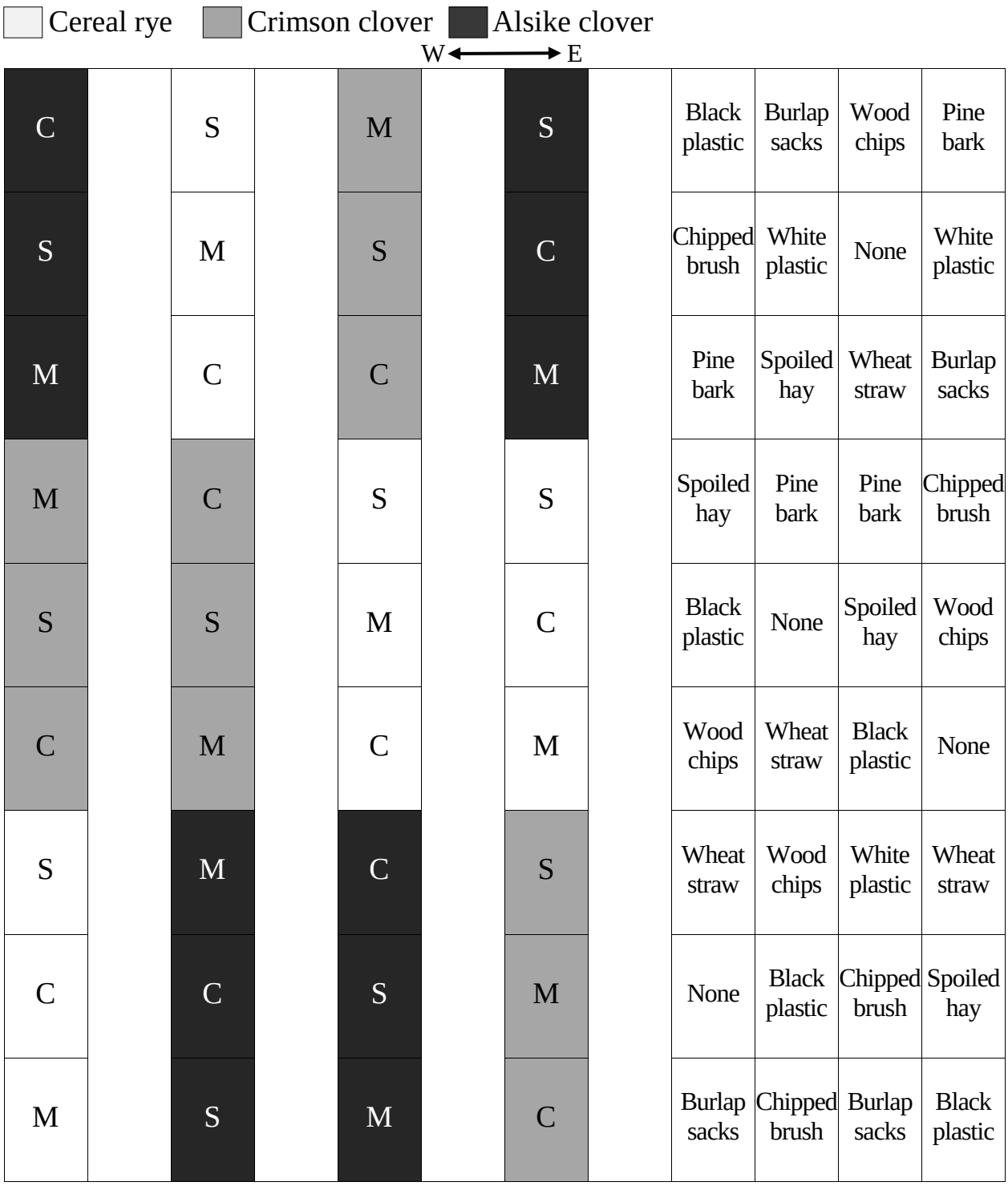


Figure 2.1. Plot layout of an organic blueberry study at the MSU Horticulture Teaching and Research Center, Holt, MI. Fertilizer sub-plots ["S"=synthetic (ammonium sulfate), "C"=blended compost, "M"=McGeary Organics 8-1-1] were arranged in a randomized complete block design (RCBD) within cover crop whole plots (legend above). Cover crops were arranged in a RCBD with north-to-south rows as blocks. Mulches were arranged in a RCBD with rows as blocks.

Table 2.4. Pre- and post-field establishment populations of soil microbes in an organic blueberry trial at the MSU Horticulture Teaching and Research Center, Holt, MI, 2007-2008.

	Population ± SE (CFU per g soil)[z]		
Microbe	**19 Oct 2007**	**30 Oct 2008**	**Increase (%)**
Total bacteria	2,979,835 ± 855,371	10,676,873 ± 585,101	258%
Fluorescent *Pseudomonas* spp.	13,287 ± 5,241	27,362 ± 2790	106%
Streptomyces spp.	27,607 ± 3,859	1,078,094 ± 95,039	3,805%
Bacillus spp.	2,048,740 ± 613,769	587,055 ± 164,900	-71%
Cultivable fungi	4,453 ± 524	83,140 ± 3,171	1,767%
Trichoderma spp.	290 ± 92	1,537 ± 106[y]	430%
Total nematodes[x,w]	210 ± 20[x]	476 ± 47[w]	126%

[z]Colony-forming units per gram of soil. SE=standard error of the mean; 0- to 10-cm soil depth; n=10 in 2007 (entire field), n=36 in 2008 (cover crop and fertilizer plots).
[y]2009 samples only.
[x]Number per 100 cm^3 soil, n=6 (entire field).
[w]Number per 100 cm^3 soil, n=9 (mean of cover crop and fertilizer trial).

Table 2.5. Mixed-model analysis of variance of the effects of cover crops and fertilizers on populations of cultivable soil microbes in an organic blueberry trial at the MSU Horticulture Teaching and Research Center, Holt, MI, 2008-2009. Soil pH is included as a covariate. Bold font indicates significance at $P < 0.05$.

Effect	**Num df**[z]	**Den df**[z]	**Fungi**	***Trichoderma* spp.**	**Bacteria**	**Fluorescent *Pseudomonas* spp.**	***Streptomyces* spp.**	***Bacillus* spp.**	**Soil pH**
					P > F				
Cover crop	2	6	0.66	0.33	**0.02**	0.15	0.27	0.94	0.25
Fertilizer	2	18	**0.0005**	0.34	0.11	**0.02**	**0.0002**	0.49	0.36
Year	4	18	0.15	-[y]	**0.001**	**0.02**	**<0.0001**	0.35	**<0.0001**[x]
Cover crop*fertilizer	1	9	0.12	0.77	0.06	**0.002**	**0.003**	0.30	**0.02**[x]
Cover crop*year	2	9	**0.0006**	-	0.84	0.06	**0.03**	0.48	**0.0001**[x]
Fertilizer*year	2	9	**0.02**	-	0.62	**0.02**	**0.0003**	0.38	**0.02**[x]
Cover crop*fertilizer* year	4	9	0.23	-	0.60	0.17	**0.005**	**0.05**	**0.009**[x]
Soil pH	1	9	0.40	0.78	0.12	**0.05**	**<0.0001**	0.03	
Soil pH*cover crop	2	9	0.80	0.40	**0.01**	0.13	0.19	0.95	
Soil pH*fertilizer	2	9	**0.002**	0.35	0.12	0.06	**0.0001**	0.62	
Soil pH*year	4	9	0.91	-	**0.006**	0.12	**0.0001**	0.65	
Soil pH*cover crop *fertilizer	1	9	0.21	0.82	0.09	**0.03**	**0.004**	0.46	
Soil pH*cover crop*year	2	9	**0.001**	-	0.78	**0.05**	**0.04**	0.37	
Soil pH*fertilizer*year	2	9	**0.02**	-	0.45	**0.02**	**0.0002**	0.26	
Soil pH*cover crop *fertilizer*year	4	9	0.19	-	0.50	0.15	**0.005**	0.07	

[z]Denominator or numerator degrees of freedom.

[y]Not applicable, variable only measured in 2009.

[x]Denominator df=27.

Table 2.6. Mixed-model analysis of variance of the effects of cover crops and fertilizers on populations of cultivable soil microbes in an organic blueberry trial at the MSU Horticulture Teaching and Research Center, Holt, MI, 2008-2009. Bold font indicates significance at $P < 0.05$.

Effect	Num df[z]	Den df[z]	Fungi	*Trichoderma* spp.	Bacteria	Fluorescent *Pseudomonas* spp.	*Streptomyces* spp.	*Bacillus* spp.	Soil pH
					P > F				
Cover crop	2	6	0.58	0.08	0.85	0.67	0.90	0.73	0.25
Fertilizer	2	18	0.79	0.66	0.28	0.07	**0.001**	0.36	0.36
Year	4	18	**<0.0001**	-[y]	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**[x]
Cover crop*fertilizer	1	27	**0.01**	0.37	0.62	0.06	**0.04**	0.65	**0.02**[x]
Cover crop*year	2	27	**0.02**	-	0.55	0.15	0.40	0.17	**0.0001**[x]
Fertilizer*year	2	27	**0.004**	-	0.21	0.98	0.33	0.14	**0.02**[x]
Cover crop*fertilizer *year	4	27	**0.02**	-	0.09	0.36	0.07	0.85	**0.009**[x]

[z]Denominator or numerator degrees of freedom.

[y]Not applicable, variable only measured in 2009.

[x]Denominator df=27.

Table 2.7. Populations of cultivable fungi, *Trichoderma* spp., bacteria, fluorescent *Pseudomonas* spp., *Streptomyces* spp., and *Bacillus* spp. in 0- to 10-cm soil as affected by cover crop and fertilizer treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009.

Cover crop	Fertilizer	Fungi (CFU[z] × 10^3 g $soil^{-1}$)	*Trichoderma* spp. (CFU × 10^3 g $soil^{-1}$)	Bacteria (CFU × 10^6 g $soil^{-1}$)	Fluorescent *Pseudomonas* spp. (CFU × 10^4 g $soil^{-1}$)	*Streptomyces* spp. (CFU × 10^5 g $soil^{-1}$)	*Bacillus* spp. (CFU × 10^5 g $soil^{-1}$)	Soil pH
				2008				
Rye	McGeary	85.5 ± 4.1[y]	-[x]	12.4 ± 1.0	2.4 ± 0.4	9.8 ± 1.8	16.0 ± 12.1	6.9 ± 0.3
	Compost	78.3 ± 5.9	-	9.9 ± 1.0	3.7 ± 1.3	15.0 ± 3.7	2.7 ± 1.5	6.9 ± 0.3
	Synthetic	92.6 ± 8.9	-	13.1 ± 3.2	2.8 ± 0.5	7.6 ± 1.6	2.6 ± 1.4	6.5 ± 0.3
Crimson clover	McGeary	60.5 ± 9.5	-	9.7 ± 0.4	1.6 ± 0.7	7.6 ± 2.2	6.8 ± 5.5	5.6 ± 0.4
	Compost	90.6 ± 7.1	-	11.8 ± 1.7	3.8 ± 1.2	14.4 ± 3.1	2.9 ± 1.0	6.8 ± 0.2
	Synthetic	82.2 ± 7.8	-	11.2 ± 0.5	3.2 ± 1.0	10.0 ± 1.6	3.5 ± 0.7	6.6 ± 0.5
Alsike clover	McGeary	76.3 ± 4.6	-	8.4 ± 3.3	2.3 ± 0.5	15.8 ± 4.3	10.5 ± 6.8	6.3 ± 0.3
	Compost	102.1 ± 15.3	-	10.4 ± 1.2	2.2 ± 0.5	11.2 ± 0.9	3.5 ± 0.6	6.3 ± 0.4
	Synthetic	80.2 ± 10.0	-	9.2 ± 1.1	2.6 ± 1.2	5.6 ± 2.0	4.2 ± 0.6	5.9 ± 0.3
				2009				
Rye	McGeary	47.8 ± 7.3	1.2 ± 0.1	5.1 ± 3.3	6.6 ± 2.3	3.5 ± 0.6	1.1 ± 0.5	5.2 ± 0.4
	Compost	38.6 ± 3.7	1.2 ± 0.2	4.6 ± 2.3	11.9 ± 3.0	5.3 ± 0.4	1.0 ± 0.4	5.5 ± 0.3
	Synthetic	40.9 ± 2.1	1.4 ± 0.2	5.2 ± 1.9	10.7 ± 1.4	2.8 ± 0.6	0.9 ± 0.5	5.4 ± 0.3
Crimson clover	McGeary	55.5 ± 4.6	1.4 ± 0.1	4.1 ± 2.0	9.4 ± 1.0	5.4 ± 1.2	0.7 ± 0.2	5.6 ± 0.3
	Compost	37.1 ± 3.1	2.3 ± 0.8	3.9 ± 1.9	11.2 ± 0.6	4.4 ± 0.4	1.0 ± 0.3	5.6 ± 0.3
	Synthetic	54.4 ± 5.5	1.6 ± 0.1	3.8 ± 1.8	10.6 ± 1.8	4.9 ± 0.9	0.7 ± 0.3	5.5 ± 0.1
Alsike clover	McGeary	51.1 ± 4.5	1.7 ± 0.3	6.4 ± 2.6	13.0 ± 2.4	4.5 ± 0.5	0.7 ± 0.4	6.0 ± 0.2
	Compost	59.3 ± 3.6	1.4 ± 0.2	1.0 ± 0.2	10.8 ± 2.4	5.3 ± 1.2	1.1 ± 0.7	5.4 ± 0.1
	Synthetic	45.6 ± 4.6	1.5 ± 0.2	6.9 ± 3.9	10.2 ± 1.3	3.2 ± 0.4	0.6 ± 0.4	5.6 ± 0.2

[z]CFU=colony-forming unit. [y]Mean ± SEM. [x]Not determined.

Table 2.8. Pearson correlation coefficients (r) between soil microbial populations and pH in a study of cover crops and fertilizers in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009.

	Fungi	***Trichoderma* spp.**	**Bacteria**	**Fluorescent *Pseudomonas* spp.**	***Streptomyces* spp.**	***Bacillus* spp.**
Trichoderma spp.	-0.13[z]					
Bacteria	0.55***	0.02				
Fluorescent *Pseudomonas* spp.	-0.55***	0.23	-0.37**			
Streptomyces spp.	0.47***	0.04	0.46***	-0.36**		
Bacillus spp.	0.27*	0.01	0.22	-0.30*	0.16	
Soil pH	0.49***	0.20	0.51***	-0.26*	0.60***	0.28*

[z]Significance levels: *P<0.05; **P<0.01; ***P<0.001.

Table 2.9. Mixed-model analysis of variance of the effect of fertilizers on populations of plant-parasitic nematodes in soil in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008.

	Lesion nematodes	**Dagger nematodes**	**Ring nematodes**	**Cyst nematodes**	**Cyst nematode eggs**	**Root-knot nematodes**	**Total plant-parasitic nematodes**
Fertilizer	**Number per 100 cm^3 soil**						
Synthetic	3	0	20	9	760	0.7	32
Compost	5	0	41	19	2027	0.0	65
McGeary	4	0	54	2	220	0.0	60
P* > *F	0.77	n/a[z]	0.43	0.09	0.10	n/a	0.55

[z]Insufficient non-zero observations for ANOVA.

Table 2.10. Mixed-model analysis of variance of the effects of fertilizers on populations of soil nematodes (tylenchs, aphelenchs, dorylaims, mononchs, bacterial feeders, total non-plant-parasitic nematodes, and total nematodes), the ratio of non-plant-parasitic to plant-parasitic nematodes (NPP:PP), oligochaetes, and arbuscular mycorrhizal fungi (AM fungi) spores in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008. Numbers in a column followed by the same letter are not significantly different at $P < 0.05$ (Tukey's HSD). Bold font indicates the ANOVA was significant at $P < 0.05$.

	Tylenchs	**Aphelenchs**	**Dorylaims**	**Mononchs**	**Bacterial-feeders**	**Total non-plant-parasitic nematodes**	**Total nematodes**	**Ratio NPP:PP[z]**	**Oligochaetes**	**AM fungi spores**
Fertilizer	**Number per 100 cm^3 soil**									
Synthetic	22	17	27b	0	348**a**	413**b**	446**b**	16	12	70
Compost	82	25	55a	0	408**a**	570**a**	635**a**	11	22	72
McGeary	25	27	23b	0	212**b**	287**b**	347**b**	11	10	45
P > F	0.09	0.79	**0.02**	n/a[y]	**0.001**	**0.002**	**0.008**	0.79	0.38	0.35

[z]Ratio of non-plant-parasitic to total (plant-parasitic + non-plant-parasitic) nematodes.

[y]Insufficient non-zero observations for ANOVA.

Table 2.11. Mixed-model analysis of variance of the effects of mulch on populations of cultivable soil microbes in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009. Bold font indicates the ANOVA was significant at $P < 0.05$.

Num df[z]	Den df[z]	Fungi	*Trichoderma* spp.	Ratio of *Trichoderma* spp. to total fungi	Bacteria	Fluorescent *Pseudomonas* spp.	*Streptomyces* spp.	*Bacillus* spp.	Soil moisture	Soil pH
						P > F				
8	24	0.14	**0.003**	**0.03**	**0.03**	**0.005**	**0.009**	**<0.0001**	**<0.0001**	**<0.0001**

[z]Denominator or numerator degrees of freedom.

Table 2.12. Pearson correlation coefficients (r) between soil microbial populations, soil pH, and soil moisture at sampling in a study of mulches in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, 2008-2009.

	Fungi	*Trichoderma* spp.	Bacteria	Fluorescent *Pseudomonas* spp.	*Streptomyces* spp.	*Bacillus* spp.	Soil pH
Trichoderma spp.	-0.19[z]						
Bacteria	0.38*	0.35*					
Pseudomonas spp.	0.33	0.13	0.54***				
Streptomyces spp.	0.23	0.06	0.25	0.34*			
Bacillus spp.	0.03	0.22	0.27	0.12	0.06		
Soil pH	0.03	0.23	0.46**	0.71***	0.17	0.24	
Soil moisture	0.01	0.35*	0.26	0.11	-0.16	0.37*	0.37*

[z]Significance levels: *P<0.05; **P<0.01; ***P<0.001.

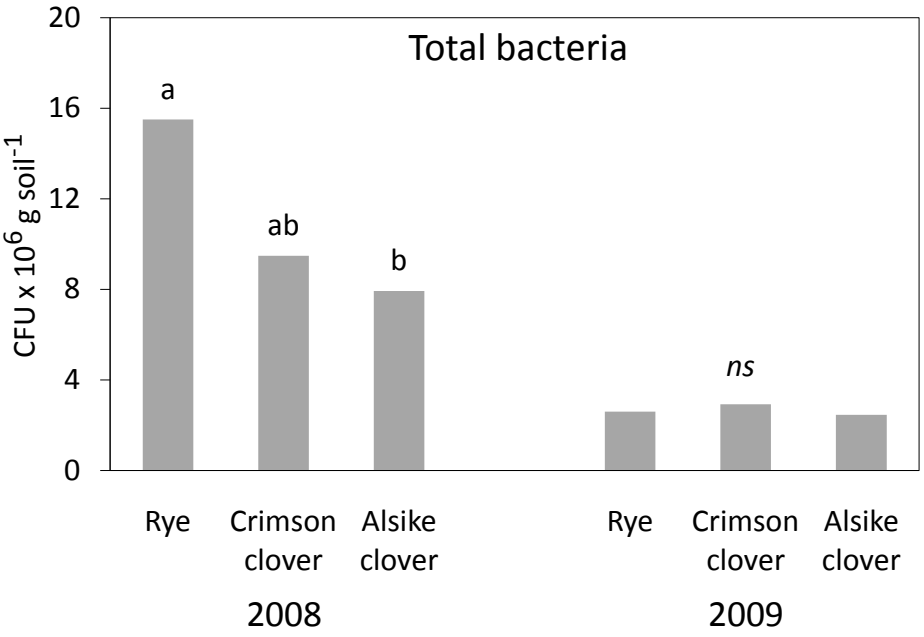


Figure 2.2. Populations of total cultivable bacteria at 0- to 10-cm soil depth as affected by cover crop treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$; *ns* indicates treatments are not significantly different at $P<0.05$ (Tukey's HSD).

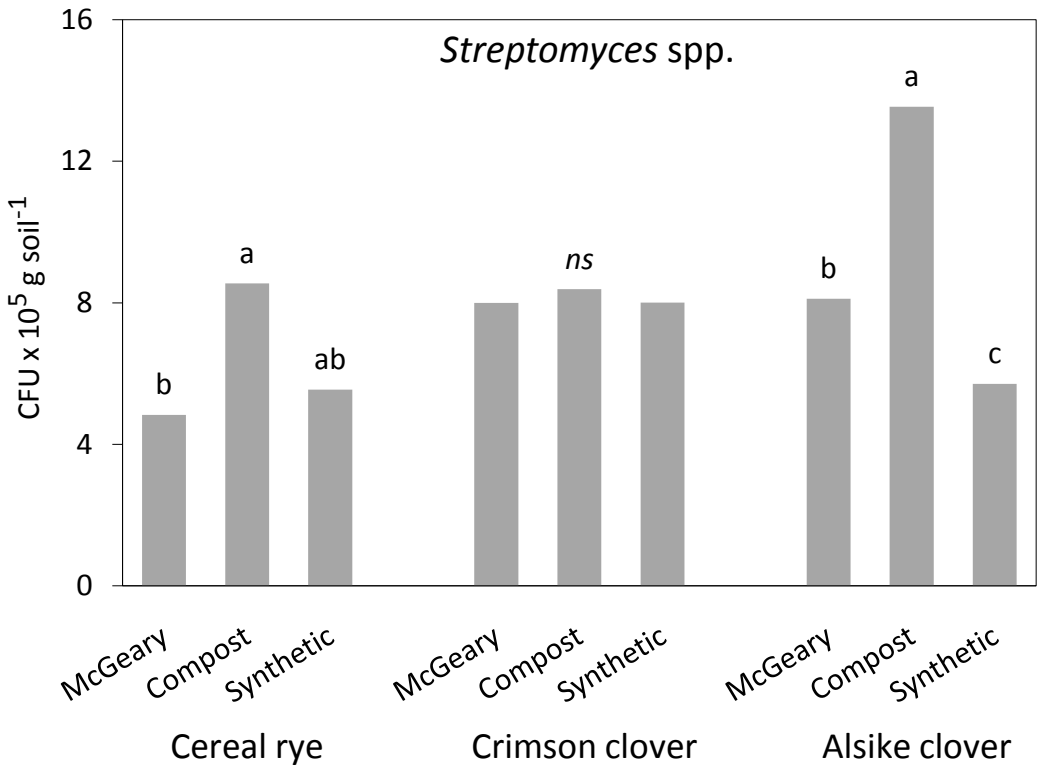


Figure 2.3. Populations of *Streptomyces* spp. at 0- to 10-cm soil depth as affected by fertilizer treatments (sub-plots) within cereal rye crimson clover, and alsike clover cover crop treatments (whole plots) averaged across 2008 and 2009 in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$ (Tukey's HSD); *ns* indicates treatments are not significantly different.

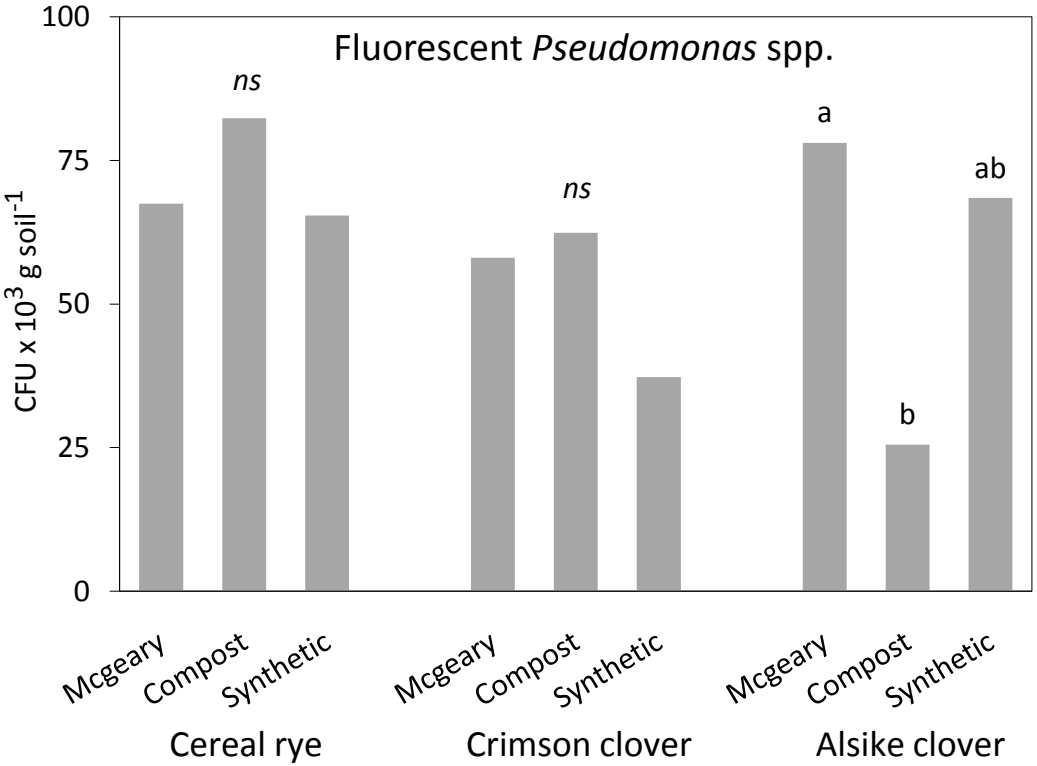


Figure 2.4. Populations of fluorescent *Pseudomonas* spp. at 0- to 10-cm soil depth as affected by fertilizer treatments (sub-plots) within cereal rye, crimson clover, and alsike clover cover crop treatments (whole plots) averaged across 2008 and 2009 in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$ (Tukey's HSD); *ns* indicates treatments are not significantly different.

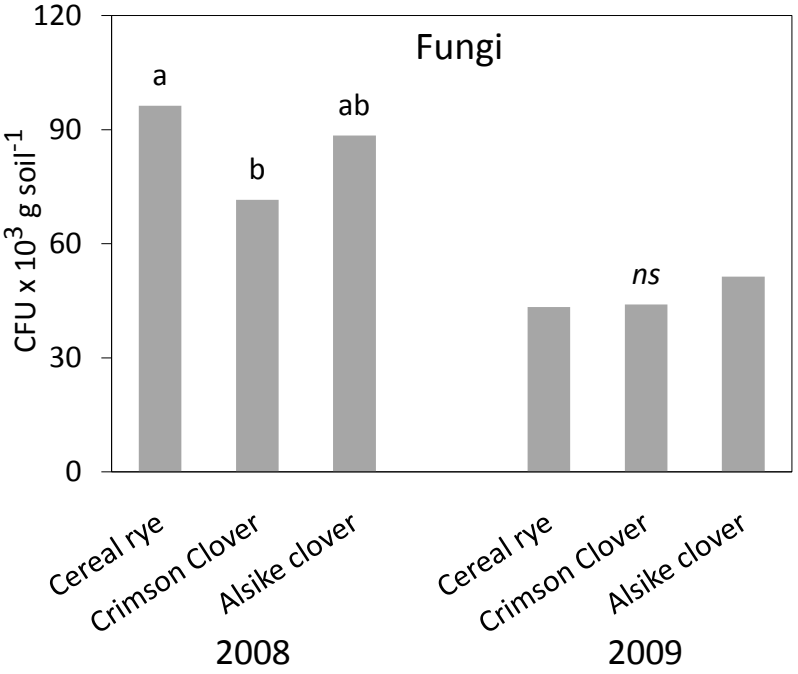


Figure 2.5. Populations of fungi at 0- to 10-cm soil depth as affected by cover crop treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$ (Tukey's HSD); *ns* indicates treatments are not significantly different.

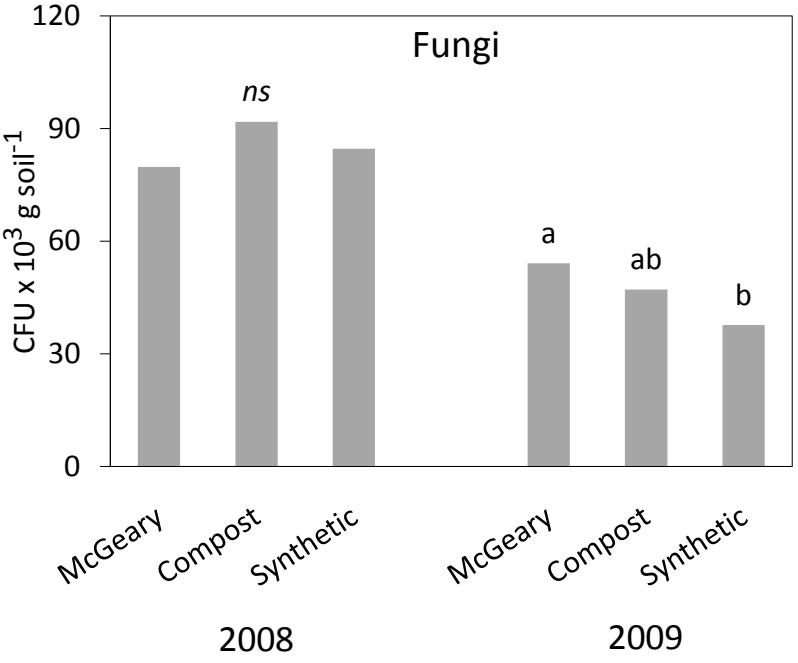


Figure 2.6. Populations of fungi at 0- to 10-cm soil depth as affected by fertilizer treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$ (Tukey's HSD); *ns* indicates treatments are not significantly different.

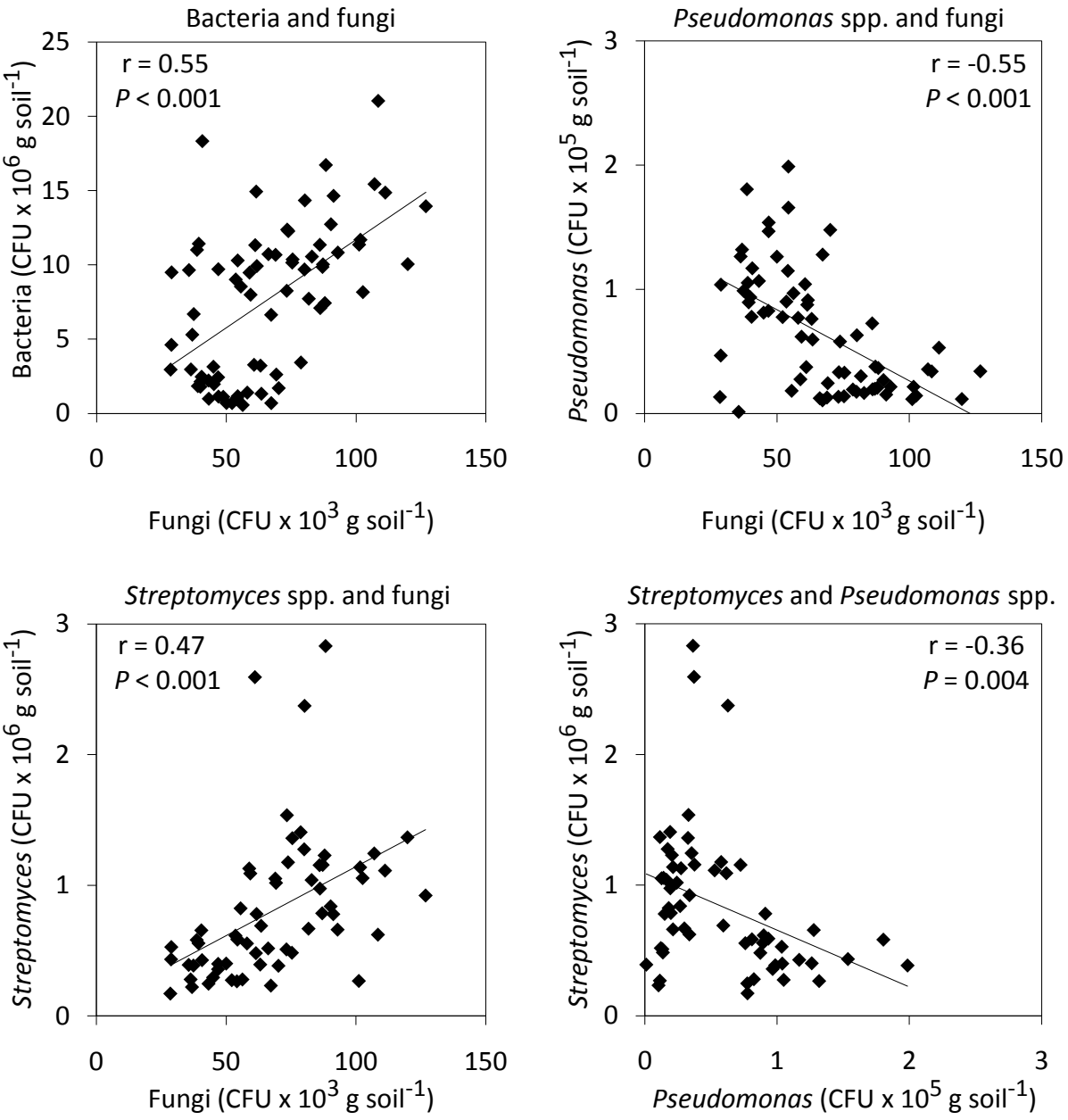


Figure 2.7. Correlations between populations of microbial groups at 0- to 10-cm soil depth in a cover crop and fertilizer study in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009. CFU = colony-forming unit. "*Pseudomonas* spp." refers to fluorescent *Pseudomonas* spp.

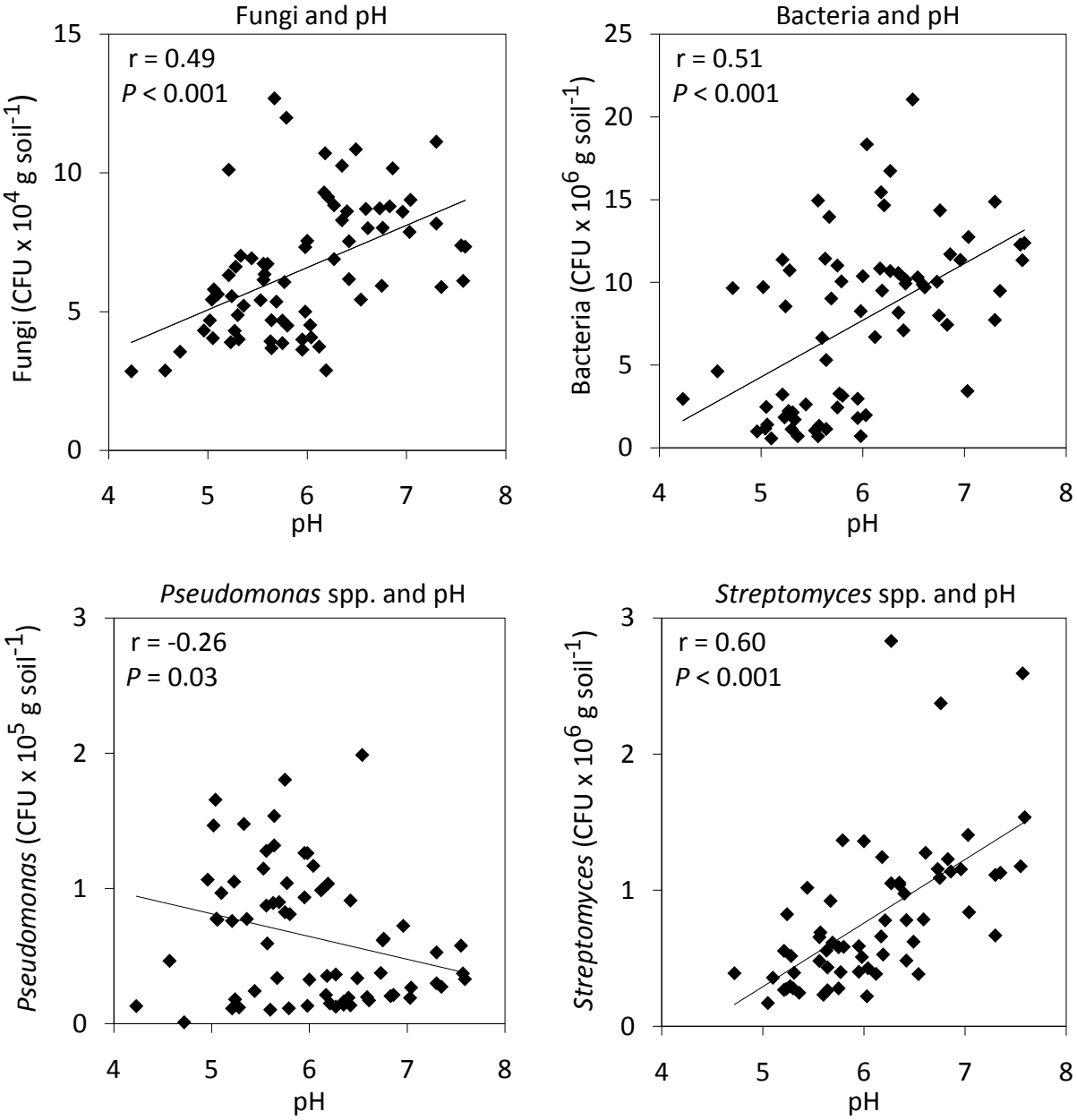


Figure 2.8. Correlations between microbial group populations and soil pH at 0- to 10-cm soil depth in a cover crop and fertilizer study in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2008-2009. CFU = colony-forming unit. "*Pseudomonas* spp." refers to fluorescent *Pseudomonas* spp.

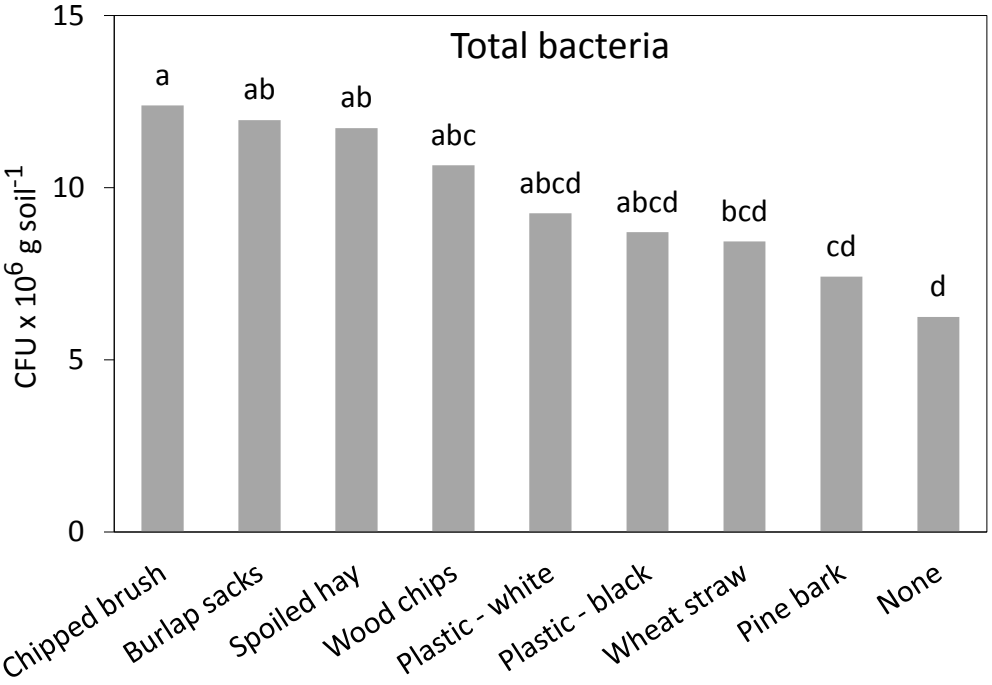


Figure 2.9. Populations of total cultivable bacteria at 0- to 10-cm soil depth as affected by mulch treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$ (Fisher's protected LSD).

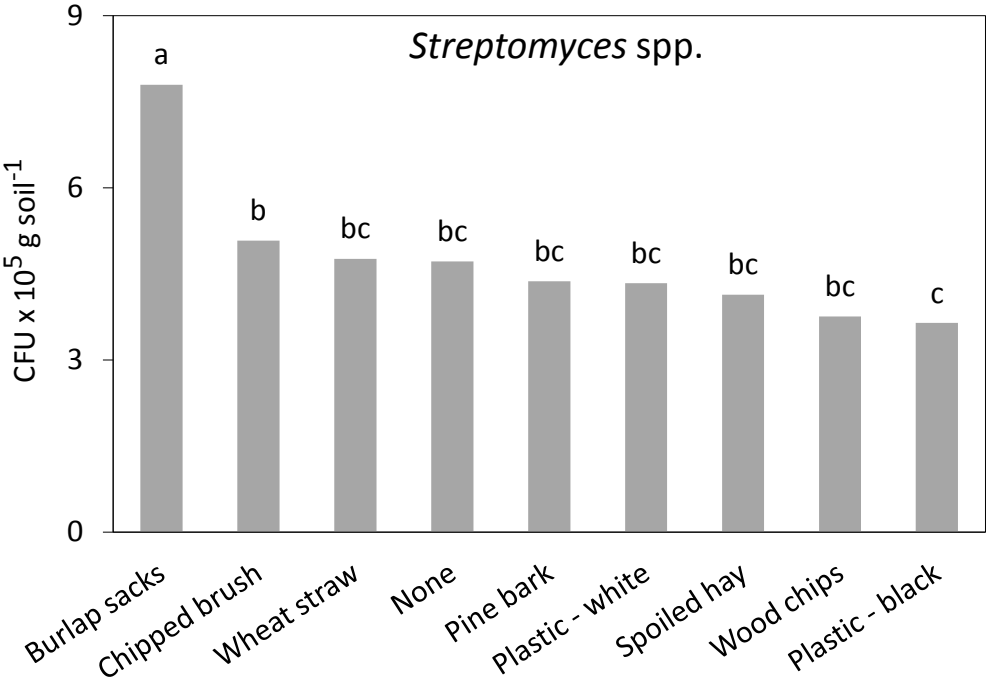


Figure 2.10. Populations of *Streptomycete* spp. at 0- to 10-cm soil depth as affected by mulch treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$ (Fisher's protected LSD).

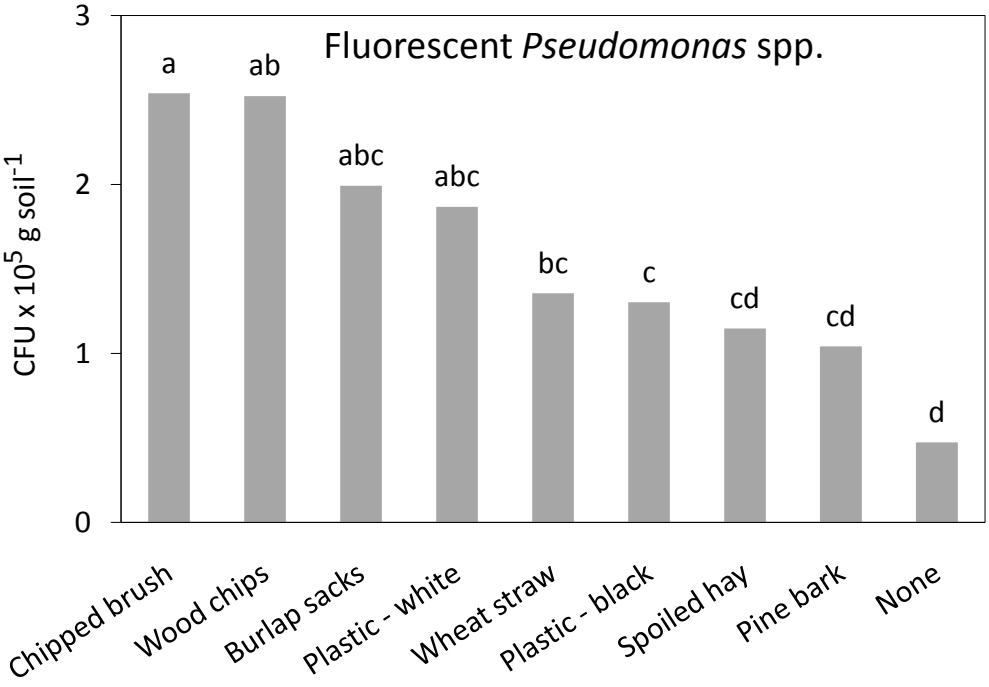


Figure 2.11. Populations of fluorescent *Pseudomonas* spp. at 0- to 10-cm soil depth as affected by mulch treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$ (Fisher's protected LSD).

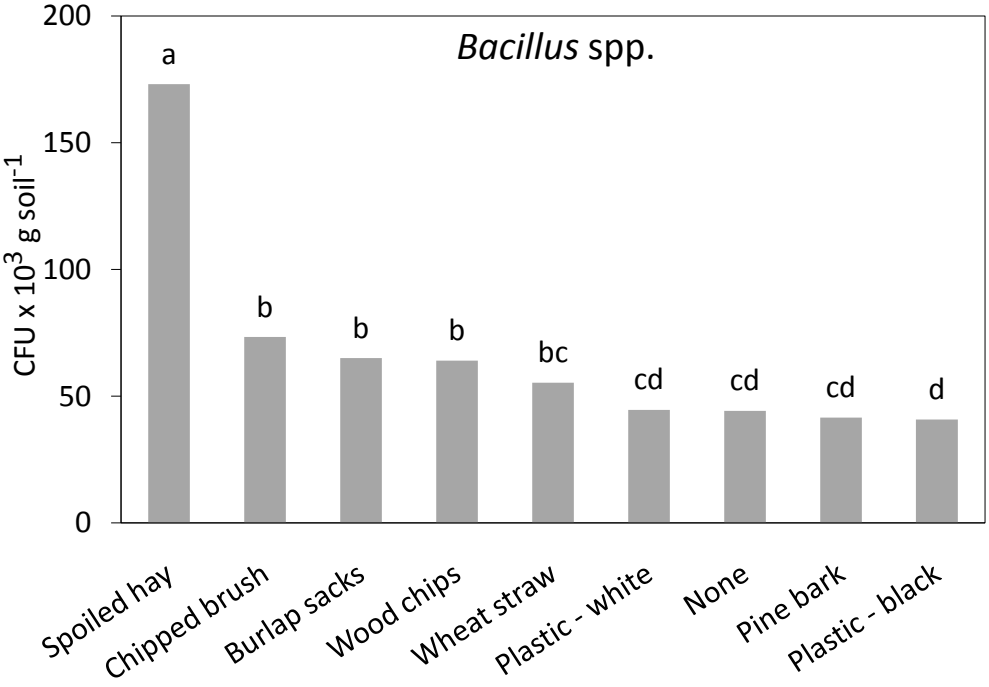


Figure 2.12. Populations of *Bacillus* spp. at 0- to 10-cm soil depth as affected by mulch treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at $P<0.05$ (Fisher's protected LSD).

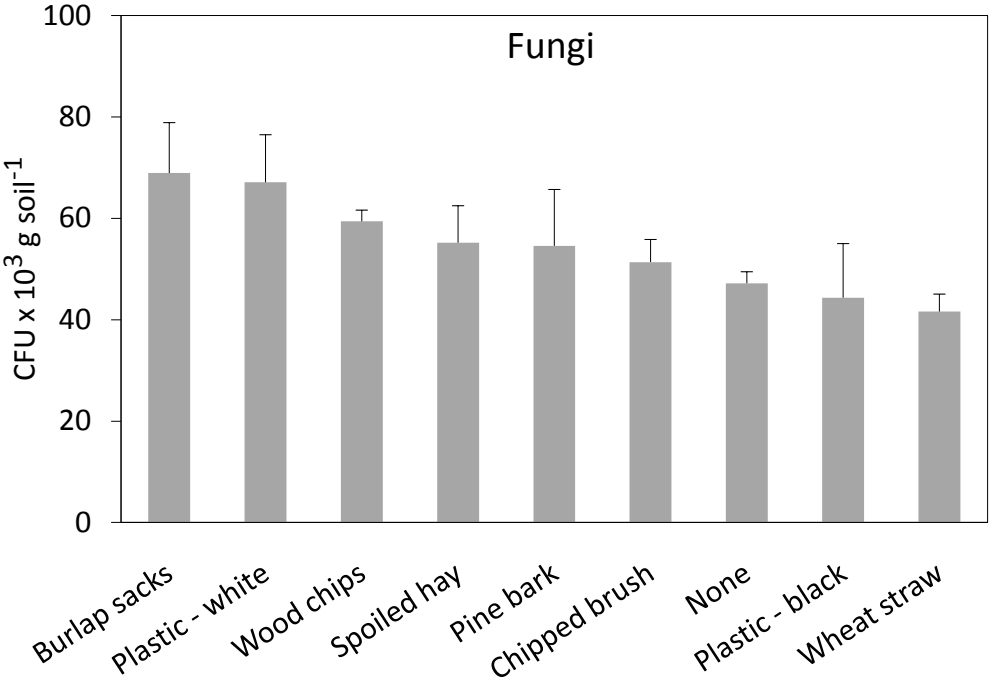


Figure 2.13. Populations of fungi at 0- to 10-cm soil depth as affected by mulch treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. CFU = colony-forming unit. Error bars are one standard error of the mean. Treatments are not significantly different (ANOVA P=0.14).

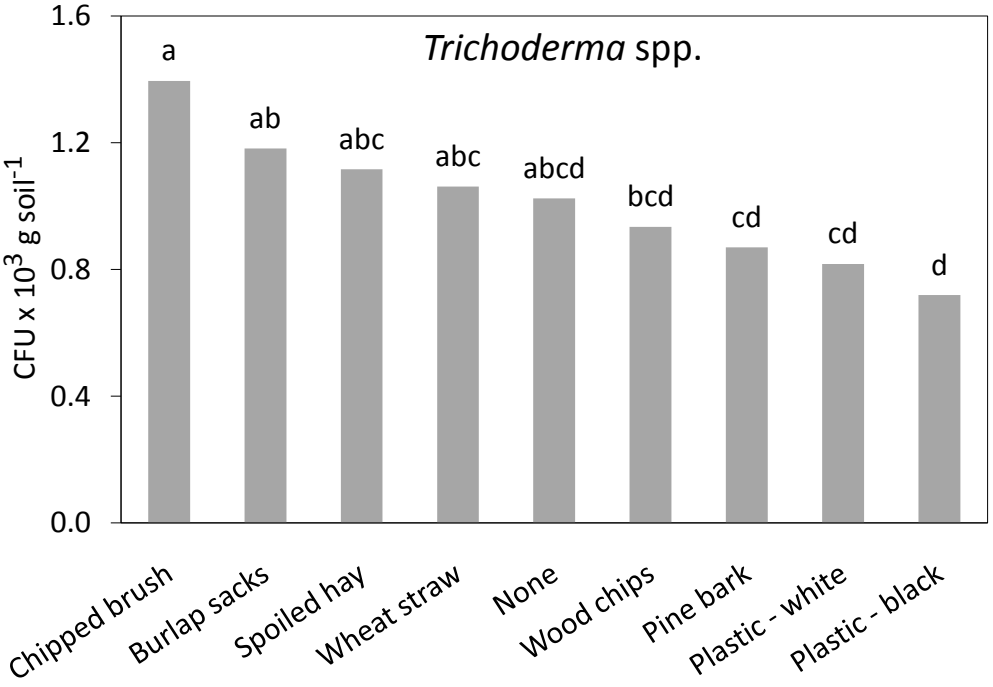


Figure 2.14. Populations of *Trichoderma* spp. at 0- to-10 cm soil depth as affected by mulch treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. CFU = colony-forming unit. Treatments with the same letter are not significantly different at P<0.05 (Fisher's protected LSD).

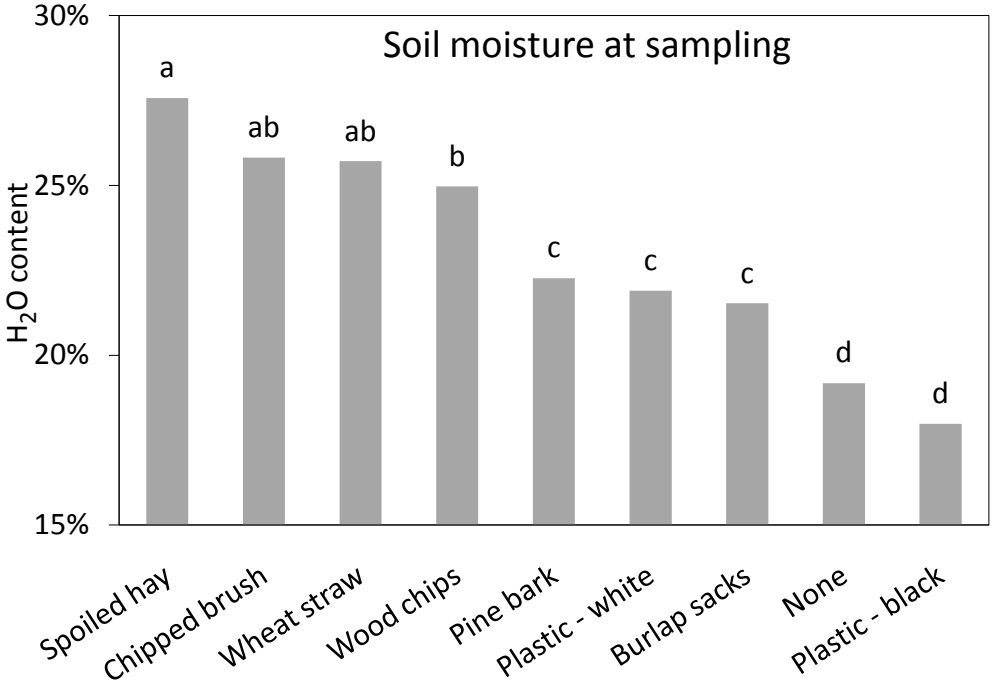


Figure 2.15. Soil moisture at sampling (29-Oct-2009) at 0- to 10-cm soil depth as affected by mulch treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. Treatments with the same letter are not significantly different at $P<0.05$ (Fisher's protected LSD).

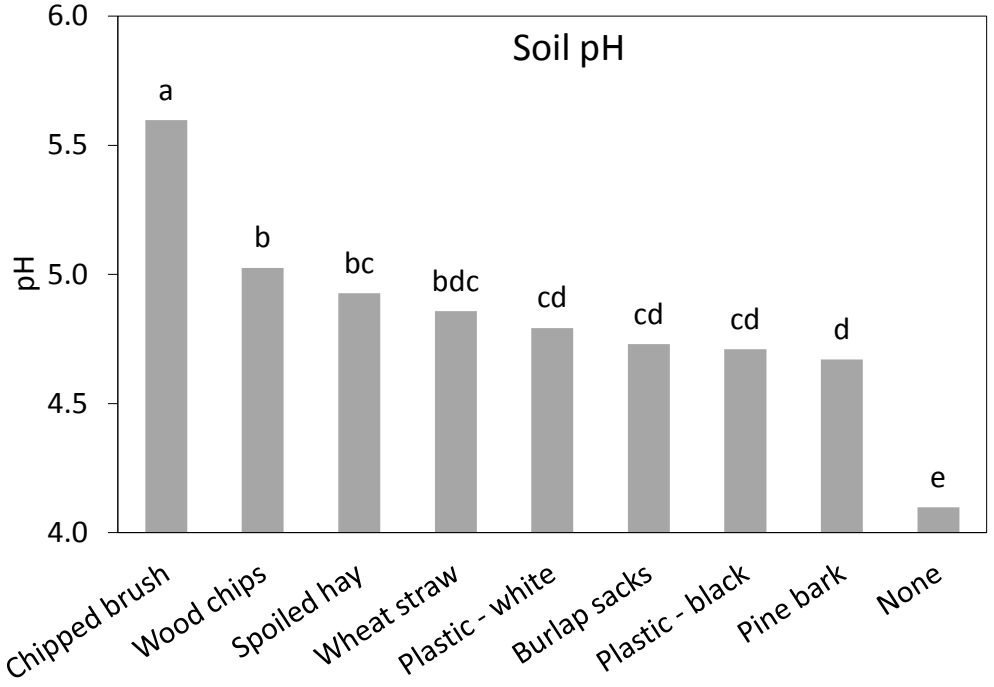


Figure 2.16. Soil pH at 0- to 10-cm soil depth as affected by mulch treatments in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. Treatments with the same letter are not significantly different at $P<0.05$ (Fisher's protected LSD).

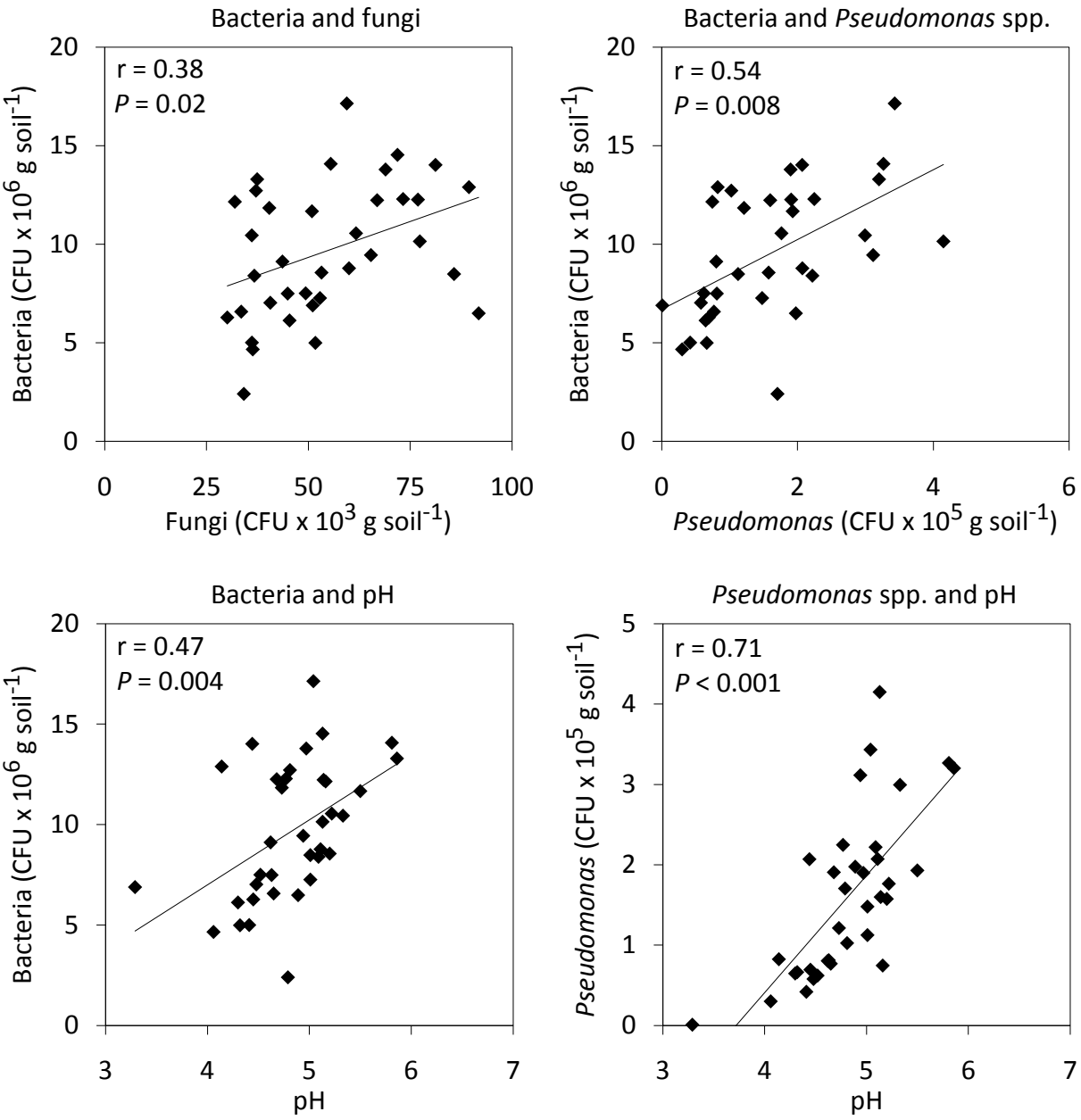


Figure 2.17. Correlations between populations of microbial groups and microbial group populations and soil pH at 0- to 10-cm soil depth in a mulch study in an organic blueberry trial at the Horticulture Teaching and Research Center, Holt, MI, 2009. CFU = colony forming unit. "*Pseudomonas* spp." refers to fluorescent *Pseudomonas* spp.

Chapter 3.

A comparison of soil biology, mycorrhizae, and plant disease in organic and conventional blueberries in Michigan.

Abstract

Michigan has almost 20,000 acres of blueberries under cultivation and ranks as the number one blueberry-producing state in the U.S. Many aspects of organic blueberry culture are not well understood due to a lack of research on best management practices in organic blueberries. This lack of knowledge is reflected by the low percentage of certified organic Michigan blueberry acreage (<0.3%) in Michigan. Measurement of plant and soil health in organic and conventional Michigan blueberries will aid in targeting future research efforts and provide insights into management effects on above- and belowground biology of perennial fruit cropping systems. Here we report on a two-year survey of eight pairs of organic and conventional blueberry fields matched by age, cultivar, and NRCS soil series. Indicators of plant and soil health assessed include labile soil carbon pools (C), potential nitrogen (N) mineralization, soil enzyme activity, soil fungal and bacterial populations, mycorrhizal colonization, and plant disease incidence. We observed a consistent pattern of higher labile C stocks, carbohydrolase and chitinase enzyme activity, populations of beneficial soil bacteria, and mycorrhizal colonization in soils under organic management, while conventionally managed soils had more slow-pool C and lower N to P enzyme activity. These findings suggest that organic management results in more microbial activity, a measure of soil health, in blueberry soils. However, a higher incidence of anthracnose fruit rot in organically grown blueberries indicates that effective organic strategies for management of fruit rot diseases are needed in organic blueberry production in Michigan.

Introduction

Organic and sustainable agriculture-minded farmers are interested in building and maintaining "soil health" (Doran and Parkin, 1996). The development of a biologically based approach to management of soil organic matter resources to meet crop needs, including nutrient supply and plant disease suppression, via an active and resilient microbial community is a longstanding goal of sustainable agriculture practitioners and proponents alike. Short- and long-term studies have investigated soil and plant responses to management intensity and cultural practices (cover cropping, reduced tillage, organic matter amendments, etc.) but the majority of these were conducted within annual cropping systems or unmanaged ecosystems (e.g., Tu et al, 2006; van Diepeningen et al., 2006; Grandy and Robertson, 2007). Relatively few studies have reported on the impacts of organic practices in perennial agroecosystems, and fewer have studied organic and conventional management practices on soil biology and plant health in temperate deciduous fruits (e.g., Sanchez et al., 2003; Yao et al., 2005; Moore-Kucera et al., 2008).

Highbush blueberries (*Vaccinium corymbosum* L.) are adapted to moist, coarse-textured acidic soils with high organic matter content, and are not easily cultivated in soils lacking these characteristics (Ballinger, 1966; Haynes and Swift, 1986). If basic cultural requirements are met, blueberry fields in Michigan remain productive for 40 years or longer (Hanson and Mandujano, 1997). This is noteworthy because there is evidence that long-term additions of fertilizer N in cropping systems deplete soil organic matter (SOM), which, in turn, may reduce crop yield potential over time (Khan et al., 2007). Additionally, recent studies have shown that synthetic N inputs intensify the activity of enzymes linked to mineralization of the most labile C pools, with resultant declines in biologically active SOM and microbial activity (Keeler et al., 2009). The remarkable longevity of Michigan blueberry fields under intensive management suggests that

unique aspects of the blueberry plant and its culture may alter feedbacks between management, soil, and plants such that conclusions drawn from studies of annual cropping or unmanaged ecosystems may not be relevant to highbush blueberry production systems.

The highbush blueberry is host to a unique symbiosis between roots and soil fungi known as ericoid mycorrhizae (ERM). ERM are thought to perform a similar function to root hairs, which facilitate water and nutrient uptake from soil in most plants but are absent from the Ericaceae, including blueberries (Vander Kloet, 1980). Ericaceous plants have hair roots with a diameter of <100 µm, with a C:N ratio of 20:1 and turnover time of 120 days (Valenzuela-Estrada et al., 2008). In the Ericaceae, hair roots constitute 40 to 90% of total root biomass, and it is common to find more than 90% of the outer layer of cells of hair roots colonized by ERM fungi in natural ecosystems (Read, 1996; Scagel, 2003b). ERM fungi can utilize nitrogen (N) in virtually all forms that are found in soil, including inorganic (nitrate and ammonium) and organic (amino acids, proteins, chitin, and tannin-precipitated protein) compounds (Read, 2004). Ericaceous plants are highly dependent on ERM for N acquisition in their native state, where soil conditions typically limit the breakdown of organic matter and accumulation of inorganic N (Read and Perez-Moreno, 2003). By synthesizing a wide array of enzymes, ERM are able to attack structural components (e.g. cellulose and lignin) of soil organic matter and mobilize biochemically protected nutrients including N and P (Read, 1996). Studies using isotope-labeled compounds demonstrated that organic N uptake by ERM-infected plants was significantly higher than by uninfected plants grown in aseptic culture (Sribley and Read, 1974) and unsterile soil (Stribley and Read, 1980). ERM play a major role in niche partitioning in natural ecosystems by allowing ericaceous plants to utilize organic forms of N in soil and detritus that is less accessible to ectomycorrhizal plants and not utilized by arbuscular or non-mycorrhizal plants (Michelsen et

al., 1996). N contained in more recalcitrant organic matter is accessed by ERM fungi via secretion of peroxide, which reacts with Fe^{+2} in acidic soils to generate hydroxyl radicals which catalyze non-specific combustion of lignin molecules (Burke and Cairney, 1998). Native *Vaccinium* spp. in a forest understory obtained 86% of N needs via ERM hyphae (Hobbie and Hobbie, 2006). The symbiosis is not without cost to the plant, as ERM are a sink for up to 20% of net photosynthetic C (Hobbie and Hobbie, 2006).

There appears to be a negative relationship between N availability and ERM colonization in laboratory studies that has not been observed under field conditions. In an early study of the ericoid mycorrhizal responses to inorganic N concentration, Stribley and Read (1976) showed that ERM colonization was reduced at high concentrations of inorganic N despite prolific mycelial growth on root surfaces. This suggested that initial establishment of the symbiosis may be regulated to some extent by the plant host. Studies under field conditions have generally found no or inconsistent effects of soil N enrichment on levels of ERM colonization (Johannson, 2000; Yang et. al., 2002), which is probably related to the complex nature of the soil environment. In a meta-analysis of studies that examined the effect of N fertilization or enrichment on mycorrhizal colonization, the percentage of root length colonized by mycorrhizae was reduced by an average of 5.8% (Treseder, 2004)

In Michigan, blueberries are a major crop, and the number of organic blueberry producers in the region is increasing steadily (pers. comm., Michigan Department of Agriculture accredited organic certifiers, list available at http://www.michigan.gov/mda/0,1607,7-125-1569_25516-55175--,00.html). However, organic blueberry culture has not been researched as extensively as conventional production systems. Many established growers have considered organic blueberries but are reluctant to transition over a major portion of their operation. This is due to an anticipated

immediate yield decline during the 3-year transition period and uncertainty associated with conversion to a somewhat unknown production system. Organic farmers with diversified farms are interested in blueberry production on a small scale but are limited by lack of cultural information on organic blueberry production. In 2008, about 60 acres of blueberries were certified organic in Michigan, which amounts to only 0.3% of the total blueberry production area. A few blueberry farms in the region have been managed with organic practices for more than 30 years, but the majority of sites were certified within the last 2-3 years. These farms provide an opportunity to compare two distinct management systems in terms of soil microorganism communities, soil biological activity, mycorrhizal colonization, and disease incidence. We hypothesize that: 1) blueberries on organic farms are more N-limited and allocate more C below-ground to increase nutrient uptake, resulting in higher ERM colonization, while ERM will be less abundant on conventional farms due to heightened levels of soil N; 2) turnover rates of labile soil C will be higher on conventional farms due to synthetic N inputs, leading to diminished labile C pool sizes compared to organic farms; 3) the turnover rate of lignin as measured by phenol oxidase and peroxidase activity, will be unaffected by management but correlated to ERM infection due to the lignin-degrading ability of ERM fungi, and 4) the N-supplying capacity of soil from conventional farms will be lower than organic farms due to synthetic N-mediated depletion of labile C pools and resultant lower biological activity and nutrient turnover; and 5) the prevalence of diseases will vary between management systems due to contrasts in pesticide use and other factors.

Methods and materials

Experimental design

We carried out an observational study to describe characteristics of plant and soil health in commercial organic and conventional blueberry production fields in Michigan. We located eight certified-organic production fields and matched each with a conventional field. Pairings were based on USDA National Resource Conservation Service (NRCS) soil type, but cultivar and field age were also matched if possible (Table 3.1). Pest (weed, insect, and disease) management, fertilization practices, and types of soil amendments applied differed sharply between the organic and conventional fields. Conventional growers relied primarily upon herbicides for managing weeds beneath bushes, while organic growers used a combination of soil cultivation, mulch, mowing, and organic herbicides. N on conventional farms was added as synthetic fertilizer (urea, ammonium, or nitrate), while N on organic farms was added as various protein-based organic fertilizers (e.g., feather meal, NatureSafe granular organic fertilizer) and composts. Insect pests and diseases on conventional farms were managed with synthetic pesticides (up to 16 sprays per calendar year), while pests on organic farms were managed to a greater extent by (e.g., pruning) and physical (e.g., insect trapping) means less frequent pesticide application.

Field sampling

We harvested one pint of ripe berries from 15 bushes by hand in late July and early August of 2008 and 2009, with disposable gloves worn to prevent cross-contamination between field sites. Soil and root samples were collected on 25-26 Sept, 3-Oct and 9-Oct 2008 and on 6-7 Jul, 21-22 Aug, and 9-10 Oct in 2009. Each site occupied approximately 0.5 ha and was sampled according to a stratified sampling plan (Dick et al., 1996). Three 2.5-cm-diameter soil samples

were taken beneath the dripline of each of 15 randomly selected blueberry bushes at 0 to 5 cm and 0 to 30 cm depths in 2008 and 0 to 5 cm and 5 to 30 cm depths in 2009 and composited by sampling depth. Large root pieces were collected at 0 to 30 cm depth from the same bushes using a narrow spade. Soil and root samples were placed in insulated coolers alongside ice packs and maintained below 4°C. Samples from each pair of organic and conventional farms were collected on the same day. Soil was passed through a 2-mm sieve within 3 days of collection. Subsamples of sieved, field-moist soil were taken and dried at 80°C for 2 days for determination of gravimetric soil moisture content, stored at 4°C, or stored in a -20°C freezer and stored for up to eight months before processing.

Fruit rot and stem disease incidence

Fifty healthy-looking berries from each site were laid out equidistantly on a raised mesh screen raised above 1 to 2 cm of water in aluminum pans, incubated for 10 days at 100% relative humidity, and then inspected for the presence of the following fruit rot pathogens: *Colletotrichum acutatum* Simmonds, *Alternaria tenuissima* (Kunze:Fr) Wiltshire, and *Phomopsis vaccinii* Shear. The incidence twig blight and cane dieback was recorded on 15 bushes at each field on 25-26 July of 2009.

Abiotic soil variables (2008 and 2009)

Soil samples collected at 0- to 30-cm depth in 2008 were air-dried and submitted to a commercial laboratory (A & L Great Lakes Laboratory, Fort Wayne, IN) for analysis of soil organic matter, Bray-1 extractable P, and extractable Ca, Mg, and K using soil test procedures recommended for the North Central Region (http://extension.missouri.edu/explorepdf/specialb/sb1001.pdf). Soil samples collected at 0- to 5-cm and 5- to 30-cm depths in October 2009 were passed through a 2-mm sieve, dried at 80°C for

3 days, ground to a fine powder with a mortar and pestle and analyzed for total C and total N by combustion (NA1500 elemental analyzer (Carlo-Erba, Milan, Italy) (courtesy of Kevin Haynes and Prof. David Rothstein, Soil Biogeochemistry Laboratory, Department of Forestry, Michigan State University).

Light-fraction SOM (2008 samples)

Light fraction soil organic matter (LF) was isolated from soil by suspending 15 g of air-dried soil in 40 ml sodium polytungstate (NaPT) solution adjusted to a density of 1.7 g per ml in 50-mL centrifuge tubes, shaking for 30 min at 190 oscillations min^{-1} on an orbital shaker, allowing the heavy fraction to settle overnight, and then vacuum-aspirating the light fraction through a 1-µm glass fiber filter (Millipore, cat. no. APFB04700) (modified from Sollins et al., 1999). After the NaPT was rinsed from the LF with at least 250 ml deionized H_2O, the LF was dried at 70°C. After drying, the LF was weighed and then ground with a mortar and pestle for determination of C and N content by combustion as describe above. Light fraction SOM was only determined on sandy soils collected at 0 to 5-cm and 0 to 30-cm depths in 2008.

C_l, k, and mrt and short-term CO_2 respiration (2008)

Labile soil C (C_l) content, along with its decomposition constant (k), and mean residence time (mrt) were determined by measuring CO_2 respiration from soil incubated for 461 days. Twenty grams (oven-dry equivalent) of field-moist soil were added to glass vials covered with laboratory film (Parafilm, Chicago, IL). Soil was maintained at 60% water-filled pore space determined gravimetrically (Linn and Doran, 1984). CO_2 evolution from soil microcosms was measured twice per week for two months, once every other week up to day 250, and then every

four weeks with a LI-820 infrared gas analyzer (Li-Cor Biosciences, Lincoln, NE). Labile C content was estimated according to the equation $R_t = (C_1 \times k)e^{-kt} + c$, where R_t is the respiration rate in µg CO_2-C g soil^{-1} day^{-1}, k is the decomposition constant for the labile C pool, t is the incubation time in days, and c is the rate of CO_2 respiration of the slow and recalcitrant SOM pools (McLauchlan and Hobbie, 2004). Modeling of C_l by long-term soil incubation fits an exponential decay function to estimate C_l. The respiration rate was nearly constant for muck soils over the course of the incubation, and similar results have been reported for other soils with high organic matter (Weintraub and Schimel, 2003). Due to the disparity in CO_2 release patterns between sand and muck soils, C_l was determined only for sandy soils, and due to the lengthy incubation required, only assessed on soil samples collected in 2008. The amount of C respired over a short-term soil incubation may be a simpler method for determination of labile soil C (McLauchlan and Hobbie, 2004) and was included for comparison with C_l.

Potential nitrogen mineralization (2008)

Field-moist soil collected in the fall of 2008 at 0- to 5-cm and 0- to 30-cm depths was sieved to 2 mm, added to 125-mL flasks, and adjusted to 60% water holding capacity (Linn and Doran, 1984). Fifteen grams of soil were added to each flask on a dry-weight basis. Flasks were covered with a double layer of laboratory film and incubated at 25°C for 14 days. There were two replicate flasks for each soil sample collected. Inorganic N was extracted in 1M KCl (1:5 m/v). I determined Nitrate-N according to Doane and Horwath (2003) and ammonium-N determined by the method of Nelson (1983).

Potential nitrogen mineralization and short-term CO_2 respiration (2009 samples)

Potential nitrogen mineralization and CO_2 respiration of soil were determined in 30-day incubations modified slightly from methods described in Robertson et al. (1999). Field-moist soil was passed through a 2-mm sieve and stored at 4°C for up to 40 days. Ten grams (oven-dry equivalent) of field-moist soil was added to glass vials and adjusted to water holding capacity (WHC) for optimal microbial activity, 55% for sandy soils (Paul et al. 2001) or 65% for muck soils (Zimenko and Revinskaya, 1972). Small vials containing soil were incubated at 25°C within humidity chambers (1-pint jars with water added to 1 cm) and covered with plastic film (Parafilm, Chicago, IL) to allow air exchange but prevent excessive moisture loss. Deionized water was added to vials about once per week to maintain a constant mass throughout the incubation. Inorganic N was extracted from 5 g of air-dry soil in 25 ml of 1M KCl after shaking on an orbital shaker at 125 rpm for 30 minutes. Soil solids were allowed to settle to the bottom of the flask and then the supernatant was poured through filter paper (Whatman No. 2) that was previously leached with 1M KCl. NO_3^--N and NH_4^+-N concentrations were determined as described above.

Enzyme activity (2008 and 2009)

One gram of soil was removed from storage at -20° C and added to 125 ml of 1M sodium acetate buffer at a pH of 5.0. Slurries were continuously mixed on a stir plate while 200-µL aliquots were withdrawn and added to 96-well plastic microplates, followed by 50 µL of prepared substrates, 4-methylumbelliferone(MUB)-β-D-cellubioside, 4-MUB-β-D-glucoside, 4-MUB-N-acetyl- β-D-glucosaminide, L-tyrosine-7-amino-4-methylcoumarin, 4-MUB-phosphate, L-dehydroxyphenylalanine, or L-dehydroxyphenylalanine plus 0.3% hydrogen peroxide for

determination of activity of the following enzymes: β -1,4-glucosidase (BG), β -D-1,4-cellobiosidase (CBH), β -1,4-N-acetyl-glucosaminidase (NAG), acid phosphatase (PHOS), tyrosine amino peptidase (TAP), phenol oxidase (POX), and peroxidase activity (PER), respectively. Buffer, buffer plus fluorescent tag, buffer plus substrate, soil slurry plus substrate, and nothing were included to correct for background fluorescence or absorbance. Plates were incubated at 15°C. Incubation times were as follows: PHOS: 2 to 4 hr; BG: 2 to 4 hr; NAG 3 to 4 hr; CBH 4 to 6 hr; PER: 3–5 hr; TAP: 5 to 7 hr, POX: 20–24 hr. Fluorescence (enzymes except PER and POX) or absorbance (POX and PER) was measured on a microplate reader (Thermo Flouroskan and Multiskan, Thermo Scientific, Hudson, NH) and then converted to activity per gram of dry soil per hour (Saiya-Cork et al., 2002) .

Cultivable bacteria, fungi, and beneficial microorganisms (2008 and 2009)

Cultivable soil microorganisms were enumerated on semi-selective media Using field-moist soil that was passed through a 2-mm sieve and stored for up to 14 days at 4°C. Soil taxa enumerated included total cultivable fungi, *Trichoderma* spp., total cultivable bacteria, *Bacillus* spp., fluorescent *Pseudomonas* spp., and *Streptomyces* spp. In 2008, fungi, bacteria, and *Trichoderma* spp. populations were assessed in soil collected at 0- to 5-cm and 0- to 30-cm depths in late September and early October of 2008. In 2009, populations of fungi, bacteria, *Trichoderma* spp. *Bacillus* spp., fluorescent *Pseudomonas* spp., and *Streptomyces* spp. were assessed in soil samples collected at 0- to 5-cm and 5- to 30-cm depths on 9-10 Oct 2009. *Trichoderma* spp. populations were assessed on Rose Bengal agar in 2008 and on a *Trichoderma* spp.-selective medium containing several fungicides (Askew and Laing, 1993) in 2009. Soil dilution plating methods are described in more detail in Chapter 2 of this thesis.

Nematode community structure

Soil and roots were collected on 6-7 June 2010 to assess nematode community structure, prevalence of root pathogens and population of oligochaetes (small earthworms). Soil was collected from all sites at a 0- to 5-cm depth using a 2.5-cm diameter soil probe. Root pieces 5 to 10 cm long were excised from soil taken at 0 to 30 cm depth using a 10-cm wide trowel. Soil and roots were stored in polyethylene zipper bags and kept cool with ice packs during transit. Roots and soil were stored at 4°C and processed within 3 and 10 days of collection, respectively. Nematode identification and counts were made by Mr. Fred Warner of the Michigan State University Diagnostic Services laboratory.

Root processing and mycorrhizal colonization assessment

Root pieces up to 30 cm long were washed of adhering soil. Hair roots were carefully excised with a forceps and scalpel blade and placed in 50% ethanol at 4°C for up to 4 months (Stackpoole et al., 2008). Hair roots were soaked for 48 hours after storage in ethanol, in 10% KOH, rinsed three times with distilled water, acidified in 1% aqueous HCl overnight, and stained at room temperature in acidified 0.05% aniline blue (Vohnik et al., 2009). Root segments were then mounted on slides and examined at 600x to 960x magnification on an Olympus IX-71 inverted microscope (Olympus America Inc., Center Valley, PA) equipped with differential interference contrast. Twenty-five 0.5-cm-long by 70-130-µm-diameter root segments were examined per root sample, which equates to about 12 cm of hair roots examined per plant. The method of McGonigle (1990) was modified to assess the number of epidermal cells colonized. The standard gridline-intersect method (Gionovenneti and Mosse, 1980) or root length method (Biermann and Linderman, 1981) was not used due to difficulty in distinguishing between dark septate endophyte (DSE) microsclerotia and ERM at low magnification (10-200x) used for these

methods. DSE colonization was recorded as intracellular microsclerotia associated with superficial or intercellular thickened non-staining hyphae (Stoyke and Currah, 1991; Jumpponen and Trappe, 1998). ERM and DSE colonization is expressed as the percentage of epidermal root cells filled with hyphal coils or microsclerotia, respectively (Figure 3.1). Colonization of individual plants was calculated as the average percentage colonization of hair root cells of 50 (2008) or 25 (2009) root segments. ERM and DSE colonization values in each field site were calculated by averaging the colonization of roots of eight plant replicates for samples collected in fall 2008 and July 2009 and four plants for samples collected in August and October 2009.

Statistical analysis

All statistical analyses were performed in SAS 9.2 (SAS Institute, Cary, NC). Analysis of variance of the fixed effects of management (conventional and organic), soil depth (0 to 5 and 0 to 30 cm in 2008, 0 to 5 cm and 5 to 30 cm in 2009), and sampling date (July, August, and October, determined only in 2009) was carried out in the GLIMMIX procedure. Pairs of conventional and organic farms (n=8) were considered random blocking effects. Six organic-conventional farm pairs were on sandy soils and two pairs were on muck soils. Years were analyzed separately. Because several parameters (C_l, mrt, k, light fraction organic matter) were not measured on muck soils, mucks were omitted from some analyses of 2008 data to allow comparison of organic and conventional management effects across variables. Dependent variables were transformed as needed, e.g., if there was a systematic pattern to the distribution of residuals or if the variances of fixed effects were unequal according to Levene's test for homogeneity of variance. In addition to inspection of residual plots and Levene's test, the Box-Cox procedure, which minimizes the residual error sums of squares, was used to determine the optimal transformation for each response variable. Soil type was added as a fixed affect for

analysis of ERM and DSE because non-zero estimates were provided for soil type and blocking effects, while these effects were not mutually estimable for analyses of variables (enzyme, microbes, labile C, potential N mineralization) assessed on a bulk-soil basis. PROC CORR was used to determine the relationship between dependent variables measured at 0- to 30-cm depth on sands (n=12) and mucks (n=4); correlation analysis was conducted separately for sand and muck soils because the values of biological measures on muck soils were frequently of an order of magnitude greater than those observed on sands. For 2009 data, responses from samples assessed at 0 to 5 cm and 5 to 30 cm were averaged on a per-unit mass basis according to bulk density values published for each soil type (NRCS soil survey, http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm) prior to correlation analysis, and correlation analyses for sand and muck soils were run separately, as in 2008. SAS PROC FACTOR was used for principal component analysis of biological response variables expressed on a bulk-density-weighted average of samples collected at 0- to 5-cm and 0- to 30-cm depths on sandy soils collected in October 2009. Factor scores were rotated to provide orthogonal contrasts between principal components (Sinsabaugh et al., 2008). Prior to performing the principal component analysis, variables were standardized to a correlation matrix. Anthracnose and Alternaria fruit rot incidence were analyzed as a completely randomized design with management and sampling year as fixed effects because fruit was collected from different field sites in 2008 and 2009 and pairs of organic and conventional farms were not matched by blueberry cultivar in all cases (Table 3.1).

Results

Plant disease measurements

Management practices significantly affected the incidence of anthracnose fruit rot ($P < 0.01$), while significant effects of management and year were observed for incidence of Alternaria fruit rot ($P = 0.02$ and $P = 0.006$ respectively) (Table 3.3). There were no significant interactions between management and year on anthracnose or Alternaria rot. Anthracnose was significantly higher on organic blueberries, while conventional blueberries had a higher incidence of Alternaria rot (Figure 3.2).

Abiotic variables (2008 and 2009)

There was a significant effect of management on soil pH ($P = 0.03$) and significant interactive effect of management and depth on soil water content ($P = 0.05$, Table 3.4). Conventional farms had significantly higher soil water content ($P = 0.03$) and lower soil pH ($P=0.02$) than organic farms at 0- to 5-cm depth in the fall of 2008 (Table 3.8). Sandy soils collected at 0- to 30-cm depth from organic farms had significantly higher Ca ($P = 0.006$, Table 3.4. and Figure 3.4). In 2009, soil organic carbon, water content, total soil N, and soil C:N were not affected by management, while soil water content varied significantly by sampling date (Table 3.5).

Labile soil C fractions (2008)

The overall effect of management on labile C fractions was not significant (Table 3.6). Light fraction soil organic matter content, light fraction C content, light fraction N content, light fraction N concentration, light fraction C:N ratio, 100-day CO_2 respiration, labile C, and respiration of slow plus recalcitrant C pools varied significantly by depth (Table 3.6). Significant

management by sampling depth interactions were observed for contents of light fraction soil organic matter ($P = 0.02$), light fraction C ($P = 0.009$, and light fraction N ($P = 0.004$, Table 3.10). CO_2 respiration ($P = 0.03$) and labile C content ($P = 0.04$) were significantly higher on samples collected from organic farms at 0- to 5- cm depth (Table 3.10 and Figure 3.6), while slow plus recalcitrant pool C tended to be higher on conventional farms ($P = 0.10$, Figure 3.6).

Potential nitrogen mineralization (2008 and 2009)

In 2008 management significantly affected nitrification, while sampling depth affected potential N mineralization, nitrification, and relative nitrification (Table 3.7). At 0- to 5- cm soil depth, nitrification was significantly higher on organic farms ($P = 0.008$, Figure 3.7). Across sampling dates in 2009, soils collected from organic farms had significantly higher potentially mineralizable N at 0- to 5-cm soil depth ($P = 0.01$, Figure 3.8). There were significant interactions between management and sampling date on all soil nitrogen cycling variables (Table 3.8). At 0 to 30 cm depth in July, conventional farms had higher inorganic soil N ($P = 0.04$, Figure 3.9), while incubated soil from organic farms had higher potentially mineralizable N ($P = 0.003$, Figure 3.10) and net nitrification ($P = 0.05$, Figure 3.11). Organic farms had significantly higher inorganic soil N in October ($P = 0.001$, Figure 3.9).

Soil enzyme activity and short-term CO_2 respiration (2008 and 2009 samples)

In 2008 there were no significant effects of management or soil depth on BG, CBH, NAG, or PHOS activity (Table 3.9), but in 0- to 5-cm-depth soil, NAG activity tended to be higher in response to organic management ($P = 0.08$, Figure 3.13). TAP, POX, and PER enzyme activity tended to vary significantly by date, while BG, CBH, NAG, PHOS, and TAP varied significantly by sampling depth in 2009 (Table 3.10). Specific BG and NAG activity and specific

CO_2 respiration were significantly higher in soil collected from organic farms at 0- to 5-cm soil depth in 2009 (P = 0.05, 0.004, and 0.05, respectively; Table 3.13 and Figures 3.14, 3.15 and 3.20). On a per gram of soil basis, the ratio of C:N enzyme activity was significantly affected by management and date (P = 0.05 for both factors, Table 3.11), while specific C:N enzyme activity varied significantly by management (P = 0.03, Table 3.11). The ratio of C:P enzyme activity on a soil and soil organic carbon SOC basis was significantly affected by soil depth (Table 3.11). The ratio of N:P enzyme activity on a soil and SOC basis varied significantly by management, date, and depth (Table 3.11). The ratio of C:N was significantly lower in soils from organic farms compared to conventional farms collected at 5- to 30-cm depth on a per gram of soil basis (P = 0.05, Figure 3.18) and at 0- to 5-cm on a per gram SOC basis (P = 0.02, Figure 3.14). C:P enzyme activity ratios were significantly higher on organic farms at 0- to 5-cm depth on a bulk soil and SOC basis (P = 0.01 and 0.03, respectively, Figure 3.18). The ratio of N:P enzyme activity on organic farms was significantly higher than conventional farms at 0- to 5-cm soil depth on a bulk soil and per SOC basis (P = 0.008 and 0.005, respectively, Figure 3.19).

Cultivable bacteria, fungi, and beneficial microorganisms (2008 and 2009)

In 2008, populations of cultivable soil bacteria were significantly affected by management (P = 0.04, Table 3.12) and significantly higher in soil samples collected at 0- to 5-cm depth from organic farms (P = 0.04, Figure 3.17). In 2008, populations of cultivable fungi varied by sampling depth (P = 0.001, Table 3.12), while *Trichoderma* spp. populations varied by sampling depth (P = 0.04) and the interaction between management and sampling depth (P = 0.004, Table 3.13).

In 2009, populations of *Bacillus* spp., *Streptomyces* spp., and fungi varied significantly by depth (P = 0.009, 0.0007, and 0.002, respectively, Table 3.13), while there was a marginally

significant effect of management on fluorescent *Pseudomonas* spp. populations which were higher in soils collected from organic farms at 0- to 5-cm sampling depth ($P = 0.06$, Figure 3.23).

Root colonization by ericoid mycorrhizae and dark septate endophyte microsclerotia

In 2008, there was a significant effect of soil type on DSE colonization ($P = 0.001$, Table 3.14). In 2009, ERM colonization was significantly affected by management and the interaction between management and NRCS soil type ($P = 0.05$ and 0.03, respectively, Table 3.14 and Figure 3.24), while DSE colonization was significantly affected by sampling date, NRCS soil type, and the management by soil type interaction ($P = 0.003$, 0.04, and 0.05, respectively, Table 3.14 and Figure 3.24). Matched pairs of organic and conventional farms tended to have higher colonization on organic farms, while DSE colonization was especially low on muck soils (Figure 3.25). ERM colonization levels varied from 8% to 50% among samples taken from individual plants.

Beneficial and plant-parasitic nematodes

Root lesion nematodes were more numerous on organic farms at 0- to 5-cm soil depth ($P = 0.02$, Table 3.16). Populations of tylenchs, aphelenchs, and total nematodes were higher in soils collected from organic farms ($P = 0.02$, 0.01, and 0.04, respectively, Table 3.16). Bacterial-feeding and total plant-parasitic nematodes tended to be higher on organic farms at 0- to 5-cm soil depth ($P = 0.06$ and 0.07 respectively, Figure 3.28).

Correlations in sandy soils in 2008

Among the abiotic variables measured on sandy soils in 2008, there were positive correlations between pH and Ca, pH and Mg, and Ca and Mg (Table 3.18). Bray P was negatively correlated with water content, LF C:N, and CO_2 respired in a 100-day laboratory

incubation but positively correlated with net nitrification and C_l mean residence time (Table 3.18). Nitrification was negatively correlated with soil moisture and Mg content and positively correlated with potential N mineralization (Table 3.18). LF C:N was positively correlated with water content and Mg, but negatively correlated with C_l mean residence time (Table 3.18). CO_2 respired in a 100-day laboratory incubation correlated positively with soil Mg and potentially mineralizable N and negatively correlated to Bray P and relative nitrification (Table 3.18). Hydrolase enzymes were generally correlated with one another (Table 3.18). Populations of cultivable bacteria were positively correlated with Ca, BG and NAG activity, cultivable fungi and *Trichoderma* spp. (Table 3.18). Cultivable fungi and populations were positively correlated with BG and NAG activity and bacteria, while *Trichoderma* spp. populations were positively correlated with LF C:N, C_l and NAG activity (Table 3.18). Ericoid mycorrhizal colonization was positively correlated with soil pH and negatively correlated with the mrt of C_l (Table 3.18; Figure 3.27 in the latter combination). Dark septate endophyte colonization was negatively correlated with water content and LF C:N but positively correlated with nitrification (Table 3.18).

Correlations in muck soils in 2008

In muck soil samples collected at 0- to 30-cm depth in 2008, there were significant positive correlations between Mg and Ca; potential nitrogen mineralization and net nitrification; net nitrification and PHOS; ERM and pH; ERM and potential N mineralization; ERM and net nitrification; and DSE and BG activity (Table 3.19). Bray-1 extractable P was negatively correlated with soil organic matter and PHOS enzyme activity (Table 3.19).

Correlations in sandy soils in 2009

On sandy blueberry soils in 2009, we observed significant positive correlations between water content at sampling and soil organic C; soil pH and ERM colonization (Figure 3.27); soil organic C and total soil N; soil organic C and soil C:N; soil organic C and PHOS; soil N and NH_4^+-N; CO_2 and BG; CO_2 and CBH; CO_2 and NAG; CO_2 and fluorescent *Pseudomonas* spp. populations; Inorganic N and net nitrification; BG and CO_2; BG and CBH; BG and NAG; BG and PER; CBH and CO_2 respiration; CBH and NAG; PHOS and soil organic C; POX and PER; TAP and DSE; *Bacillus* spp. populations and net nitrification; populations of *Bacillus* spp. and bacteria; *Bacillus* spp. and *Streptomyces* spp. populations (Table 3.20). Significant negative correlations were observed between soil organic C and specific CO_2; total soil N and specific CO_2; soil C:N and net nitrification; net nitrification and POX; PHOS and fungi; TAP and *Streptomyces* spp. populations; TAP and fungi; ERM and NH_4^+- N; ERM and BG; ERM and PHOS (Figure 3.27); ERM and PER; and DSE and POX (Table 3.20). A marginally significant negative correlation between total soil N and ERM was also observed (Figure 3.27).

Correlations in muck soils in 2009

On muck soils in 2009, there were significant positive correlations between water content at sampling and specific CO_2; soil organic C and net nitrification; soil organic C and PHOS; soil organic C and *Trichoderma* spp.; soil C:N and *Bacillus* spp. populations; CO_2 and PER; net nitrification and PHOS; net nitrification and TAP; net nitrification and bacteria; net nitrification and fungi; BG and CBH; PHOS and PER; PHOS and TAP; TAP activity and DSE colonization;

bacteria and *Streptomyces* spp.; bacteria and fungi; and *Streptomyces* spp. and fungal populations (Table 3.6). Significant negative correlations were observed between water content and fungal populations; total soil N and net nitrification; CO_2 and bacterial populations; specific CO_2 and bacteria; specific CO_2 and fungi; inorganic N and *Bacillus* spp. populations; NAG and fungi; *Streptomyces* spp. populations and ERM colonization; and *Streptomyces* spp. and fungal populations (Table 3.6).

Principal component analysis

A principal component analysis was used to reduce the large number of soil variables to a lower number of orthogonal linear combinations of biological variables. The first principal component, accounting for 29% of the variance in the data, was a mainly a function of carbohydrolase enzyme activity but also CO_2 respiration and PER, while the second principal component accounted for 20% of the observed variance and was a function of beneficial bacteria and ERM colonization contrasted with PHOS and TAP activity and (Figure 3.29). As factor scores for carbohydrolase activity increased among field sites, there tended to be a wider separation among organic and conventional farms in terms of beneficial microbes and PHOS and TAP activity (Figure 3.29).

Discussion

Biological activity in soil on organic and conventional farms tended to diverge in patterns of C, N, and P acquisition, as assessed in enzyme activity assays and soil incubations. Higher NAG activity, which measures the release of N-acetylglucosamine from polymers in cell walls of fungi, bacteria, nematodes, and soil arthropods, was observed on organic farms at 0- to 5-cm soil depth in both 2008 and 2009, although the effect was not quite statistically significant. Although

NAG was not correlated with inorganic N or potentially mineralizable N, NAG may be important in releasing N for plant uptake in organically managed soils. Tabatabai et al. (2010) observed highly significant correlations between NAG activity and potentially mineralizable N in soils collected from a corn and soy rotation field in Iowa and concluded that NAG is a good indicator of N mineralization in soil.

Another significant finding was that management systems differ in the availability of labile C, as determined by measurement of soil respiration in short and long-term soil incubations, which provides information on the quantity of C available to soil microbes. This effect of management was most pronounced in 0- to 5-cm soil, indicating soil surface C inputs on organic farms may promote microbial activity to a greater extent than those on conventional farms. In addition to the soil respiration data, the notion of greater lability of soil C inputs on organic farms is further supported by significantly higher BG activity, expressed on a SOC basis, at 0- to 5-cm soil depth. Extensive use of herbicides may limit soil organic matter inputs on conventional farms to blueberry litter (both roots and shoots), as opposed to a more diverse array of organic matter on organic farms, including weed biomass, compost, organic fertilizers and sod residues. Blueberry leaves have a C:N ratio near 80:1 (Kourtev et al., 2002) and thus provide a relatively low quality C substrate to saprotrophic soil organisms. Litter inputs of the ericaceous plant *Kalmia angustifolia* L. tend to suppress microbial activity (Joanisse et al., 2007).

Slow + recalcitrant pool C tended to comprise a more significant portion of total soil C in conventional fields than organic fields. This finding may be related slower breakdown of lignin-C by fungi in response to enrichment with mineral N (Sinsabaugh et al., 2002). As fields are transitioned from conventional to organic, breakdown of this more recalcitrant pool of soil C may resume as mineral N-mediated suppression of lignin decomposition is alleviated. However,

the breakdown of high C:N litter likely requires exogenous N for breakdown. N-immobilized as previously accumulated low-quality (high C:N ratio) organic matter is broken down may partially explain the lagging growth and plant N deficiency previously reported by Michigan blueberry growers who have transitioned mature, previously conventional fields to organic management.

Soil nitrogen availability was affected by management practices in fall 2008 and in July and October in 2009. In July 2009, inorganic N availability was higher on conventional farms, while potentially mineralizable N and net nitrification were higher in soils collected from organic farms. Additionally, in fall of both years, organic farms had higher levels of inorganic soil N. The observation of higher potentially mineralizable N in response to organic management was anticipated, as organic fertilizers typically release N over an extended period (Agehara and Warncke, 2005) and the majority of organic growers applied N at the same rate as conventional growers. Likewise, the observation of higher inorganic N availability on conventional farms in July was not surprising because mineral N fertilizers are completely soluble and immediately enrich inorganic N.

Divergence in the quality of C inputs between organic and conventional farms may explain the observed higher rates of nitrification on organic farms. Wurzburger and Hendrick (2007) observed that polyphenolic-rich litter of ericaceous plants may limit nitrate accumulation in soil by suppressing microbial breakdown of organic soil N. There is increasing recognition that heterotrophic bacteria and archaea contribute to nitrification in acidic soils (Prosser and Nicol, 2008) nitrifiers. More abundant labile C in soil on organic farms may provide more energy for heterotrophic nitrification.

One of the most striking differences was a highly significant effect of management on the ratio of N:P enzyme activity at 0- to 5-cm soil depth. Analysis of records of blueberry soils submitted by growers the Michigan State University soil testing lab over the years hint that soils are becoming increasingly P-depleted (Hanson, 1989, 2007). Plants are a major sink for soil P (Lindahl et al, 2005) and it is possible that soils of conventionally managed blueberries are relatively P-depleted at shallow depth, especially considering that dense root mats at shallow depth were observed frequently in conventional fields but only in organic fields where dense mulches were employed. It is important to note that prior to the organic transition, organic farms were previously managed with conventional practices. The observed N:P enzyme ratios suggest a shift from a P-limited soil microbial community in conventional fields to one that is more N-limited under organic management.

Currey et al. (2009) reported that N-enrichment suppressed NAG and stimulated PHOS and this scenario may have played out in response to synthetic fertilizer enrichment on conventional blueberry farms. Significant positive relationships between PHOS and N availability have been reported in forest soils (Treseder and Vitousek, 2004). Sinsabaugh et al. (1993) proposed a resource availability model for enzyme activity, such that microbial investment in extracellular enzymes will shift in response to limiting resources, because release of exozymes by microbes is their primary means for acquiring energy and nutrients from the surrounding environment. High N-degrading enzyme activity on organic farms may be important in the breakdown of complex N in organic fertilizers to simpler organic or mineral N available for plant uptake. Reduced N:P enzyme ratios in conventionally managed soils may be related to suppression of N-acquiring enzymes by synthetic N fertilization, high midseason N availability, and relatively higher P demand. Although inorganic N was higher in conventionally managed

soils only on the July sampling date, a more pronounced midseason spike in inorganic N may have had a cascading effect on soil enzyme activity over the remainder of the growing season. According to our enzyme assays, N was the most limiting resource in soils on organic farms, while P was more limiting than N in conventional blueberry soils.

Between phenol oxidase and peroxidase, the activity of peroxidase appears to be the main oxidative enzyme in blueberry soils. Sinsabaugh (2010) showed that PER is associated with decomposition of recalcitrant C and is induced by phenolic compounds. For instance, five times higher PER activity was found in biological crusts (surface communities of autotrophic microbes and non-vascular plants) in a burned area compared to an unburned area, and the author attributed the response to reduced supplies of labile C (Sinsabaugh, 2010). It would be interesting to examine whether the peroxidase activity assessed here, with added peroxide to optimize enzyme activity, is of biological origin or instead is a non-biological reaction between added peroxide and soluble Fe (II) ions (Burke and Cairney, 1998). Fe (II) accumulates in acidic soils under waterlogged conditions (McFarlane, 1999). With excess precipitation, fields with shallow water tables are prone to flooding, which was observed in some of the fields sampled in this study. Timonen and Sen (1998) assessed the presence of enzymes in mycorrhizal and non-mycorrhizal roots of Scots pine (*Pinus sylvestris* L.) grown in non-sterile soil and observed that peroxidase originated primarily from plant roots rather than mycorrhizal fungi, while PHOS enzymes were localized in inter- and extraradical fungal tissue of mycorrhizal roots. This suggests that plant roots may be a significant source of peroxidase activity. Dense mats of roots near the soil surface were observed in some blueberry fields in the current study. To my knowledge, the relative contribution of soil microbes, root-associated fungi, and plant roots to enzyme activity in soil has not been widely studied.

ERM colonization was, overall, about 5% higher on organic farms. This suggests a that a positive effect of organic N on ERM colonization, observed previously in experiments conducted under laboratory conditions (Xiao and Berch, 1999) and with containerized plants (Scagel, 2005; Montalba et al. 2010) may also occur in Michigan blueberries. ERM play a prominent role in uptake of organic nitrogen by blueberries and other ericaceous plants (Stribley and Read, 1974; Sokolovski et al. 2002; Yang et al.2002; Walker et al. 2010). ERM colonization may aid in direct plant uptake of N supplied by organic fertilizers.

Studies conducted under controlled conditions have suggested that ERM colonization is reduced as inorganic N availability increases (Stribley and Read, 1976), and contribute relatively less to plant nutrition as N availability increases (Stribley et al. 1975). Conventionally managed soils had higher levels of inorganic N in July, which is a period of peak N demand in blueberries. ERM colonization may compensate to some extent for lower soil inorganic N levels on organic farms than conventional farms that were observed in July by increasing plant utilization of organic soil N, which is at least 50 times more abundant than inorganic N in all soils under study. Determining the levels and composition of dissolved organic N (DON) in soil, whether organic fertilization alters the composition or relative amount of DON to inorganic N, and whether higher levels of ERM colonization facilitate increased uptake of organic N may add to the current understanding soil N cycles in blueberry fields.

A significant management by soil type interaction on ERM colonization indicates that management effects were not consistent across soil types. On the two organic fields with substantially lower ERM colonization than their conventional counterparts on the same soil series, grass sod was maintained in the planting row, while the remainder of organic and conventional fields on sandy soils utilized mulch, cultivation, or herbicides instead of sod culture

in the planting rows. In the two organic fields with low ERM colonization levels, grass roots occupied the upper soil profile, and blueberry roots were largely relegated to soil below the sod layer. ERM colonization was reduced at 15-30-cm compared to 0-15 cm soil depth in Oregon blueberry fields (Scagel and Yang, 2005). Extrapolating from these observations provides circumstantial evidence for a negative effect of grass sod on ERM colonization.

Muck soils with nearly 100% organic matter content did not always support higher mycorrhizal colonization compared to sandy soil types, indicating that organic matter content alone is a poor predictor of ERM colonization, as reported previously by Goulart et al. (1993). However, the magnitude of the difference in ERM colonization between matched pairs of organic and conventional fields was greatest in muck soils. The reason for this is not known. However, it may be inappropriate to generalize these findings to other blueberry fields planted on muck soils due to the relatively low number of organic and conventional farms on muck soils that were compared in this study.

Highbush blueberries are found in areas with perched water tables (Vander Kloet, 1980) and tolerate transient periods of flooding (Korcak, 1989; Abbott and Gough, 1987b). Wild highbush blueberry stands typically have high levels of ERM colonization (Goulart et al. 1993). ERM fungi may be tolerant of flooding and protect colonized roots from high concentrations of organic acids and other phytotoxic compounds that accumulate under waterlogged, anoxic conditions. Evidence of this was provided by, Leake and Read (1991), who reported that the length of hair roots of the ericaceous plant *Calluna vulgaris* L. grown in solution at 50 and 100 ppm of acetic acid was significantly reduced in non-mycorrhizal plants but remained unaffected in plants inoculated with and colonized by ericoid mycorrhizae. It is possible that ERM colonization is instead suppressed by excess water in Michigan blueberries due to extended

periods of flooding occurring in some fields, which limits root elongation and initiation of new hair roots (Abbott and Gough, 1987b), a prerequisite to initial ERM colonization (Kelley, 1950; Valenzuela-Estrada et al., 2008). Colonization by ERM often lags shortly behind rain events in arid climates (Hutton et al., 1994). ERM in Michigan blueberries should not be limited by soil water availability because most fields are irrigated during dry spells. An inverse relationship between ERM and soil water content was reported by Johansson (2000). We are not aware of any studies that have evaluated the effects of anoxic, waterlogged soils on ERM fungi, or whether ERM colonization protects plant roots under flooded conditions.

ERM colonization was negatively correlated with total soil nitrogen, soil NH_4^+-N, BG and PHOS activity, and the residence time of labile carbon, and positively correlated to soil pH. Scagel and Yang (2005) also reported that ERM colonization in blueberry production fields in Oregon was negatively correlated with NH_4^+-N concentration in soil. The inverse relationship between ERM colonization and soil N observed here provides evidence that ERM may be inhibited by high N availability under field conditions, as was previously found in studies carried out under controlled conditions (Stribley and Read, 1976). The observed positive correlation between soil pH and mycorrhizal colonization may be related to higher microbiological activity with increasing soil pH. It appears that ERM colonization is especially important for plant uptake of organic N when microbial activity immobilizes mineral forms of N (Walker et al. 2010). Contrary to our results, Stevens et al. (1997) reported that soil pH and mycorrhizal colonization in cultivated and native blueberries in Pennsylvania were negatively correlated. ERM colonization of native blueberry plants was not assessed in the current study, and it is possible that intensively managed blueberry soils diverge significantly from those under natural conditions. The negative correlation between PHOS activity and mycorrhizal colonization was

likewise unexpected, as ericoid mycorrhizal fungi produce phosphatases which are thought to be important in P nutrition of the host plant (Lemoine et al., 1992). As mentioned above, PHOS activity has been shown to be directly influenced by nitrogen enrichment. Thus, reduced ERM colonization at elevated PHOS levels indirectly may have been a result of high nitrogen availability. The negative correlation between ERM colonization and labile carbon mean residence time is more difficult to interpret than other significant correlations between ERM and soil variables. However, a more lengthy residence time of labile C inputs indicate slower turnover, which may be related to the quality of organic matter inputs entering the soil and the ability of indigenous soil microbial communities to degrade these inputs. It is reasonable to expect that where labile carbon persists for longer periods, biological activity is lower, either because deposited litter is of lower quality and less accessible to microbes, or because the functioning of soil microbes involved in decomposition of labile carbon has been suppressed by some factor unrelated to management practices. Competition between plants and microbes for available soil N will be lower (or less intense) when active carbon pools turn over more slowly. Under these circumstances, ERM colonization would provide less advantage in plant N acquisition because N immobilized by soil microbes as labile C is decomposed would be minimized compared to soils were labile C turns over more rapidly. However, a longer mean residence time of labile C in soil incubations may also indicate more rapid depletion of labile C in situ. We hope that further investigations will be able to unravel the causal factors underlying the significant relationships between the soil parameters and ERM colonization observed in this study.

Future studies might address the benefit provided by ERM colonization in blueberry fields using labeled isotope tracers to determine if increased levels of colonization are related to

increase plant uptake of organic nutrients. In addition, investigation of functional aspects of ericoid mycorrhizae, for example assessing enzyme activities of mycorrhizal roots as was carried out with ectomycorrhizal roots by Courty et al. (2005) may help to elucidate the role of ERM in blueberry production and their response to various management practices. Additionally, it would be of value to match the diversity of ERM fungi with their functions in soil and plants. Cairney et al. (2000) and Grelet et al. (2005) demonstrated that ericoid fungi show inter- and intraspecific differences in the ability to utilize various forms of organic and inorganic N.

Less is known about the role of DSE than ERM in Ericaceous plants. Our study confirms that DSE are nearly ubiquitous in Michigan blueberries. DSE occurrence in blueberry roots was significantly affected by soil type and sampling date, with very low root colonization observed on muck soils and higher colonization in July than in August or October. Our results concur with those of Hambleton and Currah (1997), who found that DSE occurred at low levels in roots collected from bog soils. Jumpponen and Trappe (1998) noted that DSE colonization is more abundant on aged, or senescent root tissue and may benefit plants by reducing C demand by degrading non-functional roots or root cells.

The effects of organic and conventional management on populations of cultivable soil microbes were less pronounced than on soil C and N cycling, soil enzyme activity, and ERM colonization levels. This may be due to relatively imprecise estimates of cultivable microbes in comparison to the aforementioned variables because microbes were only assessed in the fall in each year of the study. In 2009 we observed that fluorescent *Pseudomonas* spp. populations were, in general, enhanced by organic management both at both shallow and deeper soil depths, but highly variable among organic fields. In a study of microorganism populations on organic and conventional tomato farms in Virginia and Maryland, Bulluck et al. (2002) recorded higher

populations of cultivable bacteria and *Trichoderma* spp. on organic farms. However, in a subsequent study in North Carolina, *Trichoderma* spp. populations were more abundant on conventional farms (Liu et al. 2008). Therefore, it is possible that populations of readily cultivated soil microbes may not reliably responsive to the broad categorization of organic and conventional management practices. Additionally, temporal fluctuations in microbial populations due to variability in soil inputs over the growing season may contribute to high variability in observational studies. It is advisable that future investigations into soil microorganism populations include more rigorous sampling to characterize changes in population sizes over time.

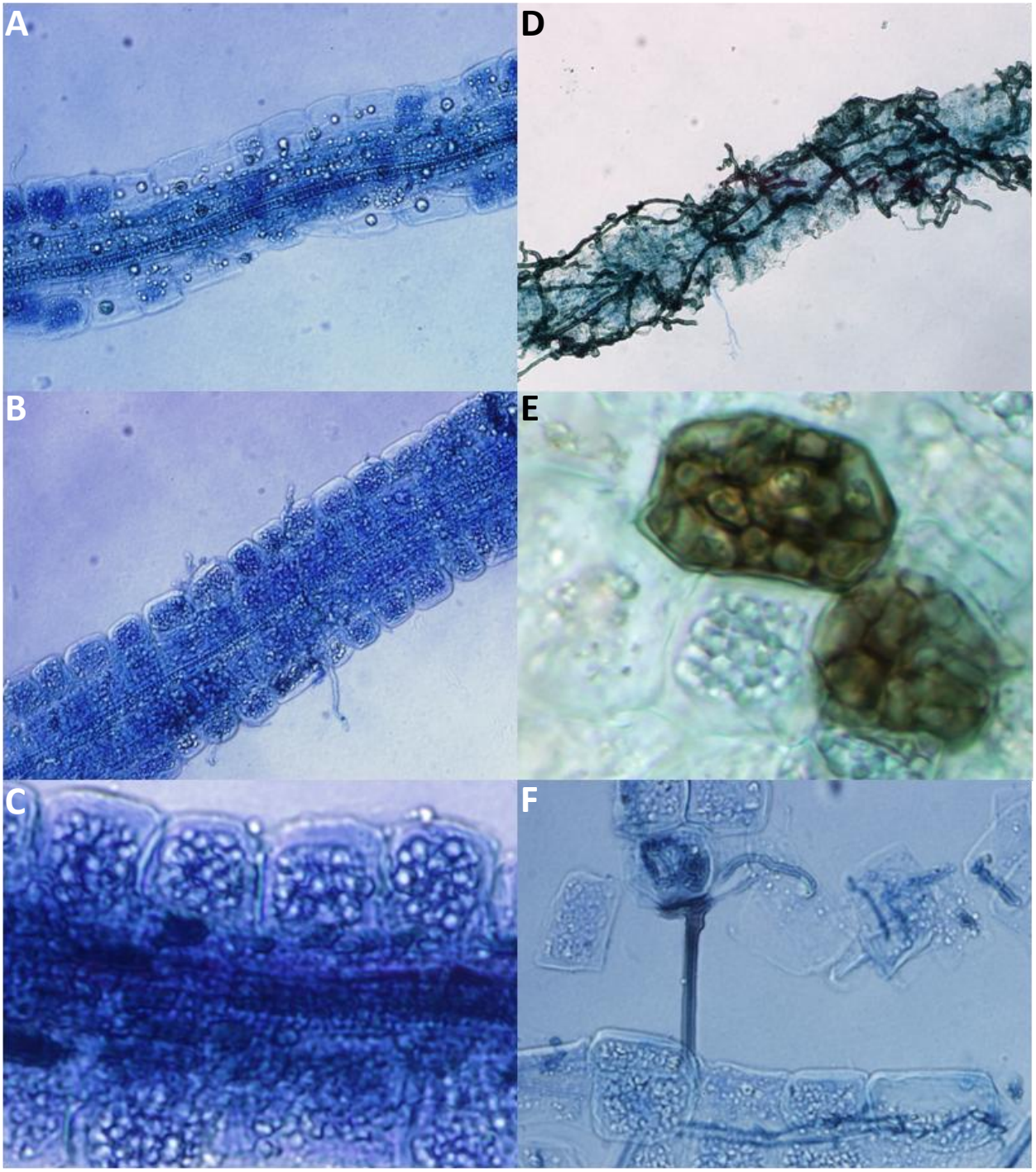
A principal component analysis used to reduce the number of measured variables to two dimensions clearly separated sandy blueberry soils by management practices. The first component represents general carbohydrolase activity while the second component is a contrast between beneficial microbes and PHOS and TAP enzyme activity. A plot of factor scores by management type indicates the soils on organic and conventional farms are more similar at low levels of general biological activity, but at higher biological activity, diverge toward mobilization of N from proteins and increased phosphorus demand (TAP and PER activity) on conventional farms, and more abundant populations of beneficial soil bacteria on organic farms. Interestingly, ERM colonization was only partially correlated with beneficial microbe factor coefficients in principal component two, but was opposed to TAP and PHOS, suggesting factors in soil which contribute to high TAP and PHOS activity (which release amino acids from protein and liberate of phosphate from organic matter, respectively) may be inversely related to ERM colonization.

Lastly, although organic farms tended to have higher populations of beneficial soil bacteria and bacterivorous nematodes, there were also significantly higher populations of root

lesion nematodes in soil. Lesion nematodes were detected on roots collected from four out of eight organic farms but not detected in roots collected from conventional farms. The reasons for this finding are not known. It is possible that pathogenic nematodes collected at 0- to 5-cm depth on organic farms were more associated with herbaceous weeds and grasses than blueberry roots, but the observation of lesion nematodes in roots of blueberries may be cause for concern.

Conclusion

These preliminary results indicate that labile N acquisition, incidence of ERM colonization, and labile C pools are higher in fields under organic management. Ratios of enzyme activity as indices of microbial investment in nutrient acquisition indicate that P is less available in conventionally managed soils while N is more limiting in organically managed soils. ERM colonization was found to be inversely related to total soil N and PHOS activity but positively correlated with soil pH in Michigan blueberry soils. ERM colonization varied among fields and was not always stimulated by high soil organic matter content or organic management practices. The role of ERM in blueberry production fields remains uncertain but further investigation into the function ERM and soil biology in blueberries may contribute to a more sustainable means of blueberry production in Michigan.



Figures 3.1. Light micrographs of blueberry hair roots colonized by ericoid mycorrhizal fungi and dark septate endophytes (DSE). **A.** Root segment with approximately 35% of hair root cells colonized by ericoid mycorrhizae (ERM). **B.** Root segment with 100% ERM colonization. **C.** Close-up of ERM hyphal coils. **D.** Moribund root tissue with DSE hyphae. **E.** DSE microsclerotia. **F.** DSE hyphae penetrating a blueberry hair root cell. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Table 3.1. Site descriptions of matched pairs of organic and conventional blueberry farms. Gray shading designates conventional fields.

Soil taxonomic class	NRCS soil series	Cultivar	Location (°N, °W)	County	Years org. or conv. mgt in 2009	Within-row soil and soil surface observations
Coarse-loamy, mixed, superactive, mesic Typic Endoaquolls	Gilford sandy loam	Elliott	42.3, 86.0	Van Buren	10	Herbicide strip and wood mulch.
	Gilford sandy loam	Bluecrop	41.7, 86.1	St. Joseph, IN	20	Sod maintained at 1- to 6-cm height within rows. Blueberry roots mainly at > 5 cm soil depth, below dense grass sod roots.
Sandy, mixed, mesic Typic Endoaquods	Pipestone-Kingsville complex	Elliott	42.4, 86.3	Van Buren	20	Herbicide strip and wood mulch. Blueberry roots concentrated at < 5 cm soil depth. 2- to 4-cm blueberry leaf litter on soil surface.
	Pipestone sand	Elliott	42.2, 86.3	Berrien	6	Wood mulch. Blueberry roots concentrated at < 5 cm soil depth. Rodent tunnels in mulch and soil.
Sandy, mixed, mesic Typic Endoaquolls	Granby loamy sand	Elliott	43.0, 86.3	Ottawa	10	Herbicide strip, planting rows alternate between wood mulch and no mulch.
	Granby loamy fine sand	Elliott	42.2, 86.2	Berrien	6	Wood mulch and white clover. Anoxic, waterlogged soil at 5- to 30-cm depth on some sampling dates.
	Granby loamy sand	Bluecrop	42.8, 86.1	Ottawa	25	Herbicide strip. Blueberry roots concentrated at soil surface.
	Granby loamy sand	Bluecrop	43.0, 86.2	Ottawa	7	Cultivated soil. Few blueberry roots in upper 20 cm of soil.
	Granby loamy sand	Bluecrop	42.2, 86.3	Ottawa	20	Herbicide strip. Dense mat of blueberry roots at 0- 5-cm soil depth.
	Granby loamy sand	Duke	42.9, 86.1	Ottawa	6	Soil cultivated at 0- 20-cm depth 1 to 2 times per year. Comparatively few blueberry roots in upper 20 cm of soil.

Table 3.1 (cont'd).

Soil taxonomic class	NRCS soil series	Cultivar	Location (°N, °W)	County	Years org. or conv. mgt in 2009	Within-row soil and soil surface observations
Mixed, mesic Aquic Udipsamments	Morocco-Newton complex	Jersey	42.4, 86.0	Allegan	30	Herbicide strip. Blueberry roots concentrated near soil surface. Some soil mottling at shallow depth.
	Morocco complex	Bluecrop	41.7, 86.1	St. Joseph, IN	20	Sod maintained at 1- to 10-cm height.
Euic, mesic Typic Haplosaprists	Houghton muck	Rubel	42.6, 86.1	Allegan	25	Herbicide strip. Anoxic, water-logged soil at 5-30 cm depth.
	Houghton muck	Rubel	42.6, 86.1	Allegan	25	Sod mowed once per year. Few roots in upper 20 cm of soil. Anoxic, waterlogged soil at 5- to 30-cm depth.
	Houghton muck	Rubel/ Jersey	42.4, 86.0	Van Buren	25	Very few weeds. Up to 3 cm leaves and stems accumulated. Anoxic soil at 5-30 cm depth on some sampling dates.
	Houghton muck	Jersey	43.0, 83.0	St. Clair	12	Grass clippings beneath bushes. Patchy sod and bare soil within rows.

Table 3.2. Variables assessed in a comparison of organic and conventional blueberry farms in Michigan in 2008 and 2009.

Parameter	Description	Unit	Dates
H_2O	Soil water content at sampling	g H_2O g soil^{-1}	2008, 2009
SOM	Soil organic matter content	%	2008
SOC	Soil organic carbon	%	2009
Ca, K, Mg	Extractable K, Mg, K	mg kg soil^{-1}	2008
Bray P	Bray-extractable P	mg kg soil^{-1}	2008
Soil N	Total N	%	2009
Nmin	14- or 30-day potential N mineralization[y]	NH_4^+-N + NO_3^--N kg soil^{-1} d^{-1}	2008, 2009
Nitr	14- or 30- day net nitrification[y]	mg NO_3^--N kg soil^{-1} d^{-1}	2008, 2009
Rnitr	Relative nitrification[y]	Nitr Nmin^{-1}	2008, 2009
LF-SOM	Light fraction soil organic matter	mg g soil^{-1}	2008
CO_2	Soil respiration[y]	µg CO_2 g soil^{-1}d^{-1}	2008, 2009
CO_2 C^{-1}	Soil respiration per g SOC	µg CO_2 g SOC^{-1}d^{-1}	2009
C_l	Labile C determined by 460-day soil incubation	mg g soil^{-1}	2008
k	Decomposition constant of labile C	d^{-1}	2008
mrt	Mean residence time of labile C	d	2008
c	Respiration rate of slow + recalcitrant C	µg CO_2 g soil^{-1}d^{-1}	2008
BG	β-D-1,4-glucosidase	nmol h^{-1} g soil^{-1}	2008, 2009
CBH	β-D-1,4-cellobiosidase	nmol h^{-1} g soil^{-1}	2008, 2009
NAG	β-1,4-*N*-acetylglucosaminadase	nmol h^{-1} g soil^{-1}	2008, 2009
PHOS	Acid phosphatase	nmol h^{-1} g soil^{-1}	2008, 2009
TAP	Tyrosine aminopeptidase	nmol h^{-1} g soil^{-1}	2009
POX	Phenol oxidase	nmol h^{-1} g soil^{-1}	2008, 2009
PER	Peroxidase	nmol h^{-1} g soil^{-1}	2009
Enzyme g C^{-1}	Enzyme activity expressed per g soil C	nmol h^{-1} g SOC^{-1}	2009

Table 3.2 (cont'd).

Parameter	Description	Unit	Dates
ERM	Ericoid mycorrhizae	Hair-root epidermal cells containing hyphal coils (%)	2008, 2009
DSE	Dark septate endophytes	Hair-root epidermal cells containing microsclerotia (%)	2008, 2009
Fungi	Cultivable fungi	CFU^{x} g $soil^{-1}$	2008, 2009
Trich	*Trichoderma* spp.	CFU g $soil^{-1}$	2008, 2009
Bact	Cultivable bacteria	CFU g $soil^{-1}$	2008, 2009
Baci	*Bacillus* spp.	CFU g $soil^{-1}$	2009
Pseu	Fluorescent *Pseudomonas* spp.	CFU g $soil^{-1}$	2009
Stre	*Streptomyces* spp.	CFU g $soil^{-1}$	2009

[z]2008 samples collected late Sept and early Oct; 2009 samples collected 6-7 Jul, 21-22 Aug, and 9-10 Oct.

[y]Soils incubated at 25°C and 60% water holding capacity (WHC) for 14 (Nmin, Nitr, Rnitr) or 100 days (CO_2) in 2008 and 30 days at 55% soil WHC for sands and 65% WHC for mucks in 2009.

[x]CFU=Colony forming units; assessed on the last sampling date in 2008 and 2009.

Table 3.3. Analysis of variance of the effects of organic and conventional management and year on anthracnose and Alternaria fruit rot of blueberries collected from farms in Michigan in 2008 and 2009. Bold font denotes significance at $P \leq 0.05$. ANOVA was conducted as a completely randomized design because the sampled field sites differed in 2008 and 2009.

	Num df[z]	Den df[z]	Anthracnose fruit rot	Alternaria fruit rot
Effect			***P > F***	
Management	1	24	**0.01**	**0.02**
Year	1	24	0.13	**0.006**
Management*year	1	24	0.37	0.65

[z]Numerator or denominator degrees of freedom.

Table 3.4. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management and soil depth on soil organic matter (SOM), water content (H_2O), calcium (Ca), magnesium (Mg), potassium (K), and Bray-1 extractable P (P) on sandy soils collected at 0−5 cm and 0−30 cm depth from Michigan blueberry farms on 25 and 26 Sept 2008. Bold font denotes significance at $P \leq 0.05$.

	Num df[z]	Den df[z]	SOM	H_2O	pH	Ca	Mg	K	P
Effect					***P > F***				
Management	1	5	0.12	0.10	**0.03**	**0.006**	0.94	0.93	0.25
Depth	1	10	-[y]	0.71	0.78	-	-	-	-
Management*depth	1	10	-	**0.05**	0.31	-	-	-	-
Preplanned contrasts									
Management at 0−5 cm	1	14	-	**0.03**	**0.02**	-	-	-	-
Management at 0−30 cm	1	14	-	0.22	0.16	-	-	-	-

[z]Numerator or denominator degrees of freedom.

[y]Not determined.

Table 3.5. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management, soil depth, and date on organic carbon (SOC), total N, C:N ratio (C:N), water content (H_2O), and pH of sandy soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009. Bold font denotes significance at $P \leq 0.05$.

	Num df[z]	Den df[z]	SOC	Total N	Soil C:N	H_2O	pH
Effect					***P > F***		
Management	1	7	0.43	0.51	0.29	0.26	0.17
Date	2	56	-[y]	-	-	**0.0002**	**0.005**
Management*date	2	56	-	-	-	0.80	**0.03**
Depth	1	14	**0.0001**	**0.0002**	0.21	**0.008**	0.44
Management*depth	1	14	0.92	0.69	0.87	0.78	0.32
Depth*date	2	56	-	-	-	0.44	0.73
Management*depth*date	2	56	-	-	-	0.80	0.77
Preplanned contrasts							
Management at 0−5 cm	1	14	0.61	0.58	0.20	0.34	0.09
Management at 5−30 cm	1	14	0.34	0.24	0.35	0.23	0.61

[z]Numerator or denominator degrees of freedom.

[y]Not determined.

Table 3.6. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management and soil depth on light fraction organic matter (LF), light fraction organic matter C content (LF C), light fraction organic matter N content (LF N), light fraction C as a percentage of soil organic carbon (LF C %), light fraction N as a percentage of total soil N (LF N %), light fraction organic matter C to N ratio (LF C:N ratio), 100-day CO_2 respiration, labile C (C_l), decomposition constant of C_l (k), mean residence time of C_l (mrt), and respiration rate of slow + resistant C pools (c) in sandy soils collected at 0−5 cm and 0−30 cm depth from Michigan blueberry farms on 25 and 26 Sept 2008. Bold font denotes significance at $P \leq 0.05$.

	Num df[z]	**Den df**[z]	**LF**	**LF C (µg g soil^{-1})**	**LF N (µg g soil^{-1})**	**LF C %**	**LF N %**	**LF C:N ratio**	**100-day CO_2**	**C_l**	***k***	**mrt**	***c***
Effect							***P > F***						
Management	1	5	0.49	0.60	0.58	0.65	0.71	0.36	0.12	0.12	0.33	0.33	0.39
Depth	1	10	**<0.001**	**<0.001**	**<0.001**	0.12	**<0.001**	**0.001**	**<0.001**	**0.006**	0.67	0.67	**0.02**
Management*depth	1	10	**0.02**	**0.009**	**0.004**	0.90	0.79	0.75	0.31	0.08	0.39	0.39	0.10
Preplanned contrasts													
Management at 0−5 cm	1	10	0.10	0.14	0.15	0.64	0.65	0.31	**0.03**	**0.04**	0.19	0.19	0.10
Management at 0−30 cm	1	10	0.66	0.59	0.68	0.72	0.81	0.42	0.71	0.68	0.81	0.81	0.50

[z]Numerator or denominator degrees of freedom.

Table 3.7. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management and soil depth on nitrate (NO_3^--N), ammonium (NH_4^+-N), inorganic N (NO_3^--N + NH_4^+-N), potential N mineralization (Nmin), nitrification (Nitr), and relative nitrification (Rnitr) in sandy soils collected at 0−5 cm and 0−30 cm depth from Michigan blueberry farms on 25 and 26 Sept 2008. Bold font denotes significance at $P \leq 0.05$.

	Num df[z]	Den df[z]	NO_3^--N	NH_4^+-N	Inorganic N	Nmin	Nitr	Rnitr
Effect					***P > F***			
Management	1	5	0.27	0.18	0.15	0.19	**0.02**	0.28
Depth	1	10	0.18	**0.05**	**0.01**	**0.03**	**0.001**	**0.05**
Management*depth	1	10	**0.001**	0.95	0.25	0.46	0.08	0.49
Preplanned contrasts								
Management at 0−5 cm	1	10	**0.005**	0.24	0.06	0.13	**0.008**	0.11
Management at 0−30 cm	1	10	0.22	0.27	0.50	0.51	0.09	0.54

[z]Numerator or denominator degrees of freedom.

Table 3.8. Mixed-model analysis of variance of the effects of organic and conventional management, soil depth, and date on nitrate (NO_3^--N), (NH_4^+-N), inorganic N (NO_3^--N + NH_4^+-N), potential N mineralization (Nmin), nitrification (Nitr), and relative nitrification (Rnitr) in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009. Bold font denotes significance at $P \leq 0.05$. Preplanned contrasts of management at 0−5 and 5-30 cm soil depth averaged across sampling dates are not included because the management by sampling date interaction was significant for all variables.

	Num df[z]	Den df[z]	NO_3^--N	NH_4^+-N	Inorganic N	Nmin	Nitr	Rnitr
Effect					***P > F***			
Management	1	7	0.44	0.66	0.60	**0.02**	0.13	**0.04**
Date	2	56	**<0.001**	**<0.001**	**<0.001**	0.57	**0.001**	**<0.001**
Management*date	2	56	**0.01**	**0.002**	**<0.001**	**0.005**	**0.02**	**0.04**
Depth	1	14	**0.002**	**<0.001**	**<0.001**	**0.01**	**0.003**	0.29
Management*depth	1	14	0.54	0.08	0.16	0.09	0.33	0.70
Depth*date	2	56	**0.04**	**0.05**	0.19	0.56	**0.03**	0.14
Management*depth*date	2	56	0.83	0.10	0.87	**0.05**	0.08	0.23

[z]Numerator or denominator degrees of freedom.

Table 3.9. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management and soil depth on enzyme activity in soils collected at 0−5 cm and 0−30 cm depth from Michigan blueberry farms in late September and early October of 2008.

	Num df[z]	Den df[z]	BG g soil^{-1}	CBH g soil^{-1}	NAG g soil^{-1}	PHOS g soil^{-1}
Effect				***P > F***		
Management	1	7	0.28	0.43	0.10	0.69
Depth	1	14	0.16	0.21	0.08	0.17
Management*depth	1	14	0.85	0.97	0.82	0.65
Preplanned contrasts						
Management at 0−5 cm	1	14	0.40	0.52	0.07	0.55
Management at 5−30 cm	1	14	0.30	0.50	0.23	0.96

[z]Numerator or denominator degrees of freedom.

Abbreviations: BG (β -1,4-glucosidase); CBH (β-D-1,4-cellobiosidase); NAG (β-1,4-*N*-acetylglucosiminadase); PHOS (phosphatase).

Table 3.10. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management, soil depth, and date on enzyme activity and CO_2 respiration rate on a soil and soil organic carbon basis in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009. Bold font indicates significance at $P \leq 0.05$.

	Num df[z]	Den df[z]	BG[y] g soil^{-1}	BG g C^{-1}	CBH g soil^{-1}	CBH g C^{-1}	NAG g soil^{-1}	NAG g C^{-1}	PHOS g soil^{-1}	PHOS g C^{-1}	TAP g soil^{-1}	TAP g C^{-1}	POX g soil^{-1}	POX g C^{-1}	PER g soil^{-1}	PER g C^{-1}	CO_2 g soil^{-1}	CO_2 g C^{-1}
Effect																		
								P > F										
Management	1	7	0.76	0.13	0.48	0.14	0.12	**<0.01**	0.55	0.46	0.69	0.53	0.52	0.60	0.58	0.84	0.53	**0.03**
Date	2	56	0.14	0.88	0.28	0.57	0.06	**0.02**	0.33	**<0.01**	**<0.01**	**<0.01**	0.05	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Management *date	2	56	0.27	0.98	0.92	0.98	0.33	0.33	0.76	0.14	0.31	0.66	0.65	0.37	0.17	**0.04**	0.67	0.74
Depth	1	14	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.99	**0.03**	0.30	0.34	0.56	0.43	**<0.01**	**<0.01**	**<0.01**
Management *depth	1	14	0.64	0.24	0.74	0.38	0.33	0.10	0.32	0.88	0.53	0.99	0.89	0.99	0.07	0.21	0.79	0.73
Depth*date	2	56	0.37	0.09	0.08	0.11	0.41	0.50	0.14	0.32	**0.05**	0.57	0.20	0.27	0.74	0.41	0.09	0.07
Management *depth*date	2	56	0.93	0.70	0.78	0.77	0.98	0.99	0.49	0.71	0.43	0.59	0.62	0.57	0.98	0.98	0.75	0.76
Preplanned contrasts																		
Management at 0−5 cm	1	14	0.65	**0.05**	0.39	0.07	0.06	**<0.01**	0.30	0.55	0.96	0.96	0.57	0.68	0.13	0.37	0.62	**0.05**
Management at 5−30 cm	1	14	0.94	0.58	0.64	0.47	0.25	**0.01**	0.99	0.63	0.52	0.52	0.72	0.72	0.30	0.58	0.55	0.09

[z]Numerator or denominator degrees of freedom.

[y]Abbreviations: BG (β-D-1,4-glucosidase); CBH (β-D-1,4-cellobiosidase); NAG (β-1,4-*N*-acetylglucosiminadase; PHOS (phosphatase); TAP (tyrosine aminopeptidase); POX (phenol oxidase); PER (peroxidase); CO_2 (soil respiration).

Table 3.11. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management, soil depth, and date on the ratio of C:N, C:P, and N:P enzyme activity, on a soil and soil organic matter basis, in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009. Bold font indicates significance at $P \leq 0.05$.

			Enzyme activity					
	Num df[z]	**Den df[z]**	**C:N[y] (g soil^{-1})**	**C:N[y] (g SOM^{-1})**	**C:P[y] (g soil^{-1})**	**C:P[y] (g SOM^{-1})**	**N:P[y] (g soil^{-1})**	**N:P[y] (g SOM^{-1})**
Effect			***P > F***					
Management	1	7	**0.05**	**0.03**	0.19	0.19	**0.04**	**0.03**
Date	2	56	**0.05**	0.20	0.25	0.19	**0.02**	**0.05**
Management*date	2	56	0.55	0.33	0.44	0.51	0.87	0.56
Depth	1	14	0.08	0.95	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Management*depth	1	14	0.69	0.92	0.09	0.07	0.14	0.07
Depth*date	2	56	0.91	0.77	0.41	0.28	0.80	0.79
Management*depth*date	2	56	0.61	0.73	0.74	0.53	0.52	0.46
Preplanned contrasts								
Management at 0−5 cm	1	14	0.11	**0.02**	**0.01**	**0.03**	**0.008**	**0.005**
Management at 5−30 cm	1	14	**0.05**	0.11	0.87	0.99	0.15	0.18

[z]Numerator or denominator degrees of freedom.

[y]C:N=[LN(β-D-1,4-glucosidase):LN(β-1,4-*N*-acetylglucosiminadase + tyrosine aminopeptidase], C:P=[LN(β-D-1,4-glucosidase):LN(phosphatase)], N:P= [LN(β-1,4-*N*-acetylglucosiminadase + tyrosine aminopeptidase):LN(phosphatase)].

Table 3.12. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management and soil depth on populations of cultivable soil microorganisms in soils collected at 0−5 cm and 0−30 cm depth from Michigan blueberry farms in late September and early October 2008. Bold font indicates significance at $P \leq 0.05$.

	Num df[z]	Den df[z]	Bacteria	Fungi	Trichoderma[y]
Effect				***P > F***	
Management	1	7	**0.04**	0.33	0.96
Depth	2	14	0.11	**0.001**	**0.004**
Management*depth	2	14	0.33	0.29	**0.04**
Preplanned contrasts					
Management at 0−5 cm	1	14	**0.04**	0.67	0.34
Management at 0−30 cm	1	14	0.10	0.26	0.16

[z]Numerator or denominator degrees of freedom.

[y]Only sandy soils included in analysis due to zero-values in three of four muck field soils.

Table 3.13. Mixed-model analysis of variance and preplanned contrasts of the effects of organic and conventional management and soil depth on populations of cultivable soil microorganisms in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 9-10 Oct 2009. Bold font indicates significance at $P \leq 0.05$.

	Num df[z]	Den df[z]	Bact[y]	Baci[y]	Pseu[y]	Stre[y]	Fungi	Trich[y]
Effect					***P > F***			
Management	1	7	0.62	0.29	0.07	0.83	0.52	0.87
Depth	2	14	0.22	**0.009**	0.27	**<0.001**	**0.002**	0.13
Management*depth	2	14	0.32	0.91	0.58	0.18	0.53	0.72
Preplanned contrasts								
Management at 0−5 cm	1	14	0.97	0.45	0.06	0.35	0.29	0.93
Management at 5−30 cm	1	14	0.12	0.15	0.15	0.50	0.73	0.48

[z]Numerator or denominator degrees of freedom.

[y]Bact (bacteria); Baci (*Bacillus* spp.); Pseu (fluorescent *Pseudomonas* spp.); Stre (*Streptomyces* spp.); Trich (*Trichoderma* spp.).

Table 3.14. Mixed-model analysis of variance of the effects of organic and conventional management and soil type (NRCS soil series) on ericoid mycorrhizal (ERM) and dark septate endophyte (DSE) colonization of hair roots collected at 0−30 cm depth from Michigan blueberry farms in late September and early October 2008. Bold font indicates significance at $P \leq 0.05$.

	Num df[z]	Den df[z]	ERM	DSE
Effect			***P > F***	
Management	1	3	0.12	0.43
Soil type	4	3	0.53	**0.001**
Management*soil type	4	3	0.11	0.06

[z]Numerator or denominator degrees of freedom.

Table 3.15. Mixed-model analysis of variance of the effects of organic and conventional management, date, and soil type (NRCS soil series) on ericoid mycorrhizal (ERM) and dark septate endophyte (DSE) colonization of hair roots collected at 0−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009. Bold font indicates significance at $P \leq 0.05$.

	Num df[z]	Den df[z]	ERM	DSE
Effect			***P > F***	
Management	1	3	**0.045**	0.53
Date	2	12	0.22	**0.003**
Management*date	2	12	0.93	0.92
Soil type	4	3	0.48	**0.04**
Management*soil type	4	3	**0.03**	**0.05**
Soil type*date	8	12	0.82	0.19
Management*soil type*date	8	12	0.47	0.77

[z]Numerator or denominator degrees of freedom.

Table 3.16. Nematode populations and mixed-model analysis of variance (ANOVA) of the effect of organic and conventional management on lesion, dagger, sheath, ring, spiral, stunt, and total plant-parasitic nematodes in soil and blueberry roots collected at 0−5 cm depth from blueberry farms on 7 and 8 June 2010. Bold font denotes a significant difference between organic and conventional fields at $P \leq 0.05$.

Management	Soil	Pair (block)	Lesion[z]	Lesion (root)[y]	Dagger[z]	Sheath[z]	Ring[z]	Spiral[z]	Stunt[z]	Total plant-parasitic nematodes[z]
Organic	sand	1	40	6	0	0	55	0	0	95
Conventional	sand	1	0	0	0	0	0	0	0	0
Organic	sand	2	10	33	0	0	50	15	0	75
Conventional	sand	2	0	0	0	330	0	0	0	330
Organic	sand	3	10	6	0	0	180	0	0	190
Conventional	sand	3	0	0	0	0	0	10	0	10
Organic	sand	4	30	4	5	0	70	5	0	110
Conventional	sand	4	0	0	0	0	10	0	0	10
Organic	sand	5	5	0	0	10	15	0	0	30
Conventional	sand	5	0	0	0	0	0	0	0	0
Organic	sand	6	0	0	0	5	5	10	15	35
Conventional	sand	6	5	0	0	5	35	30	0	75
Organic	muck	7	5	0	0	5	205	0	0	215
Conventional	muck	7	0	0	0	0	0	0	0	0
Organic	muck	8	10	0	0	0	20	20	10	60
Conventional	muck	8	0	0	0	0	0	0	0	0
Management $P > F$ (H_0: Org - Conv = 0)			**0.02**	-[x]	-	-	0.34	0.78	-	0.31

[z]Number of nematodes per 100 cm^3 soil

[y]Number of nematodes per g root tissue

[x]Insufficient non-zero observations for ANOVA

Table 3.17. Nematode populations and mixed-model analysis of variance (ANOVA) of the effect of organic and conventional management on non-plant-parasitic nematodes (tylenchs, aphelenchs, dorylaims, mononchs, and bacteria-feeding nematodes), total non-plant-parasitic nematodes, total nematodes, ratios of non-plant-parasitic to total nematodes, populations of oligochaetes, and number of arbuscular mycorrhizal (AM) fungi spores in soil collected at 0−5 cm depth from blueberry farms on 7 and 8 June 2010. Bold font denotes a significant difference between organic and conventional fields at $P \leq 0.05$.

Management	Soil	Pair (block)	Tylenchs[z]	Aphelenchs	Dorylaims	Mononchs	Bacterial feeders	Total non-plant-parasitic	Total	Ratio NPP: total[y]	Oligochaetes[z]	AM fungi spores[z]
Organic	sand	1	80	20	5	0	320	425	526	0.8	135	10
Conventional	sand	1	0	15	10	0	175	200	200	1.0	110	420
Organic	sand	2	55	5	10	10	1170	1250	1358	0.9	100	40
Conventional	sand	2	20	10	5	0	90	125	455	0.3	30	30
Organic	sand	3	110	40	50	15	425	640	836	0.8	45	15
Conventional	sand	3	5	70	15	5	375	470	480	1.0	45	35
Organic	sand	4	80	20	25	5	255	385	499	0.8	210	10
Conventional	sand	4	10	55	5	0	325	395	405	1.0	20	5
Organic	sand	5	10	35	15	10	305	375	405	0.9	70	15
Conventional	sand	5	5	10	0	0	135	150	150	1.0	50	50
Organic	sand	6	25	5	40	0	430	500	535	0.9	90	70
Conventional	sand	6	25	30	5	0	390	450	525	0.9	20	25
Organic	muck	7	70	35	15	10	280	410	625	0.7	30	50
Conventional	muck	7	80	10	5	0	40	135	135	1.0	135	135
Organic	muck	8	15	20	0	0	130	165	225	0.7	40	15
Conventional	muck	8	40	35	0	0	110	185	185	1.0	90	50
Management $P > F$ (H_0: Org - Conv = 0)			0.06	0.41	**0.02**	**0.01**	0.06	0.06	**0.04**	**0.05**	0.30	0.20

[z]Number per 100 cm^3 soil. [y]Ratio of non-plant-parasitic to total (plant-parasitic + non-plant-parasitic) nematodes.

Table 3.18. Pearson's correlation coefficient (r) for correlations among variables measured on sandy blueberry soils collected at 0−30 cm depth from organic and conventional blueberry farms (n=12) in late September and early October 2008. Variables are defined in Table 3.2. Bold-italic font indicates a significant relationship at $P < 0.05$.

	SOM	H_2O	pH	Ca	Mg	K	Bray P	Nmin	Nitr	Rnitr	LF-SOM	LF-C:SOM	LF C:N	CO_2	C_l	mrt	*c*	BG	CBH	NAG	PHOS	Bact	Fungi	Trich	ERM
H_2O	0.5																								
pH	0.1	0.3																							
Ca	-0.1	0.2	***0.7***																						
Mg	0.1	***0.6***	***0.7***	***0.6***																					
K	0.1	0.3	0.4	0.4	0.4																				
Bray P	-0.2	***-0.6***	-0.2	0.1	-0.2	-0.1																			
Nmin	-0.1	-0.5	0.1	-0.1	-0.4	-0.1	0.3																		
Nitr	-0.3	***-0.6***	0.2	0.2	-0.2	0.1	***0.6***	***0.8***																	
Rnitr	-0.5	-0.2	0.1	0.2	0.4	0.3	0.3	-0.4	0.2																
LF-SOM	***0.7***	0.1	-0.3	-0.3	-0.2	-0.5	0.0	-0.2	-0.3	-0.4															
LF-C:SOM	0.5	0.0	-0.2	-0.3	-0.2	***-0.6***	0.3	-0.1	-0.2	-0.2	***0.9***														
LF C:N	0.3	***0.9***	0.4	0.2	***0.6***	0.2	***-0.6***	-0.5	-0.6	-0.1	0.0	0.0													
CO_2	0.3	0.4	0.4	0.2	***0.6***	0.2	***-0.6***	***0.7***	-0.5	***-0.6***	0.1	0.1	-0.2												
C_l	0.0	0.5	0.2	***0.7***	0.5	0.2	0.1	-0.5	-0.2	0.0	0.0	0.0	0.3	-0.1											
mrt	-0.1	-0.5	-0.5	0.0	-0.3	-0.2	***0.7***	0.3	0.5	0.0	0.2	0.1	***-0.7***	-0.1	0.2										
c	-0.6	-0.3	-0.1	-0.2	-0.2	0.1	0.4	0.1	0.2	0.3	-0.5	-0.2	-0.1	0.3	-0.2	0.0									
BG	-0.1	0.4	0.3	0.5	0.3	0.2	-0.2	-0.4	-0.2	0.1	0.0	0.0	0.5	-0.1	0.6	-0.3	0.2								
CBH	0.4	0.5	0.1	0.4	0.1	0.5	-0.4	-0.1	-0.2	-0.4	0.1	-0.2	0.2	-0.1	0.5	0.0	-0.4	0.5							
NAG	0.0	0.4	0.2	0.5	0.2	0.0	-0.2	-0.3	-0.3	-0.3	0.1	0.1	0.5	-0.1	***0.7***	-0.1	-0.3	***0.8***	0.5						
PHOS	0.3	0.3	-0.2	0.0	-0.1	0.5	-0.4	-0.3	-0.4	-0.2	0.1	-0.3	0.1	-0.2	0.2	-0.1	-0.2	0.3	***0.8***	0.2					
Bact	-0.2	0.4	0.5	***0.6***	0.4	0.3	-0.3	-0.2	-0.1	0.1	-0.2	-0.2	0.5	0.1	0.5	-0.4	0.2	***0.9***	0.4	***0.7***	0.2				
Fungi	0.0	0.2	0.1	0.4	0.2	-0.2	0.0	-0.4	-0.2	-0.1	0.3	0.2	0.2	-0.3	0.6	0.1	0.0	***0.6***	0.2	***0.7***	0.2	***0.7***			
Trich	-0.2	0.5	0.3	0.4	0.4	-0.1	-0.3	-0.3	-0.4	-0.2	-0.1	0.0	***0.7***	-0.4	***0.6***	-0.3	0.3	0.7	0.2	***0.8***	-0.1	***0.7***	***0.7***		
ERM	0.0	0.3	***0.6***	0.4	0.4	0.4	-0.4	-0.3	-0.4	0.0	-0.4	-0.4	0.5	-0.4	0.2	***-0.7***	0.0	0.2	0.1	0.2	0.1	0.2	0.0	0.4	
DSE	-0.4	***-0.7***	-0.2	0.1	-0.3	0.1	0.4	0.3	***0.6***	0.3	-0.1	-0.2	***-0.7***	-0.4	-0.2	0.5	0.3	0.1	-0.1	-0.2	0.2	0.1	0.2	-0.4	-0.5

Table 3.19. Pearson's correlation coefficient (r) for correlations among variables measured on muck blueberry soils collected at 0−30 cm depth from organic and conventional Michigan blueberry farms (n=4) in late September and early October 2008. Variables are defined in Table 3.2. Bold-italic font indicates a significant relationship at $P < 0.05$.

	SOM	H_2O	pH	Ca	Mg	K	Bray P	Nmin	Nitr	Rnitr	CO_2	BG	CBH	NAG	PHOS	Bact	Fungi	Trich	ERM
H_2O	0.71																		
pH	0.63	0.84																	
Ca	0.82	0.68	0.89																
Mg	0.68	0.42	0.78	***0.95***															
K	0.16	-0.55	-0.57	-0.14	-0.01														
Bray P	***-0.99***	-0.80	-0.67	-0.80	-0.62	-0.05													
Nmin	0.68	0.84	***0.99***	0.92	0.81	-0.51	-0.71												
Nitr	0.85	0.87	0.94	0.95	0.81	-0.31	-0.88	***0.96***											
Rnitr	0.04	-0.18	-0.66	-0.54	-0.65	0.54	-0.05	-0.63	-0.42										
CO_2	0.39	0.84	0.44	0.17	-0.14	-0.57	-0.51	0.42	0.47	0.22									
BG	***0.97***	0.55	0.53	0.81	0.73	0.34	-0.93	0.59	0.78	0.04	0.18								
CBH	0.77	0.11	0.14	0.56	0.59	0.72	-0.68	0.21	0.43	0.21	-0.21	0.89							
NAG	0.83	0.22	0.11	0.49	0.45	0.69	-0.76	0.18	0.43	0.39	0.00	0.90	***0.97***						
PHOS	***0.95***	0.88	0.80	0.86	0.68	-0.14	***-0.98***	0.83	***0.95***	-0.12	0.57	0.87	0.55	0.62					
Bact	-0.72	-0.86	-0.47	-0.39	-0.09	0.23	0.80	-0.48	-0.63	-0.34	-0.90	-0.56	-0.23	-0.43	-0.79				
Fungi	0.67	0.61	0.13	0.17	-0.09	0.12	-0.72	0.16	0.38	0.66	0.74	0.56	0.39	0.60	0.63	-0.92			
Trich	0.34	0.52	-0.03	-0.14	-0.43	-0.10	-0.43	-0.03	0.13	0.71	0.84	0.19	0.00	0.25	0.37	-0.85	0.91		
ERM	0.68	0.91	***0.99***	0.87	0.71	-0.58	-0.73	***0.99***	***0.95***	-0.54	0.56	0.56	0.14	0.15	0.86	-0.59	0.27	0.12	
DSE	***0.98***	0.55	0.51	0.78	0.69	0.35	-0.94	0.56	0.76	0.09	0.21	***0.99***	0.89	0.91	0.87	-0.59	0.60	0.23	0.54

Table 3.20. Pearson's correlation coefficient (r) for correlations among variables measured on sandy blueberry soils at 0−30 cm depth (bulk-density-weighted average of 0−5 cm and 5−30 cm) collected from organic and conventional blueberry farms (n=12) on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (mean of three dates). Variables are defined in Table 3.2. Bold-italic font indicates a significant relationship at $P < 0.05$.

	H_2O	pH	C^{yz}	N^{yz}	$C:N^{yz}$	CO_2	$CO_2 C^{-1}$	NH_4^+-N	NO_3^--N	Inorg-N	N-min	Nitr	Rnitr	BG	CBH	NAG	PHOS	POX	PER	TAP	$Bact^z$	$Baci^z$	$Pseu^z$	$Stre^z$	$Fungi^z$	$Trich^z$	ERM
pH	0.2																										
C	***0.6***	0.2																									
N	0.4	0.0	***0.8***																								
C:N	0.5	0.2	***0.7***	0.1																							
CO2	0.1	0.0	0.4	0.1	0.5																						
$CO_2 C^{-1}$	-0.5	-0.2	***-0.7***	***-0.7***	-0.3	0.4																					
NH4+	0.2	-0.4	0.4	***0.8***	-0.2	0.3	-0.2																				
NO3-	0.3	0.3	0.1	0.0	0.1	0.1	0.0	0.0																			
Inorg-N	0.3	0.1	0.2	0.3	0.0	0.2	0.0	0.4	***0.9***																		
Nmin	-0.5	0.1	-0.2	0.1	-0.5	0.2	0.3	0.2	-0.3	-0.2																	
Nitr	-0.5	-0.2	-0.2	0.3	***-0.6***	0.1	0.2	0.4	-0.4	-0.2	***0.9***																
Rnitr	0.1	-0.4	0.3	0.4	0.1	0.5	0.1	0.5	-0.3	-0.1	0.3	0.4															
BG	0.1	-0.2	0.5	0.4	0.4	***0.8***	0.1	0.4	0.3	0.4	0.2	0.2	0.5														
CBH	0.0	-0.1	0.4	0.3	0.3	***0.8***	0.2	0.3	0.2	0.3	0.4	0.4	0.5	***0.9***													
NAG	0.2	0.2	0.3	0.0	0.5	***0.9***	0.4	0.0	0.2	0.2	0.3	0.1	0.4	***0.8***	***0.8***												
PHOS	0.4	-0.5	***0.6***	0.5	0.4	0.2	-0.3	0.5	0.0	0.2	-0.2	0.0	0.5	0.5	0.3	0.2											
POX	0.5	-0.5	0.1	0.1	0.1	-0.1	-0.1	0.4	0.2	0.3	***-0.6***	-0.5	0.0	-0.1	-0.2	-0.3	0.4										
PER	0.3	-0.6	0.2	0.3	0.1	0.4	0.0	0.5	0.2	0.4	-0.3	-0.1	0.3	***0.6***	0.5	0.2	0.5	***0.7***									
TAP	-0.3	0.2	0.2	-0.2	0.4	0.2	0.0	-0.4	0.0	-0.2	0.1	0.0	0.2	0.3	0.3	0.3	0.1	-0.6	-0.4								
Bact	0.0	0.1	-0.3	-0.3	-0.2	0.1	0.3	0.2	0.1	0.2	***0.7***	0.6	0.4	0.0	0.1	0.2	-0.2	0.2	-0.1	-0.2							
Baci	-0.2	0.1	-0.1	0.0	-0.2	0.2	0.3	0.5	0.3	0.5	0.5	0.6	***0.6***	0.2	0.2	0.3	-0.2	0.0	0.2	-0.2	***0.7***						
Pseu	0.5	0.7	0.3	0.0	0.5	***0.6***	0.2	0.0	-0.1	0.0	0.0	-0.2	0.0	0.2	0.2	0.3	-0.2	0.1	-0.2	-0.1	0.4	0.3					
Stre	-0.1	0.2	-0.2	0.1	-0.3	0.1	0.3	0.4	0.2	0.3	0.2	0.3	***0.6***	0.1	0.2	0.3	-0.4	-0.2	0.0	***-0.7***	0.3	***0.6***	0.3				
Fungi	-0.1	0.5	-0.3	0.1	-0.5	-0.5	-0.2	-0.4	0.0	-0.1	-0.4	-0.3	-0.2	-0.5	-0.4	-0.4	***-0.7***	-0.5	-0.4	***-0.7***	-0.3	-0.3	-0.1	0.3			
Trich	0.5	0.1	0.2	0.0	0.3	0.2	0.1	0.6	-0.3	0.0	0.1	0.0	0.4	0.1	0.1	0.3	0.2	-0.3	-0.1	-0.1	0.3	0.3	0.2	0.4	-0.1		
ERM	0.0	***0.6***	-0.5	-0.6	-0.2	-0.3	0.3	***-0.6***	0.2	-0.1	0.0	-0.3	-0.4	***-0.6***	-0.5	-0.1	***-0.7***	-0.2	***-0.6***	-0.1	0.5	0.2	0.3	0.1	0.2	0.4	
DSE	0.0	0.4	0.4	0.4	0.2	0.2	-0.3	0.0	0.4	0.4	0.3	0.2	0.1	0.4	0.5	0.4	0.2	***-0.6***	-0.2	***0.6***	0.1	0.1	0.1	0.1	0.4	0.1	-0.1

[z]Measured only on samples collected 9-10 Oct 2009. [y]Assumed to be constant across sampling dates.

Table 3.21. Pearson's correlation coefficient (r) for correlations among variables measured on muck blueberry soils at 0-30 cm depth (bulk-density-weighted average of 0−5 cm and 5−30 cm) collected from organic and conventional blueberry farms (n=4) on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009. Variable names are defined in Table 3.2. Bold-italic font indicates a significant relationship at $P < 0.05$.

	H_2O	C^{yz}	N^{yz}	$C:N^{yz}$	CO_2	$CO_2\ C^{-1}$	NH_4^+-N	NO_3^--N	Inorg-N	Nmin	Nitr	Rnitr	BG	CBH	NAG	PHOS	POX	PER	TAP	Bact	$Baci^z$	$Pseu^z$	$Stre^z$	$Fungi^z$	$Trich^z$	ERM	DSE
C	0.5																										
N	0.8	0.7																									
C:N	-0.7	-0.2	-0.8																								
CO2	0.9	0.9	0.8	-0.5																							
CO2 C^{-1}	***0.9***	0.6	0.8	-0.7	0.9																						
NH4+	0.9	0.5	0.9	-0.9	0.8	0.9																					
NO3-	-0.6	-0.8	***-0.9***	0.6	-0.8	-0.6	-0.8																				
Inorg-N	0.3	-0.7	-0.3	-0.3	-0.2	0.2	0.1	0.5																			
Nmin	0.9	0.8	0.9	-0.6	***0.9***	0.9	0.9	-0.8	-0.2																		
Nitr	0.4	***0.9***	0.5	0.1	0.8	0.5	0.2	-0.7	-0.7	0.7																	
Rnitr	0.5	0.0	-0.2	0.1	0.3	0.5	0.0	0.3	0.6	0.2	0.1																
BG	0.8	-0.1	0.4	-0.8	0.4	0.7	0.7	-0.1	0.8	0.4	-0.3	0.4															
CBH	0.7	-0.2	0.2	-0.6	0.3	0.6	0.5	0.1	0.9	0.3	-0.3	0.7	***0.9***														
NAG	0.8	0.1	0.3	-0.5	0.6	0.8	0.6	-0.1	0.7	0.6	0.0	0.8	0.9	0.9													
PHOS	0.5	***0.9***	0.6	0.0	0.8	0.6	0.3	-0.7	-0.6	0.8	***0.9***	0.2	-0.2	-0.2	0.2												
POX	0.6	0.4	0.9	-0.9	0.6	0.6	0.9	-0.8	-0.1	0.7	0.2	-0.4	0.5	0.2	0.2	0.2											
PER	0.8	0.9	0.7	-0.2	***0.9***	0.8	0.5	-0.7	-0.3	0.9	0.9	0.4	0.1	0.1	0.5	***0.9***	0.3										
TAP	0.2	0.9	0.4	0.2	0.7	0.3	0.1	-0.6	-0.8	0.6	***0.9***	0.0	-0.4	-0.4	-0.1	***0.9***	0.1	0.8									
Bact	-0.9	-0.6	-0.6	0.5	***-0.9***	***-0.9***	-0.8	-0.2	-0.7	0.0	***0.9***	-0.6	-0.6	-0.6	-0.8	-0.7	0.1	-0.4	-0.9								
Baci	-0.8	-0.2	-0.8	***0.9***	-0.6	-0.8	-0.9	-0.6	***-0.9***	0.6	0.8	-0.1	-0.9	-0.8	-0.6	-0.1	-0.4	0.3	-0.2	0.6							
Pseu	0.8	-0.3	0.0	-0.4	0.3	0.7	0.4	0.9	0.5	-0.7	-0.6	0.8	0.8	0.9	1.0	0.0	0.7	-0.3	0.2	-0.6	-0.5						
Stre	-0.9	-0.5	-0.5	0.3	-0.9	-0.9	-0.6	-0.3	-0.6	0.0	0.9	-0.8	-0.5	-0.6	-0.9	-0.7	0.1	-0.5	-0.9	***0.9***	0.5	-0.7					
Fungi	***-0.9***	-0.3	-0.5	0.5	-0.8	***-0.9***	-0.7	-0.5	-0.8	0.3	***0.9***	-0.8	-0.7	-0.8	***-0.9***	-0.5	-0.2	-0.2	-0.7	***0.9***	0.7	-0.8	***0.9***				
Trich	0.2	***0.9***	0.7	-0.1	0.6	0.2	0.4	-0.7	0.2	0.7	-0.3	-0.3	-0.2	-0.4	-0.2	0.8	-0.8	0.7	0.7	-0.4	-0.1	-0.5	-0.3	-0.1			
ERM	0.8	0.3	0.3	-0.3	0.7	0.8	0.4	-0.1	0.4	0.6	0.3	0.9	0.6	0.7	0.9	0.5	0.0	0.7	0.2	-0.9	-0.4	0.8	***-0.9***	***-0.9***	0.1		
DSE	0.0	0.8	0.1	0.5	0.5	0.1	-0.2	-0.3	-0.7	0.3	0.9	0.1	-0.6	-0.5	-0.2	0.9	-0.3	0.7	***0.9***	-0.3	0.4	-0.4	-0.3	0.0	0.7	0.2	

[z]Measured only on samples collected 9-10 Oct.

[y]Assumed constant across sampling dates.

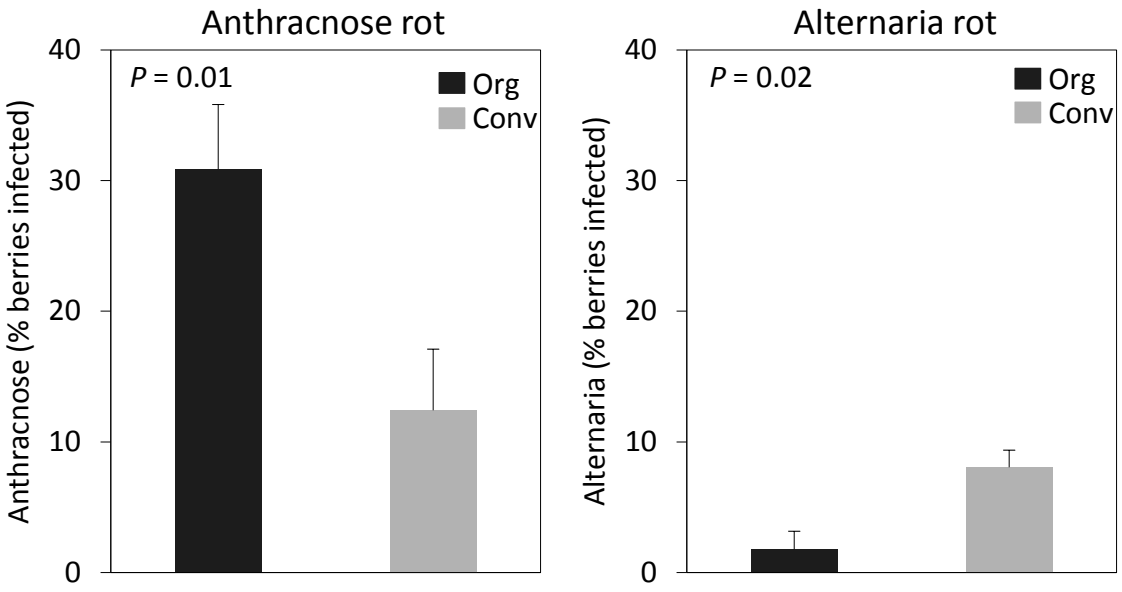


Figure 3.2. The effect of organic and conventional management on anthracnose and Alternaria rot incidence in blueberries collected from Michigan blueberry farms in 2008 and 2009. Error bars represent one standard error of the mean (n=8). Management types are considered significantly different when $P \leq 0.05$ (two-way ANOVA, management effect F-test).

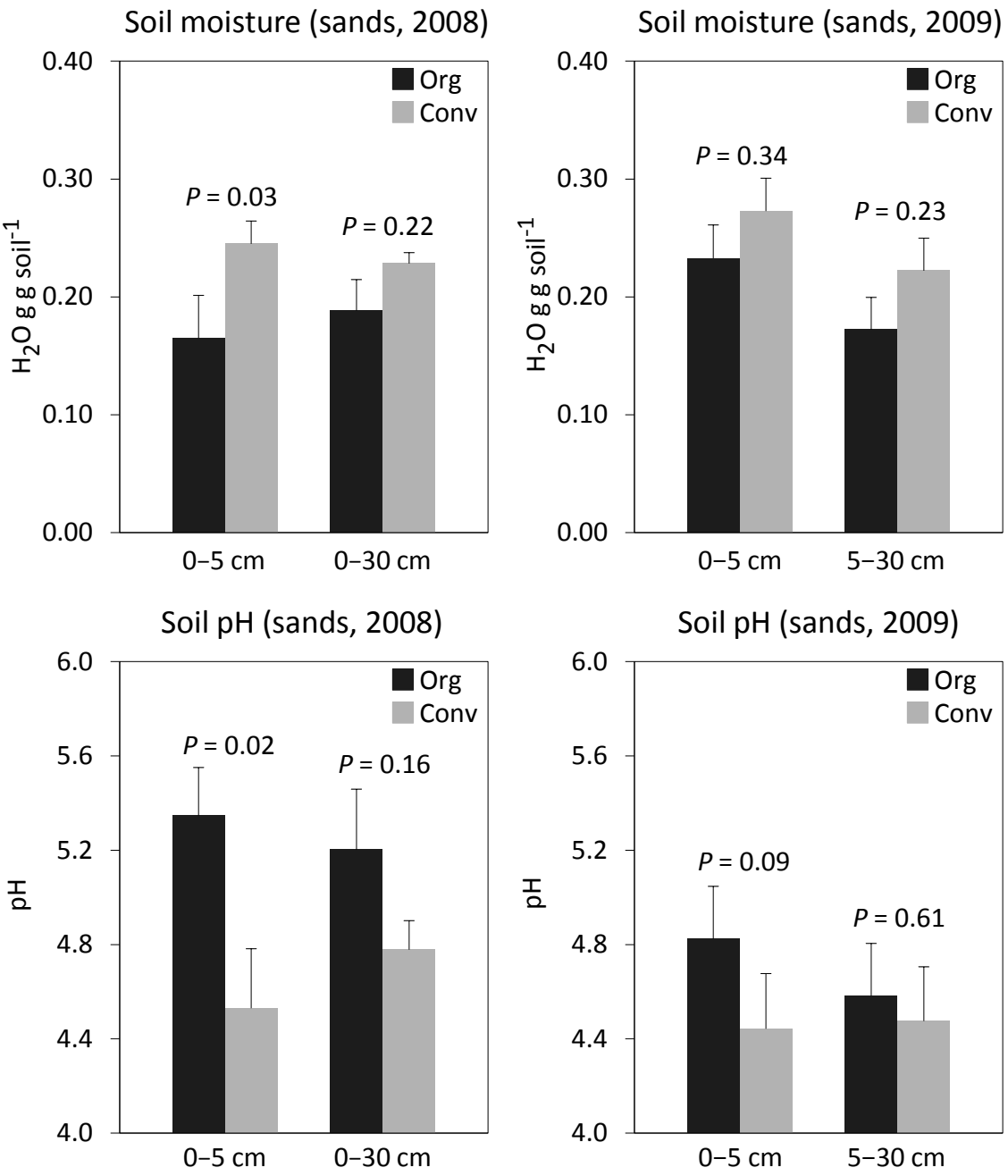


Figure 3.3. The effect of organic and conventional management on the pH and moisture content of sandy soils collected at 0−5 cm and 0−30 cm depth from Michigan blueberry farms on 25-26 Sept 2008. Error bars represent one standard error of the mean (n=6). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

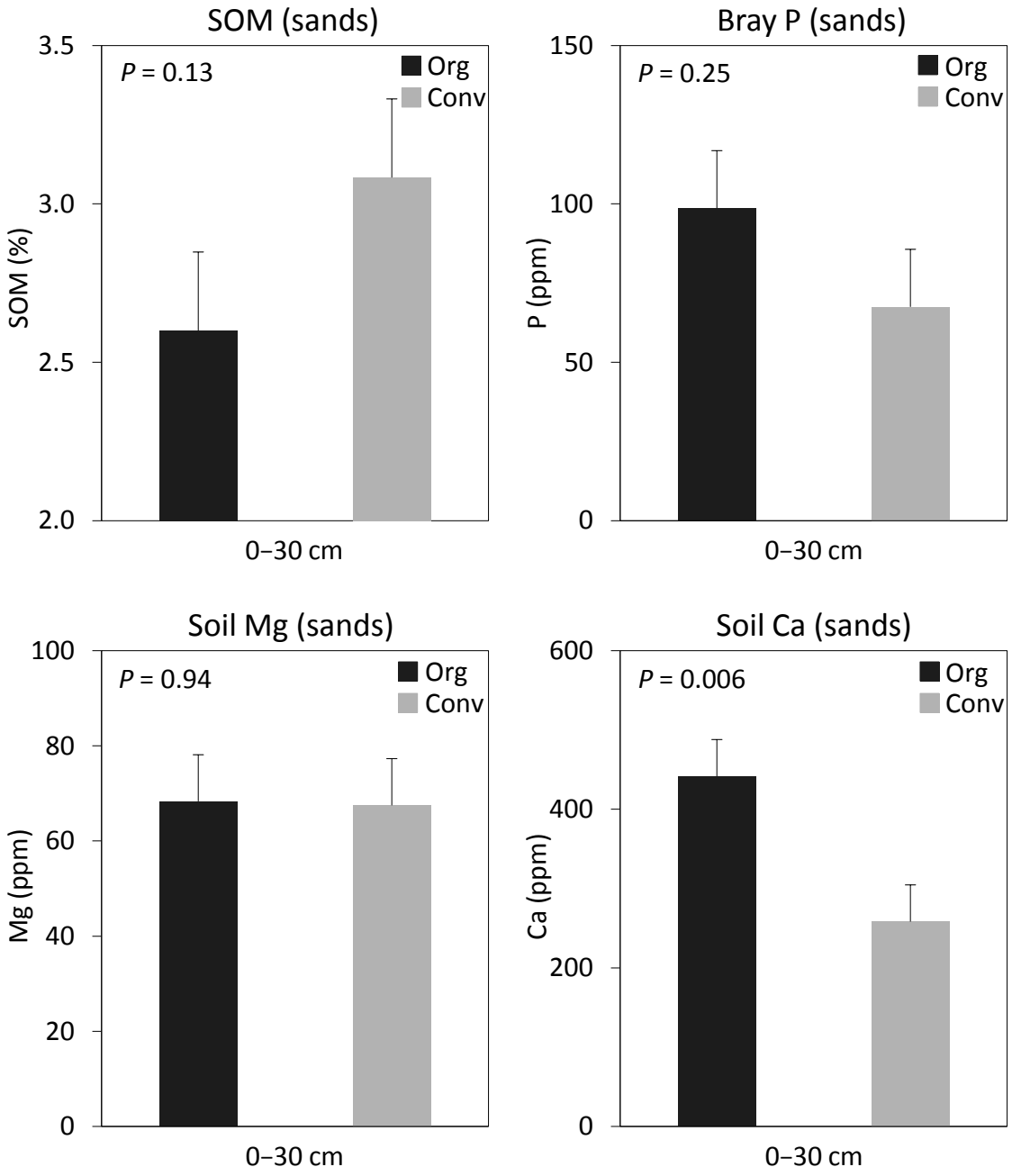


Figure 3.4. The effect of organic and conventional management on soil organic matter (SOM), P, Ca, and Mg content in sandy soils collected at 0−30 cm depth from Michigan blueberry farms collected on 25-26 Sept 2008. Error bars represent one standard error of the mean (n=6). Management types are considered significantly different when $P \leq 0.05$ (one-way ANOVA *F*-test).

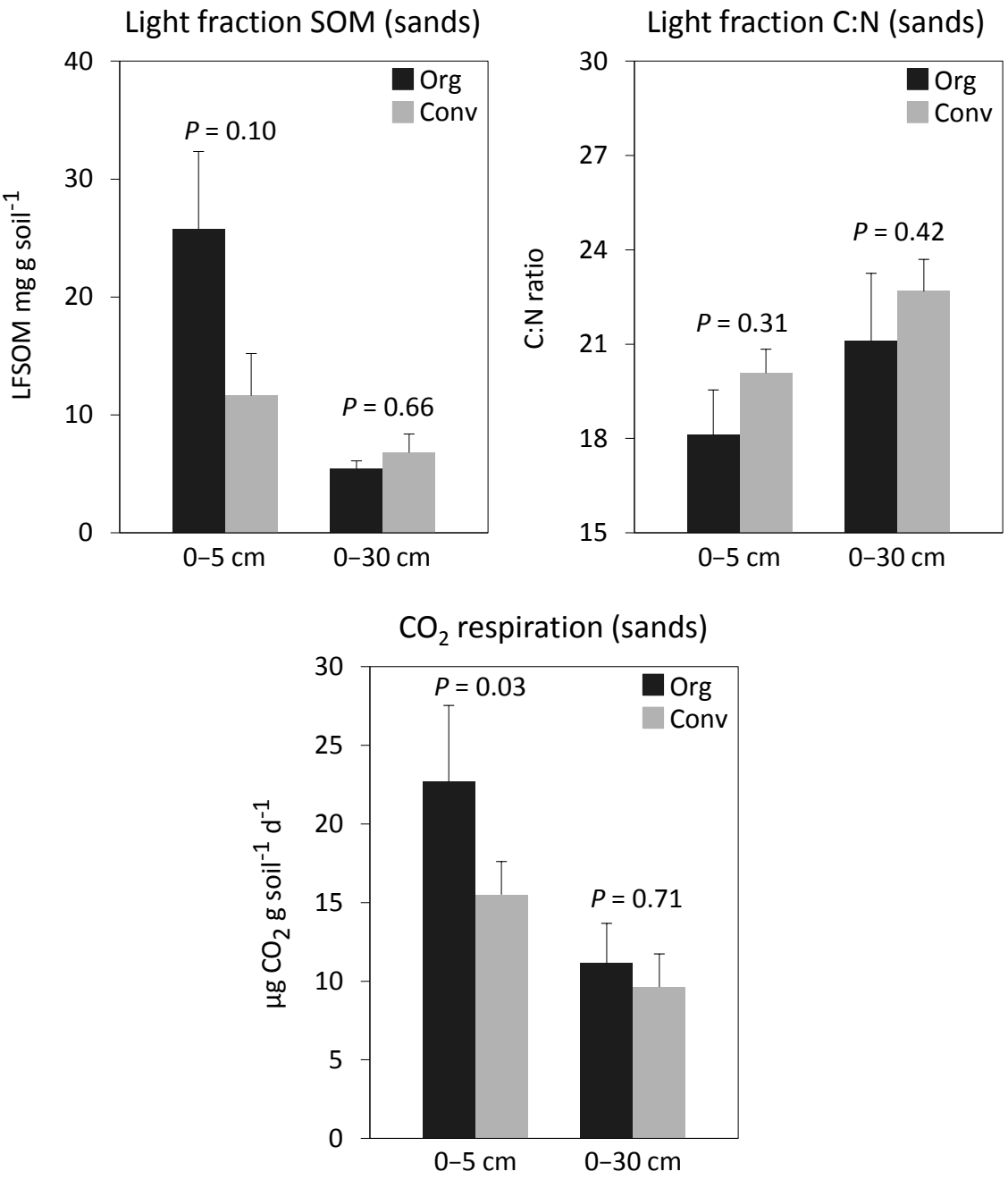


Figure 3.5. The effect of organic and conventional management on light fraction soil organic matter (SOM) content, light fraction C:N ratio and CO_2 respired in a 100-day laboratory incubation of sandy soils collected at 0−5 cm and 0−30 cm depth from Michigan blueberry farms on 25-26 Sept 2008. Error bars represent one standard error of the mean (n=6). Management types considered are significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

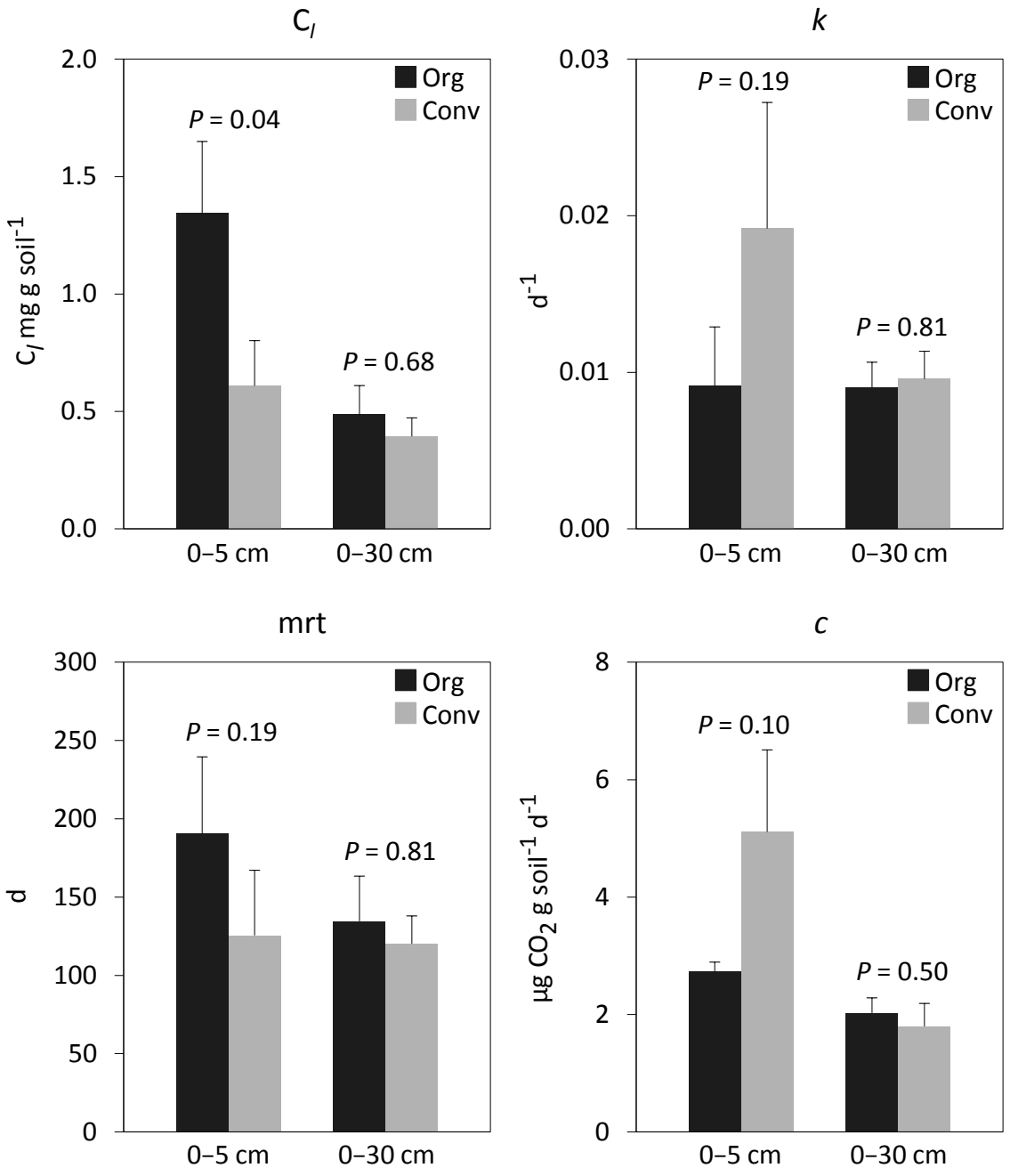


Figure 3.6. The effect of organic and conventional management on labile C (C_l) content, labile C decomposition constant (*k*), labile C mean residence time (mrt), and respiration of slow + resistant C (*c*) in sandy soils collected at 0−5 cm and 0−30 cm depth from Michigan blueberry farms on 25-26-Sept 2008. Error bars represent one standard error of the mean (n=6). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

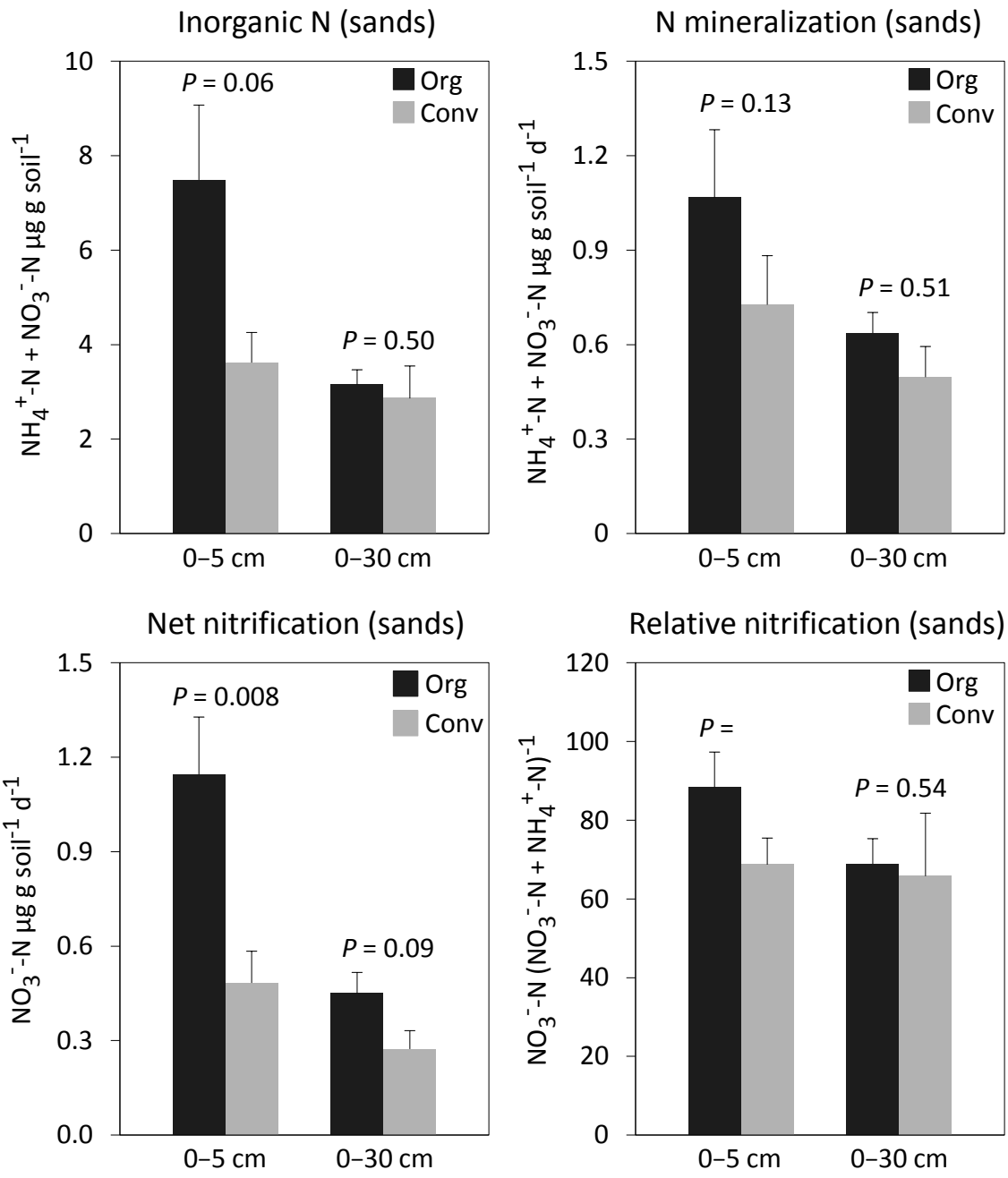


Figure 3.7. The effect of organic and conventional management on inorganic N at sampling, potential N mineralization, nitrification, and relative nitrification of sandy soils collected at 0−5 cm and 0−30 cm depths from Michigan blueberry farms on 25-26 Sept 2008. Error bars represent one standard error of the mean (n=6). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

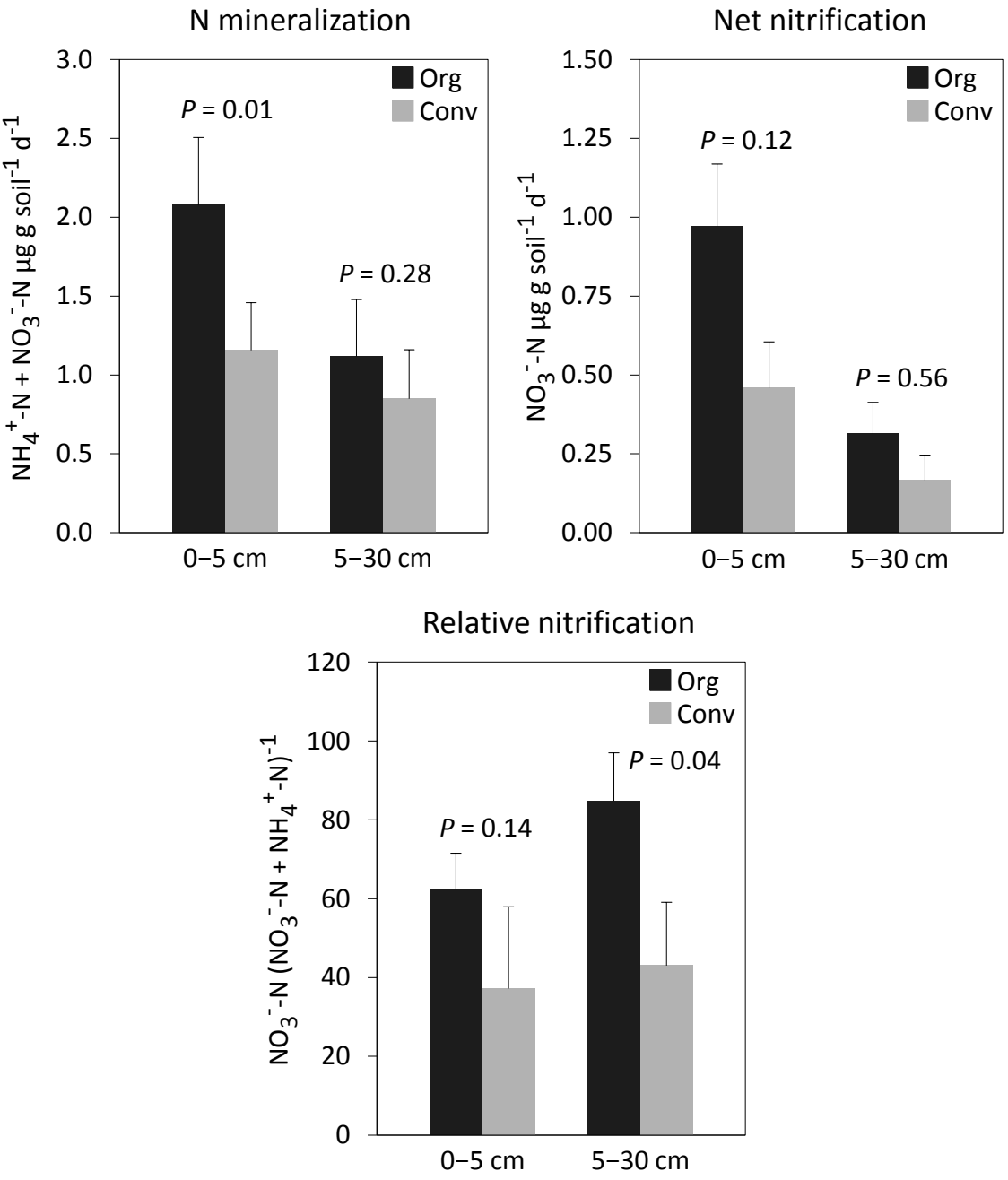


Figure 3.8. The effect of organic and conventional management on potential N mineralization, nitrification, and relative nitrification of soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 July, 21-22 August, and 9-10 October 2009 (average of three sampling dates shown). Error bars represent one standard error of the mean (n=8). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

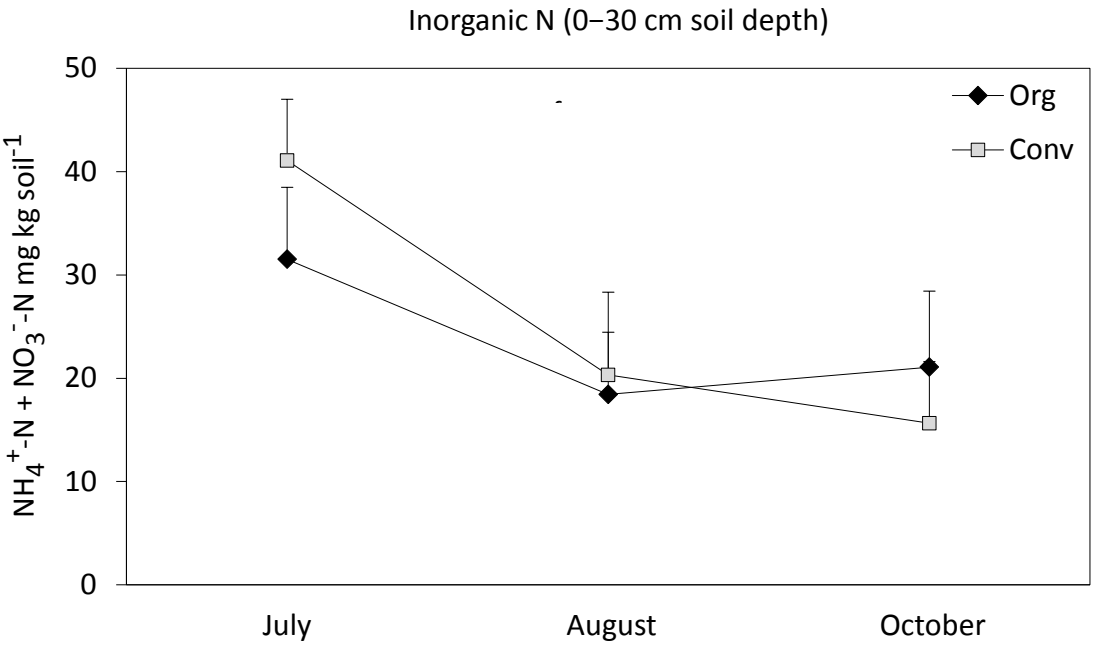


Figure 3.9. The effect of organic and conventional management on inorganic soil nitrogen (N) (nitrate + ammonium) collected at 0- to 30-cm depth (bulk-density-weighted average of 0−5 cm and 5−30 cm) from Michigan blueberry farms on 6-7 July, 21-22 August, and 9-10 October 2009. Error bars represent one standard error of the mean (n=8). The *P*-values for July, August, and October sampling dates are *P*=0.04, *P*=0.99, and *P*=0.001, respectively (pre-planned contrast *F*-test). Management types are considered significantly different when $P \leq 0.05$. Data were log-transformed prior to analysis; untransformed means and standard errors are shown.

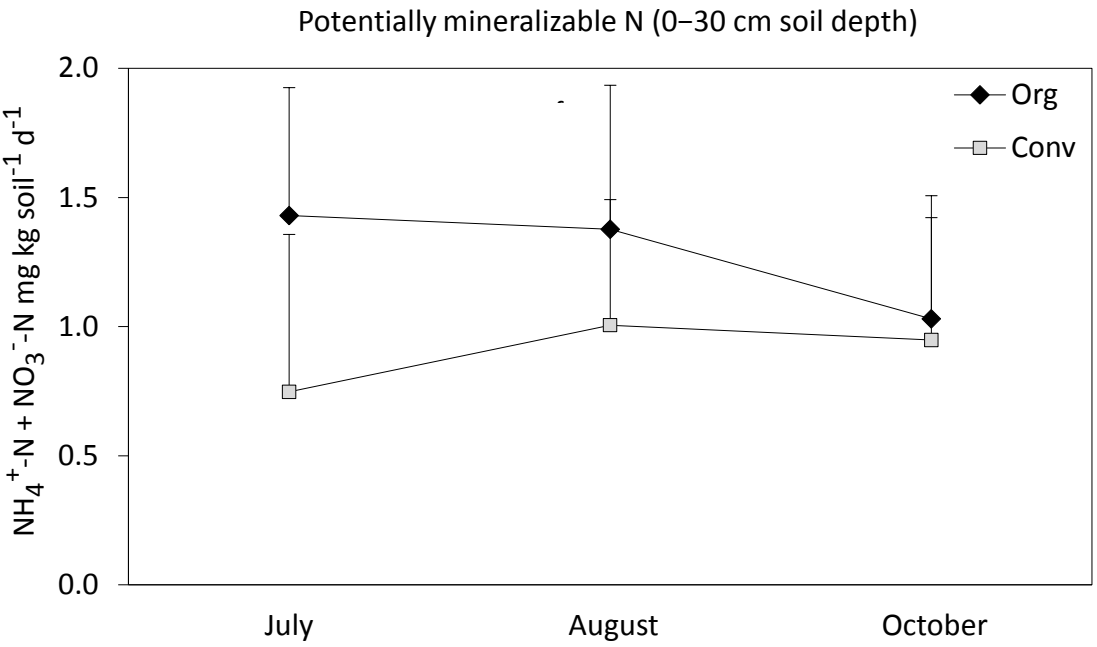


Figure 3.10. The effect of organic and conventional management on potentially mineralizable nitrogen (N) in soil collected at 0- to 30-cm depth (bulk-density-weighted average of 0−5 cm and 5−30 cm) from Michigan blueberry farms on 6-7 July, 21-22 August, and 9-10 October 2009 and incubated for 30 days at 25°C and 55% (sands) or 65% (mucks) water-holding capacity. Error bars represent one standard error of the mean (n=8). The *P*-values for July, August, and October sampling dates are P=0.003, P=0.48, and P=0.34, respectively (pre-planned contrast *F*-test). Management types are considered significantly different when $P \leq 0.05$. Data were (ln + 1)-transformed prior to analysis; untransformed means and standard errors are shown.

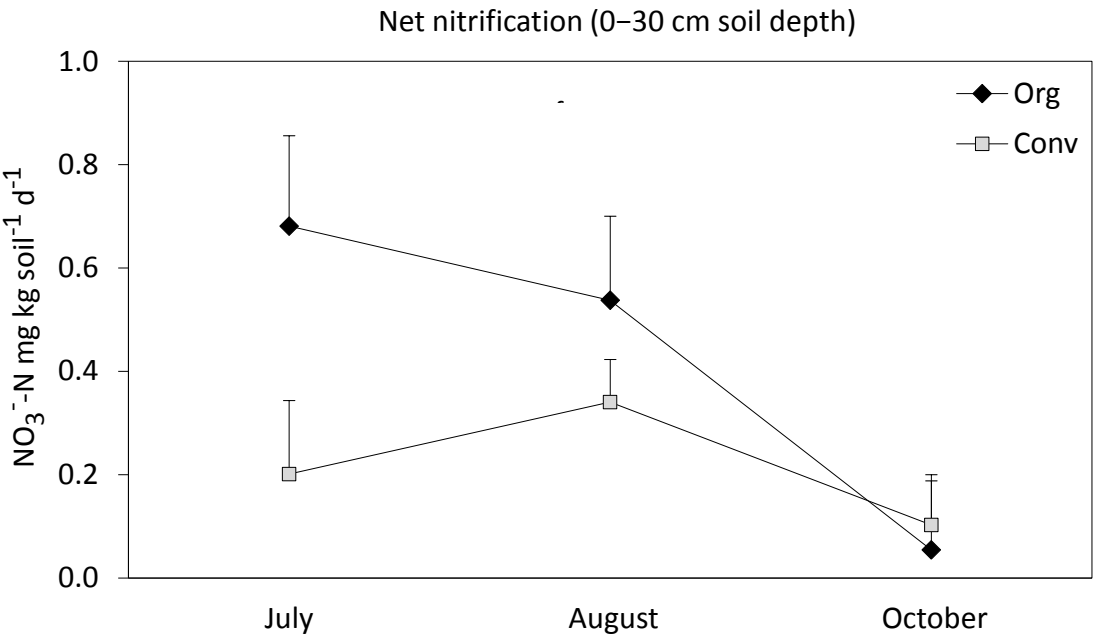


Figure 3.11. The effect of organic and conventional management on net nitrification in soil collected at 0- to 30-cm depth (bulk-density-weighted average of 0−5 cm and 5−30 cm) from Michigan blueberry farms on 6-7 July, 21-22 August, and 9-10 October 2009 and incubated for 30 days at 25°C and 55% (sands) or 65% (mucks) water-holding capacity. Error bars represent one standard error of the mean (n=8). The *P*-values for July, August, and October sampling dates are P=0.05, P=0.27, and P=0.62, respectively (pre-planned contrast *F*-test). Management types are considered significantly different when $P \leq 0.05$. Data were (ln + 1)-transformed prior to analysis; untransformed means and standard errors are shown.

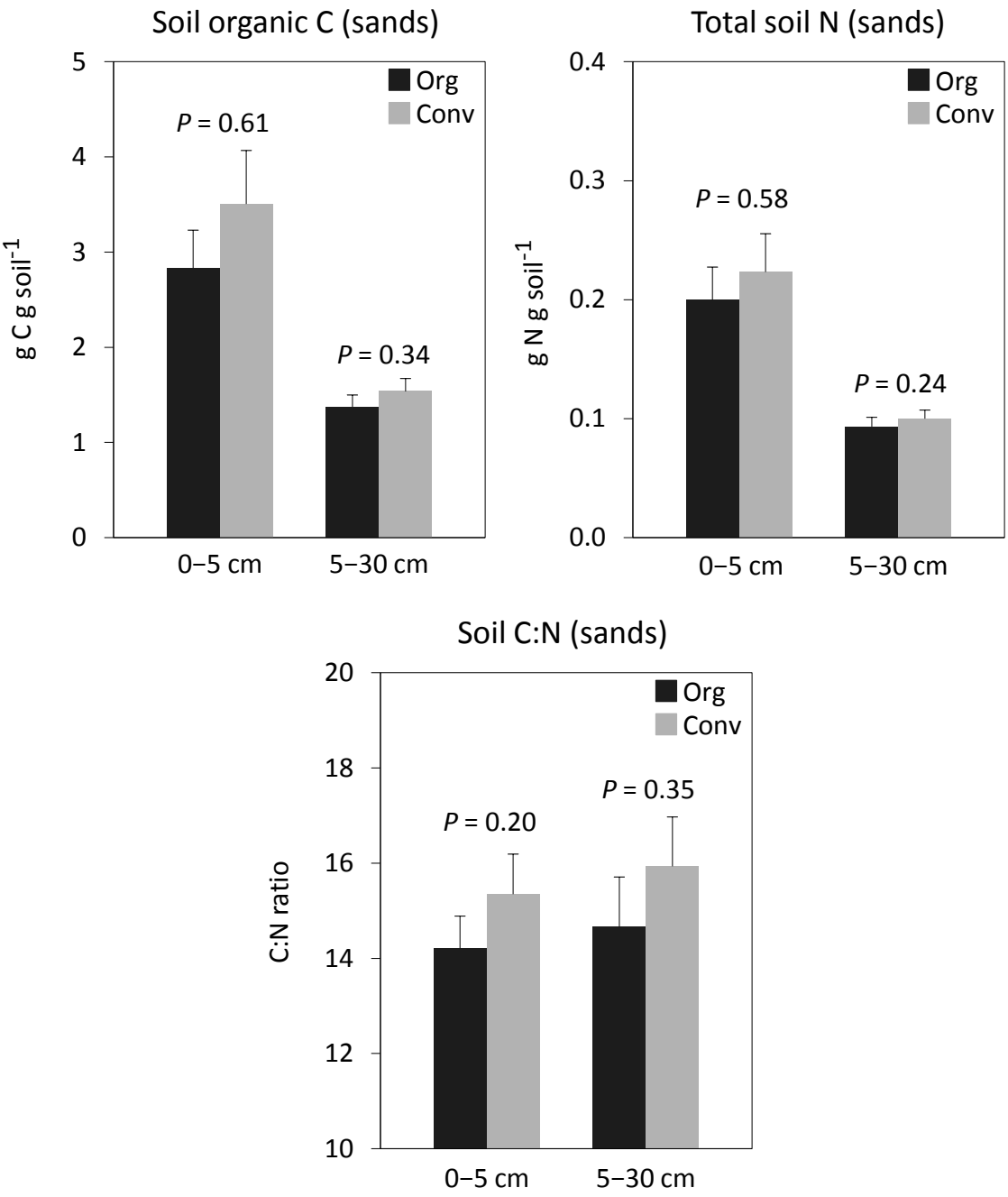


Figure 3.12. The effect of organic and conventional management on soil organic carbon, total N, C:N ratio, and water content of sandy soils collected at 0−5 cm and 5−30 cm depths from Michigan blueberry farms on 9-10 Oct 2009. Error bars represent one standard error of the mean (n=6). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast F-test).

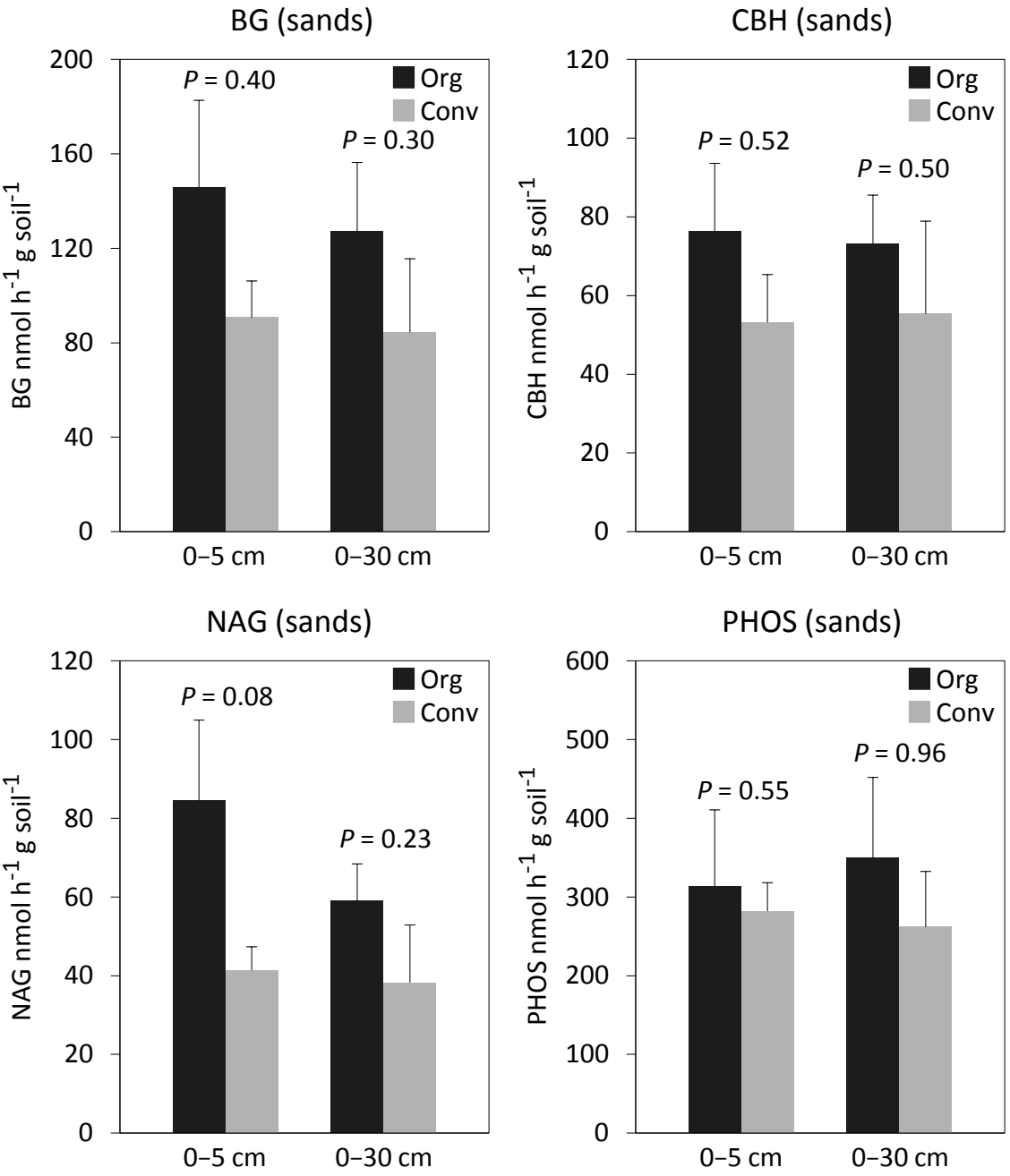


Figure 3.13. The effect of organic and conventional management on Beta-glucosidase (BG), cellobiosidase (CBH), N-acetylglucosaminidase (NAG), and acid phosphatase (PHOS) enzyme activity in sandy soils collected at 0−5 cm and 0−30 cm depth from organic and conventional Michigan blueberry farms on 25-26 Sept 2008. Error bars represent one standard error of the mean (n=6). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

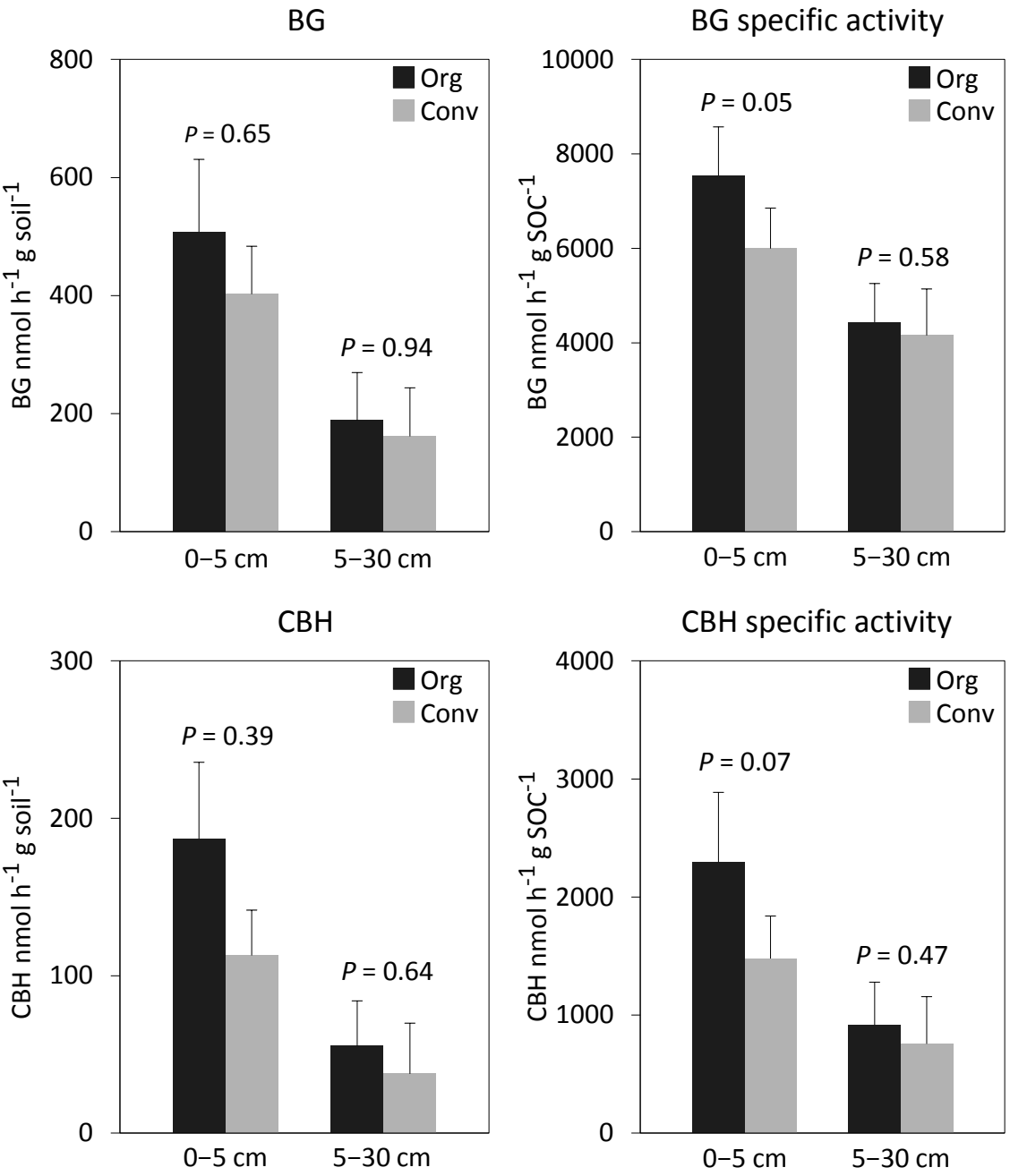


Figure 3.14. The effect of organic and conventional management on Beta-glucosidase (BG) and cellobiosidase (CBH) enzyme activity expressed per g soil and per g soil C (specific activity) in soils collected at 0−5 cm and 5−30 cm depth from organic and conventional Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (average of three sampling dates shown). Error bars represent one standard error of the mean (n=8). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

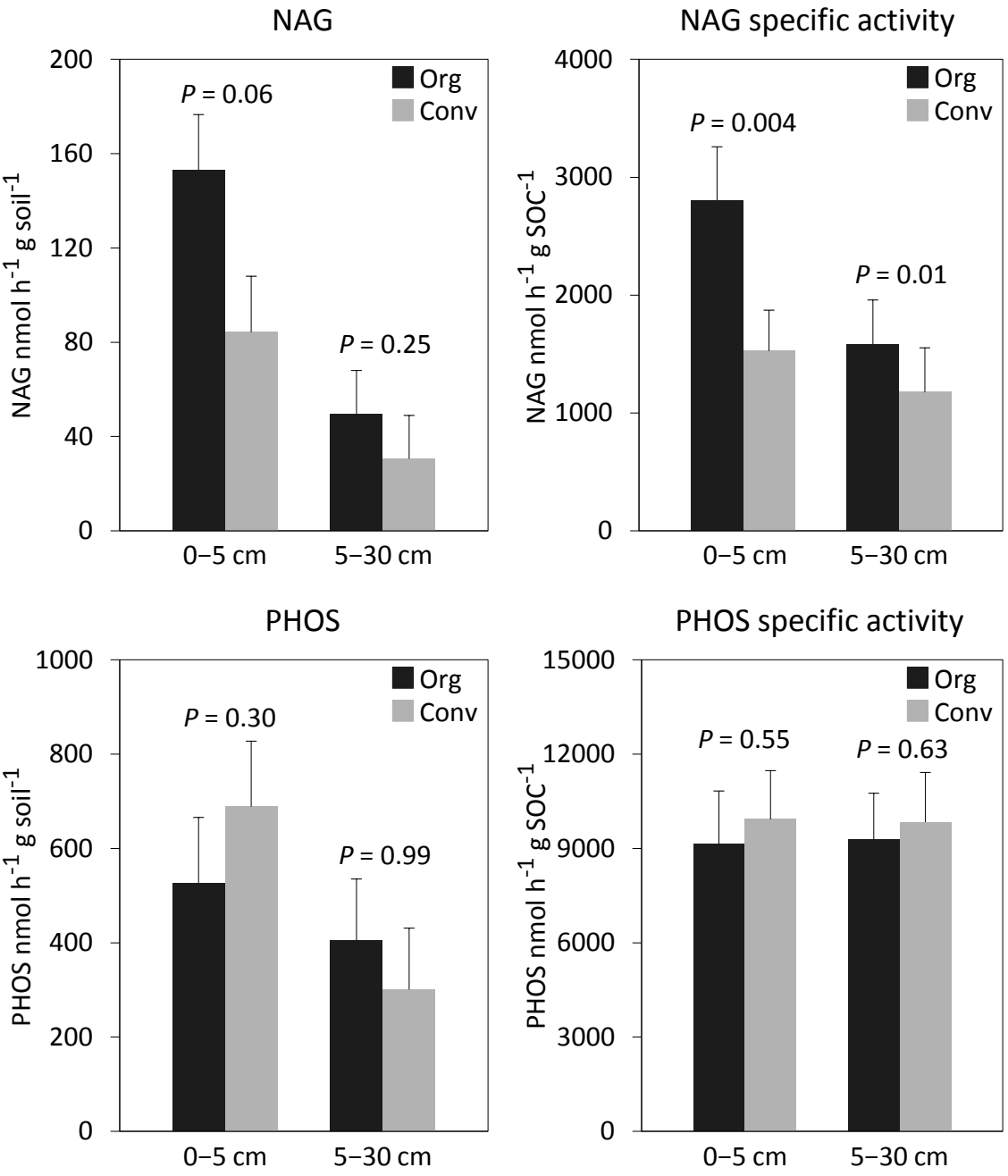


Figure 3.15. The effect of organic and conventional management on N-acetylglucosaminidase (NAG) and acid phosphatase (PHOS) enzyme activity expressed per g soil and per g soil C (specific activity) in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (average of three sampling dates shown). Error bars represent one standard error of the mean (n=8). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

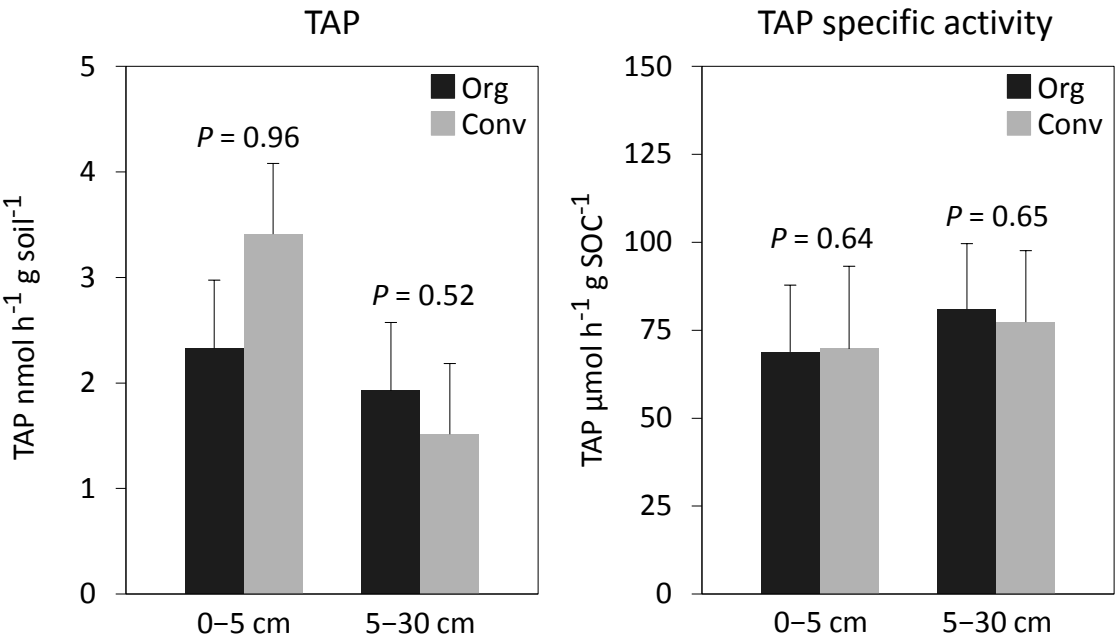


Figure 3.16. The effect of organic and conventional management on tyrosine aminopeptidase (TAP) enzyme activity expressed per g soil and per g soil C (specific activity) in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (average of three sampling dates shown). Error bars represent one standard error of the mean (n=8). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

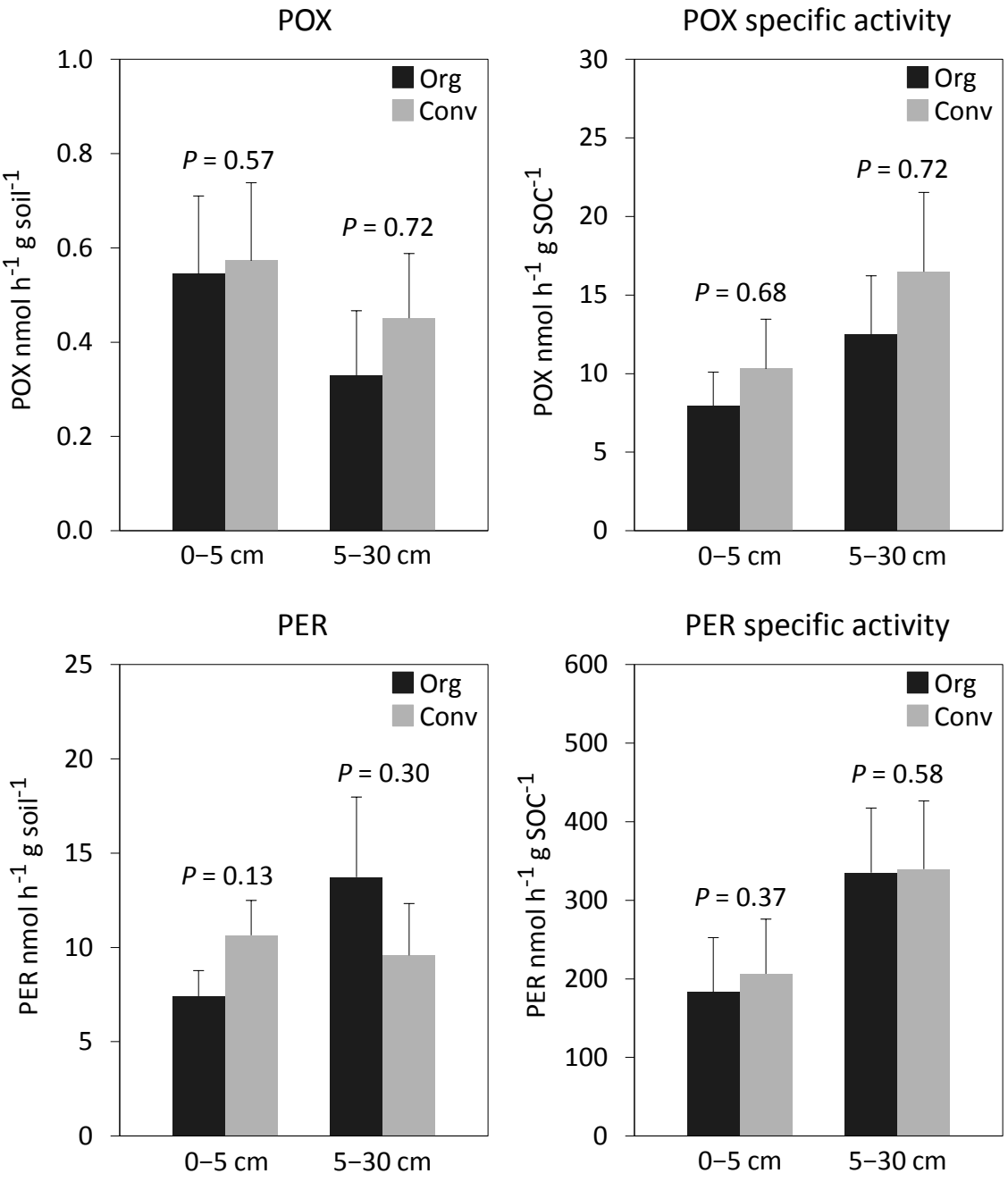


Figure 3.17. The effect of organic and conventional management on phenol oxidase (POX) and peroxidase (PER) enzyme activity expressed per g soil and per g soil C (specific activity) in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (average of three sampling dates shown). Error bars represent one standard error of the mean (n=8). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

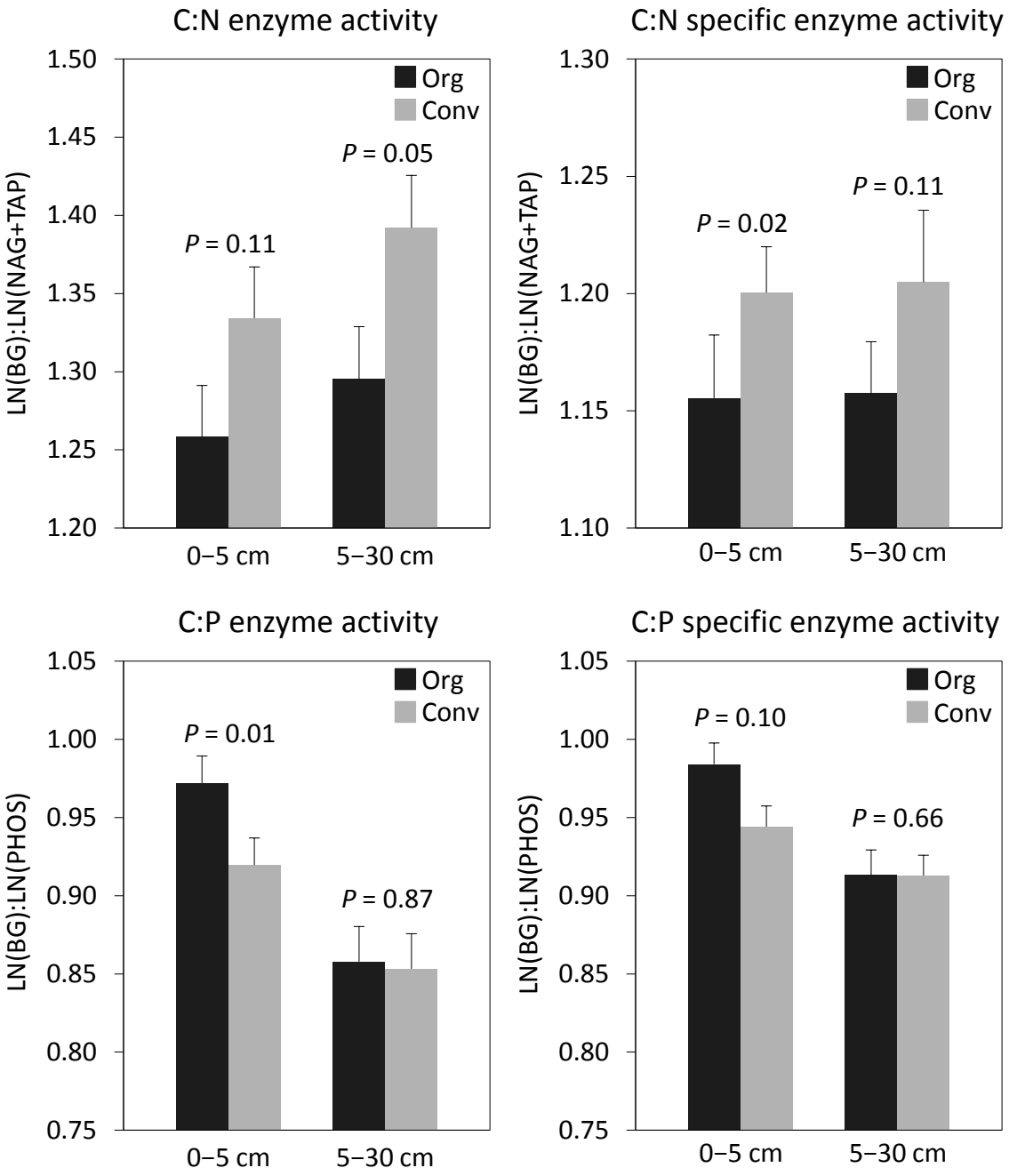


Figure 3.18. The effect of organic and conventional management on ratios of C:N enzyme activity [LN(β-D-1,4-glucosidase):LN(β-1,4-*N*-acetylglucosiminadase + tyrosine aminopeptidase] and C:P [LN(β-D-1,4-glucosidase):LN(phosphatase)] expressed per g soil and per g soil C (specific activity) in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (average of three sampling dates shown). Error bars represent one standard error of the mean (n=8). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

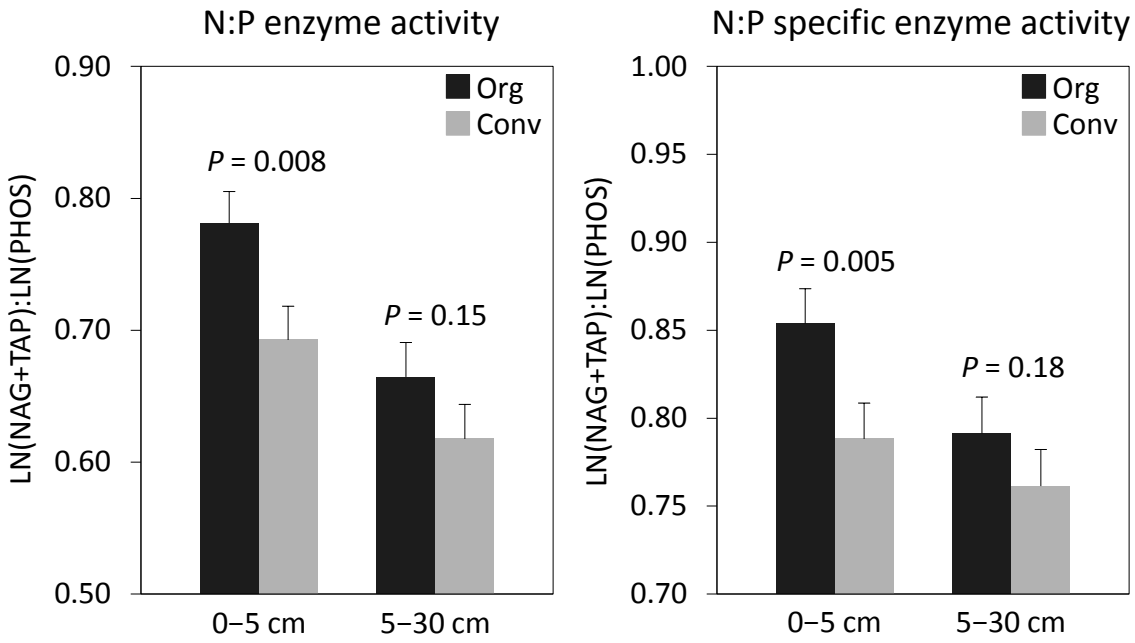


Figure. The effect of organic and conventional management on the ratio of N:P enzyme activity [LN(β-1,4-*N*-acetylglucosiminadase + tyrosine aminopeptidase):LN(phosphatase)] expressed per g soil and per g soil C (specific activity) in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (average of three sampling dates shown). Error bars represent one standard error of the mean (n=8). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

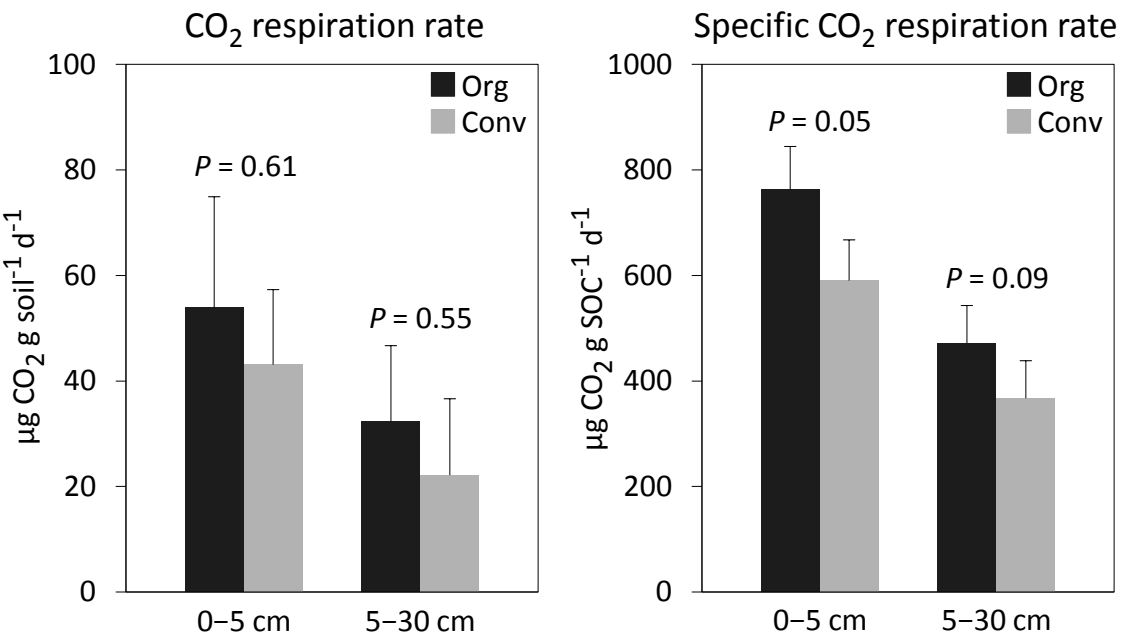


Figure 3.20. The effect of organic and conventional management on the CO_2 respiration rate in a 30-day incubation expressed per g soil and per g soil C (specific activity) in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (average of three sampling dates shown). Error bars represent one standard error of the mean (n=8). Management types considered are significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

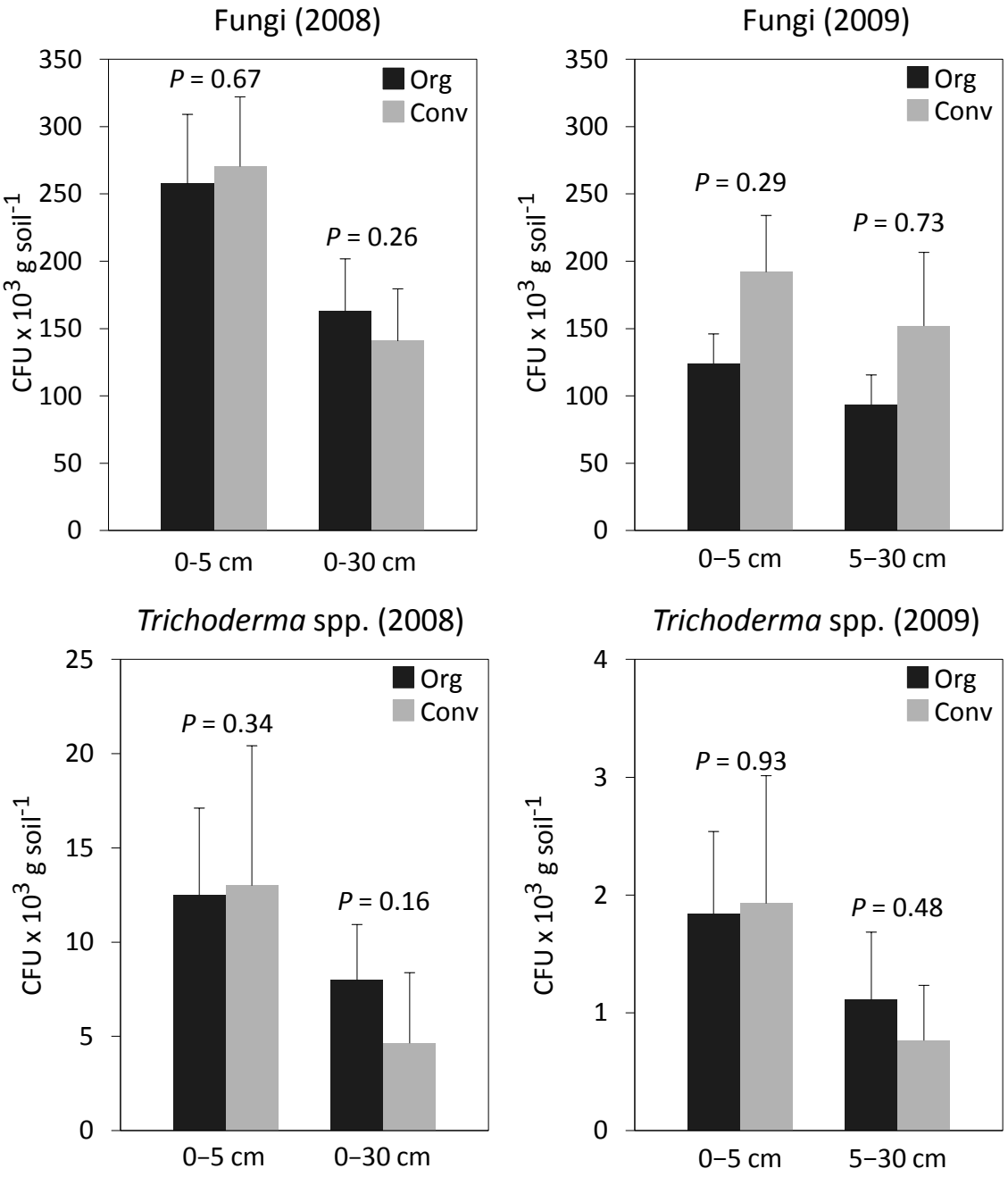


Figure 3.21. The effect of organic and conventional management on populations of cultivable fungi and *Trichoderma* spp. in soils collected at 0−5 cm and 0−30 cm (2008) and 0−5 cm and 5−30 cm (2009) depth from Michigan blueberry farms in late September and early October 2008 and 9-10 Oct 2009. Error bars represent one standard error of the mean (n=8 or n=6 for *Trichoderma* ssp.). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

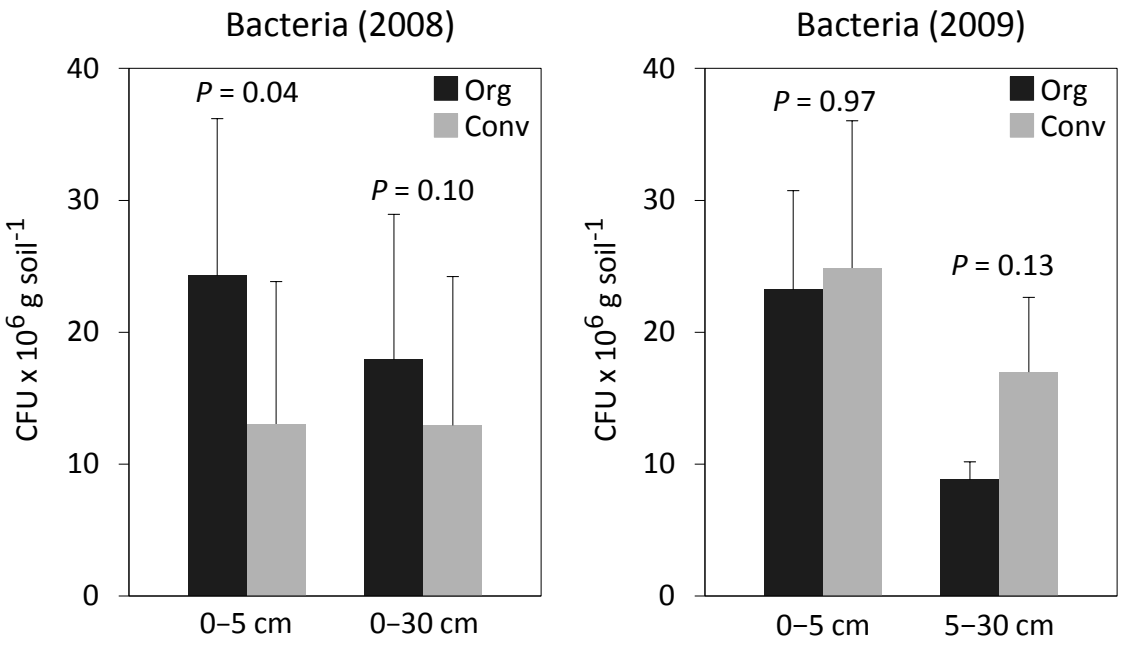


Figure 3.22. The effect of organic and conventional management on populations of cultivable bacteria in soils collected at 0−5 cm and 0−30 cm (2008) and 0−5 cm and 5−30 cm (2009) depth from Michigan blueberry farms on 25-26 Sept 2008 and 9-10 Oct 2009. Error bars represent one standard error of the mean (n=8). Management types considered are significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

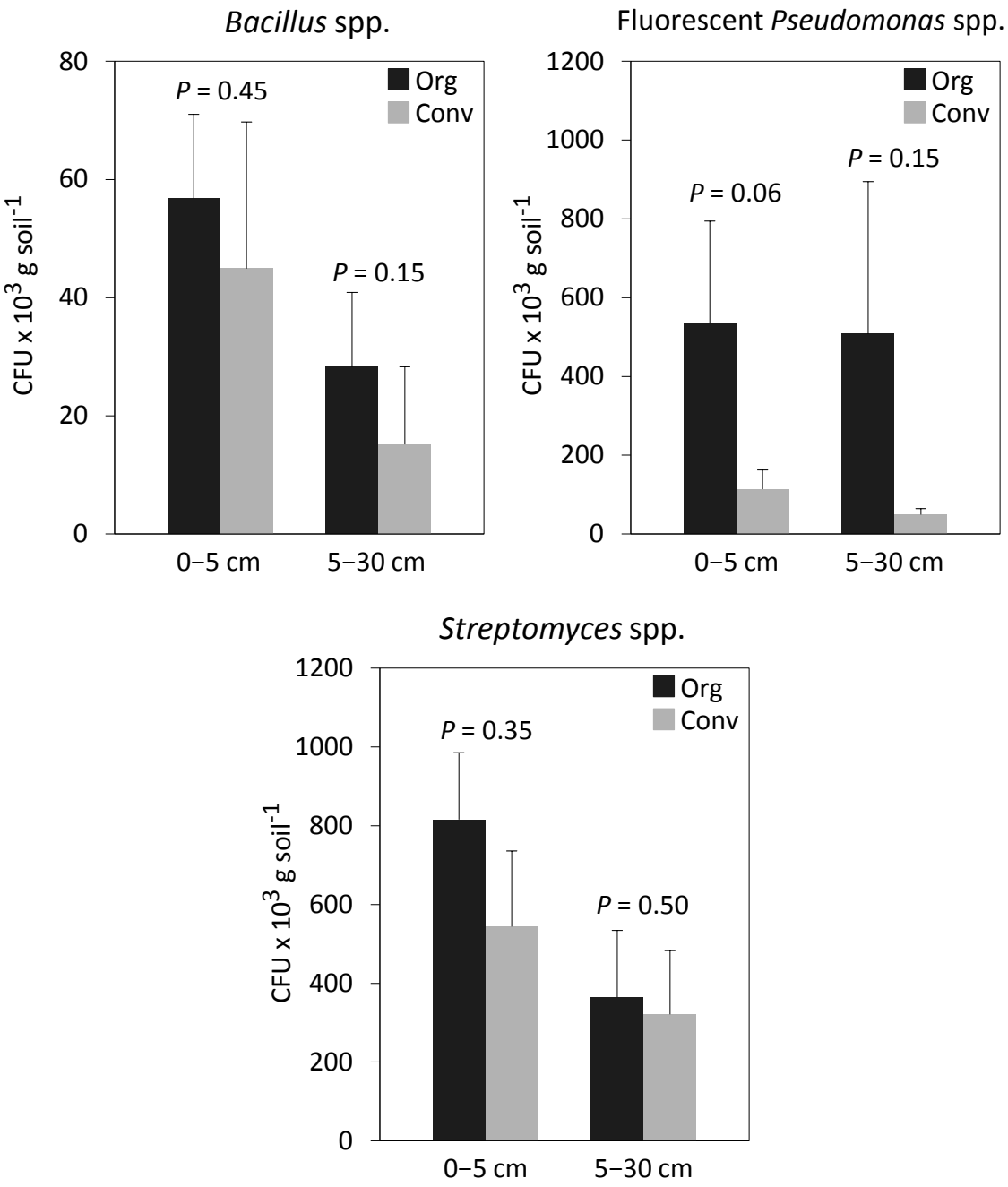


Figure 3.23. The effect of organic and conventional management on populations of *Bacillus* spp., fluorescent *Pseudomonas* spp., and *Streptomyces* spp. in soils collected at 0−5 cm and 5−30 cm depth from Michigan blueberry farms on 9-10 Oct 2009. Error bars represent one standard error of the mean (n=8). Management types are considered significantly different within a soil depth interval when $P \leq 0.05$ (preplanned contrast *F*-test).

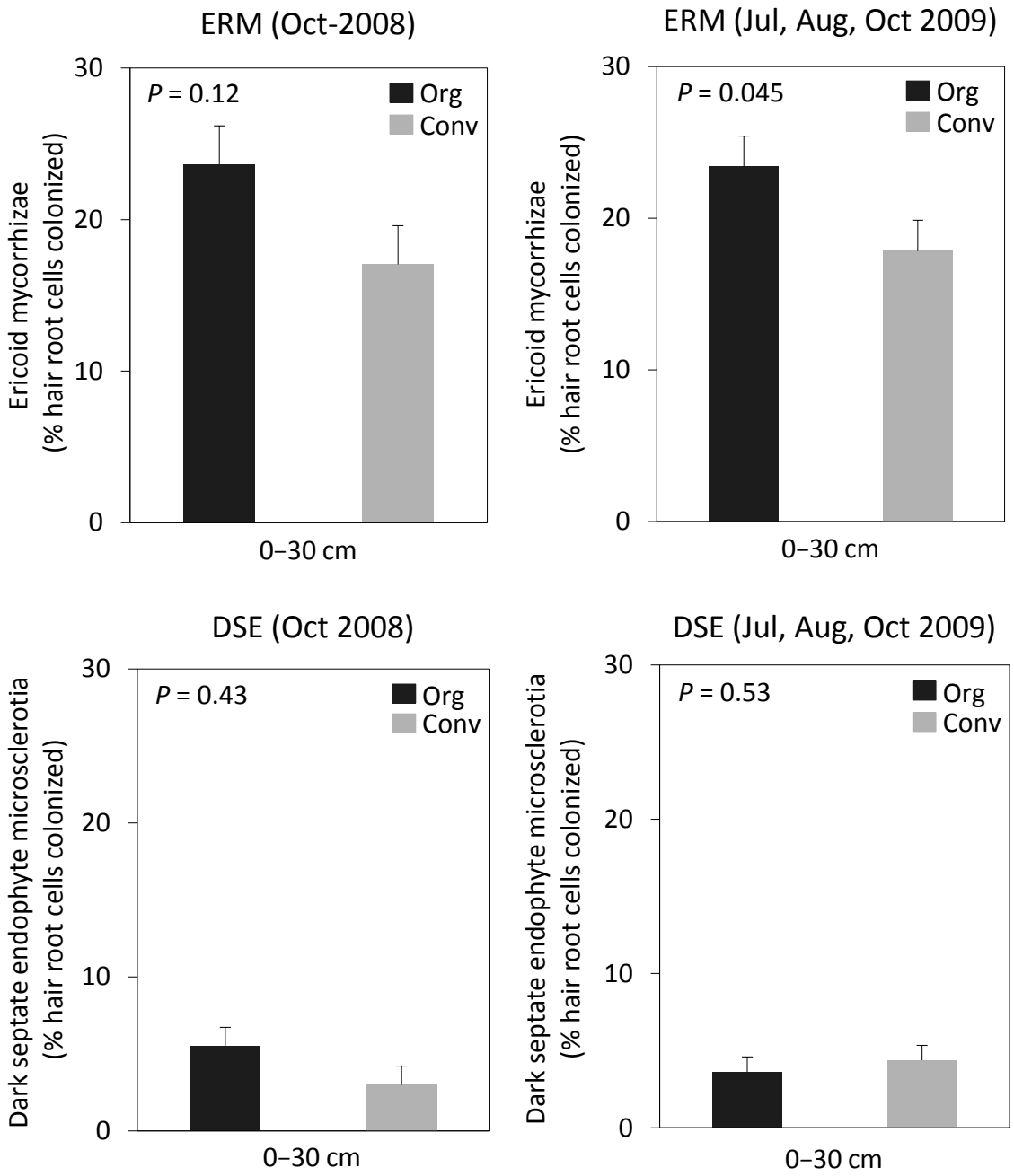


Figure 3.24. The effect of organic and conventional management on colonization by ericoid mycorrhizae (ERM) and dark septate endophytes (DSE) of roots collected at 0−30 cm depth from Michigan blueberry farms in late September and early October 2008 and 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009 (average of three sampling dates in 2009 shown). Error bars represent one standard error of the mean (n=8). Management types are considered significantly different when $P \leq 0.05$ (mixed model ANOVA, management effect F-test).

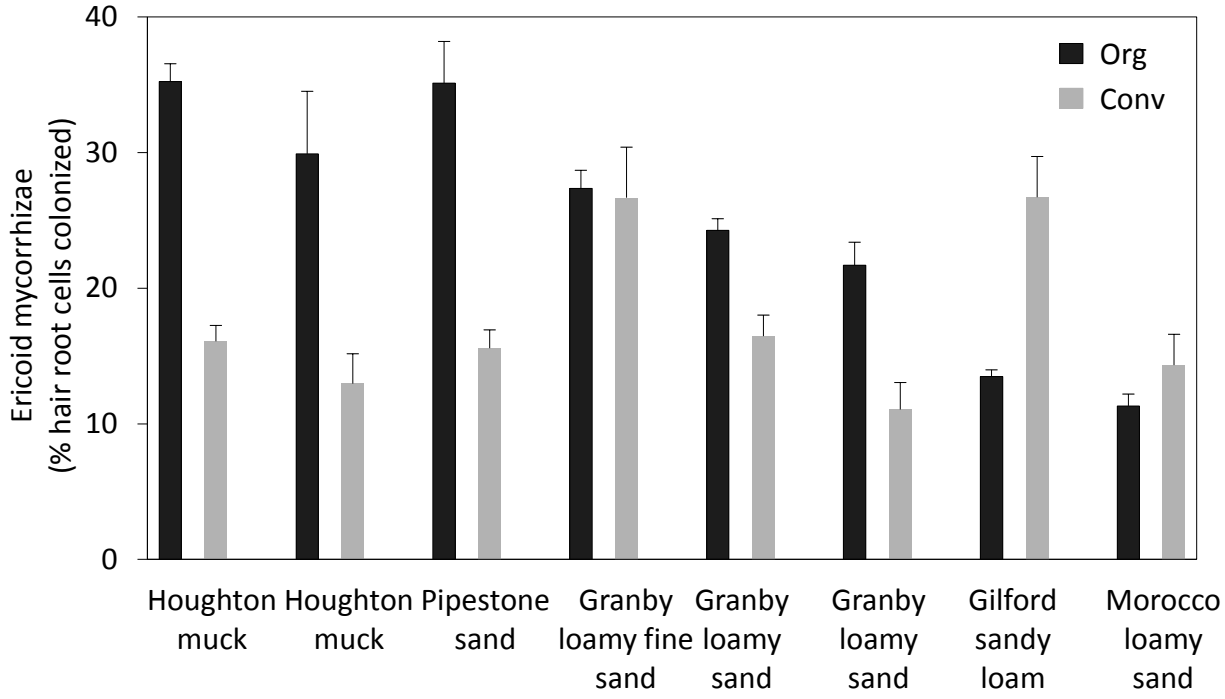


Figure 3.25. The percentage of hair root cells colonized by ericoid mycorrhizae on organic and conventional Michigan blueberry farms paired by NRCS soil series. Error bars represent one standard error of the mean of samples collected on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009.

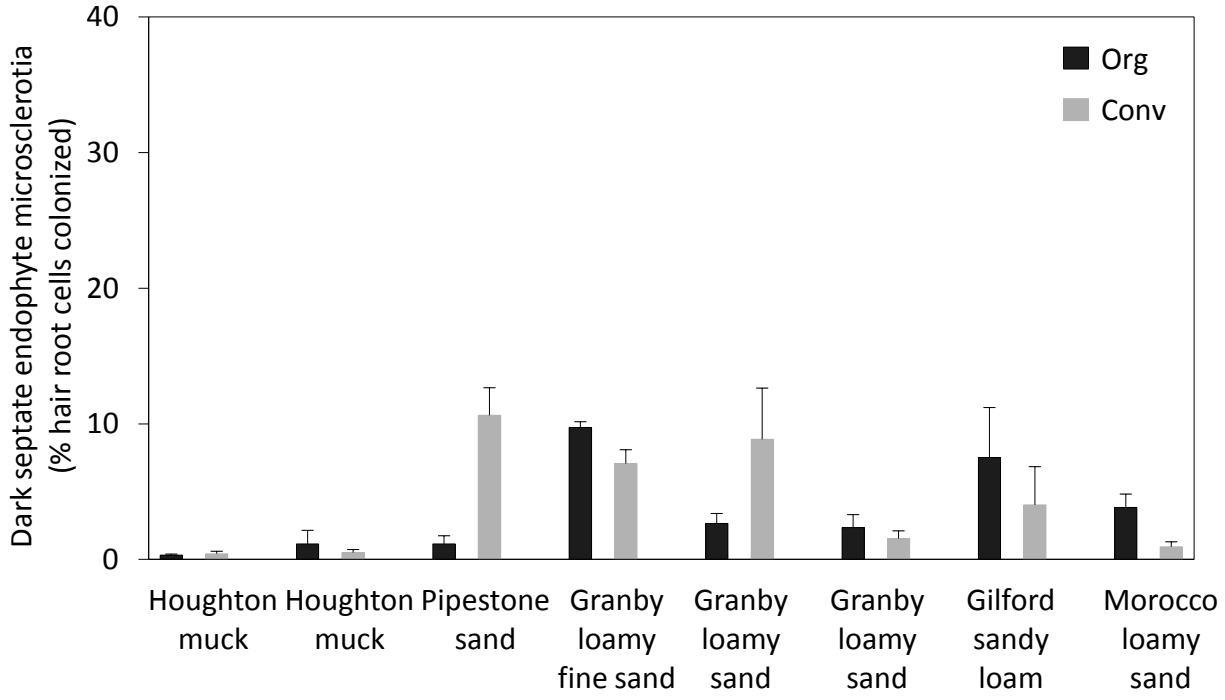
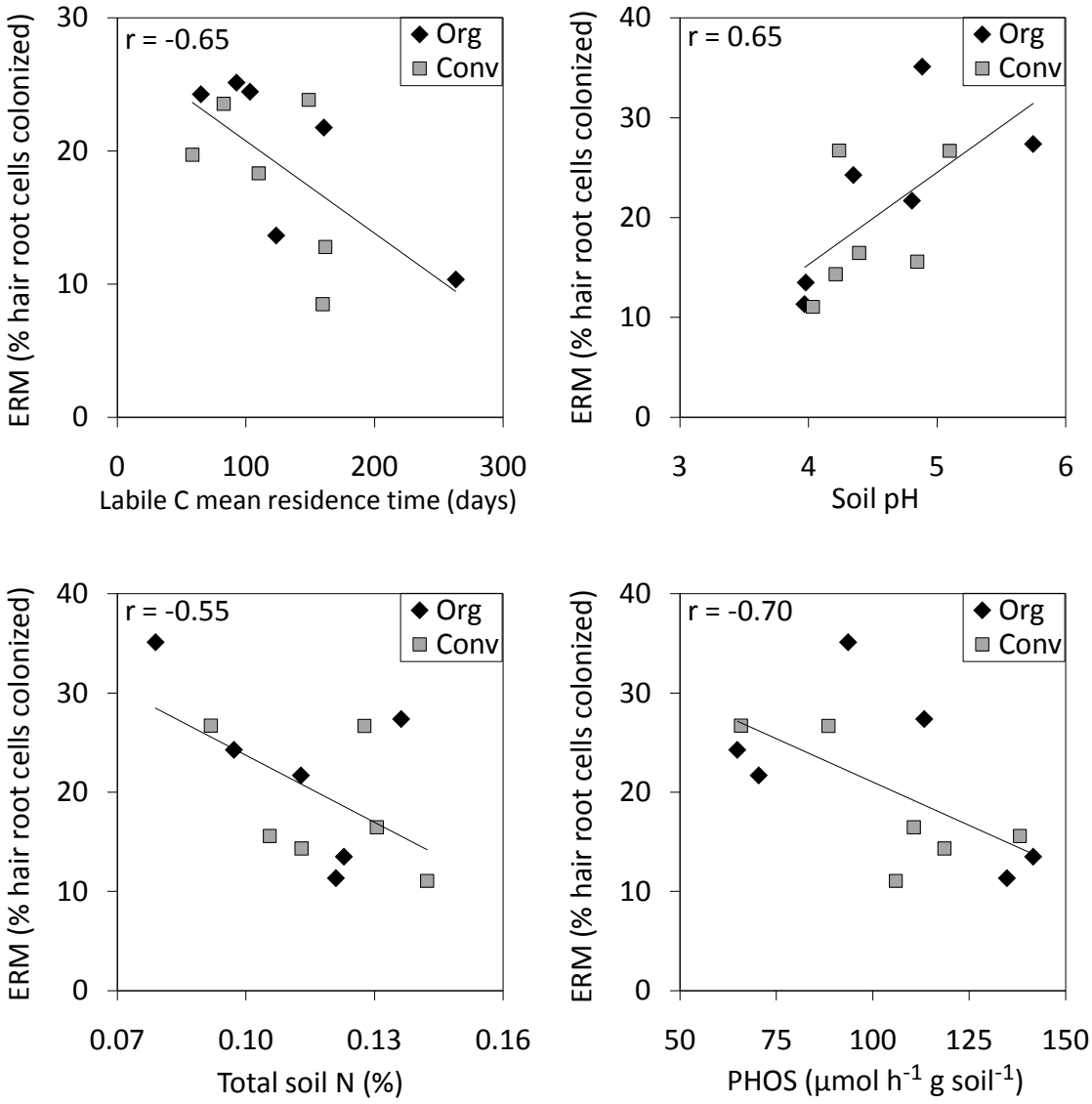


Figure 3.26. The percentage of hair root cells colonized by dark septate endophyte microsclerotia on organic and conventional Michigan blueberry farms paired by NRCS soil series. Error bars represent one standard error of the mean of samples collected on 6-7 Jul, 21-22 Aug, and 9-10 Oct 2009.



Figures 3.27. Correlations between ericoid mycorrhizal colonization (ERM) and labile C mean residence time (mrt), soil pH, total soil N, and acid phosphatase (PHOS) activity on organic and conventional blueberry farms (n=12; sands only) in Michigan. Root and soil samples were collected at 0−30 cm depth on 25-26 Sept 2008 (ERM and labile C mrt) and on 6-7 Jul, 21-22 Aug, and 9-10-Oct 2009 (ERM and soil pH, ERM and total soil N, ERM and PHOS, mean of three sampling dates).

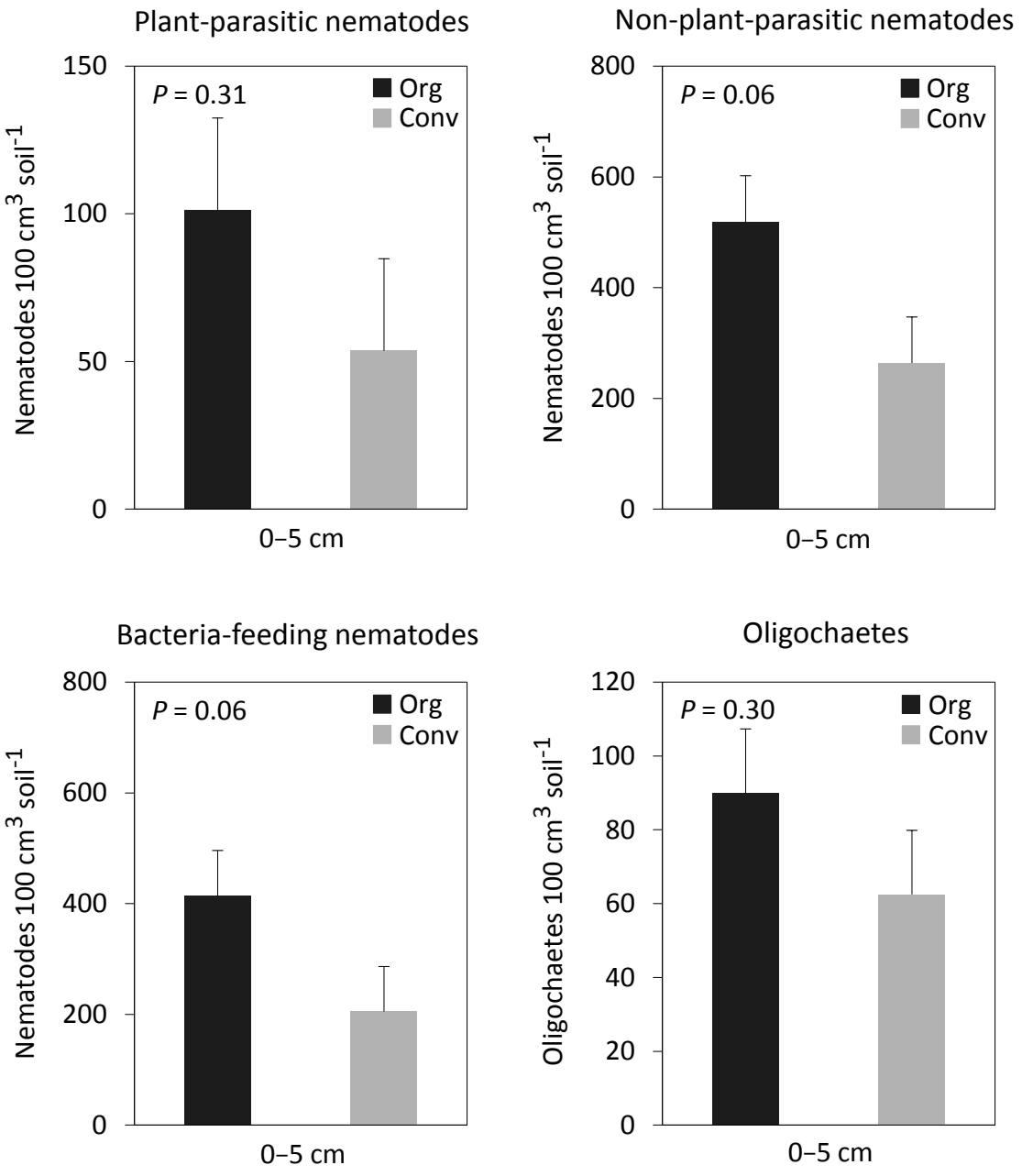
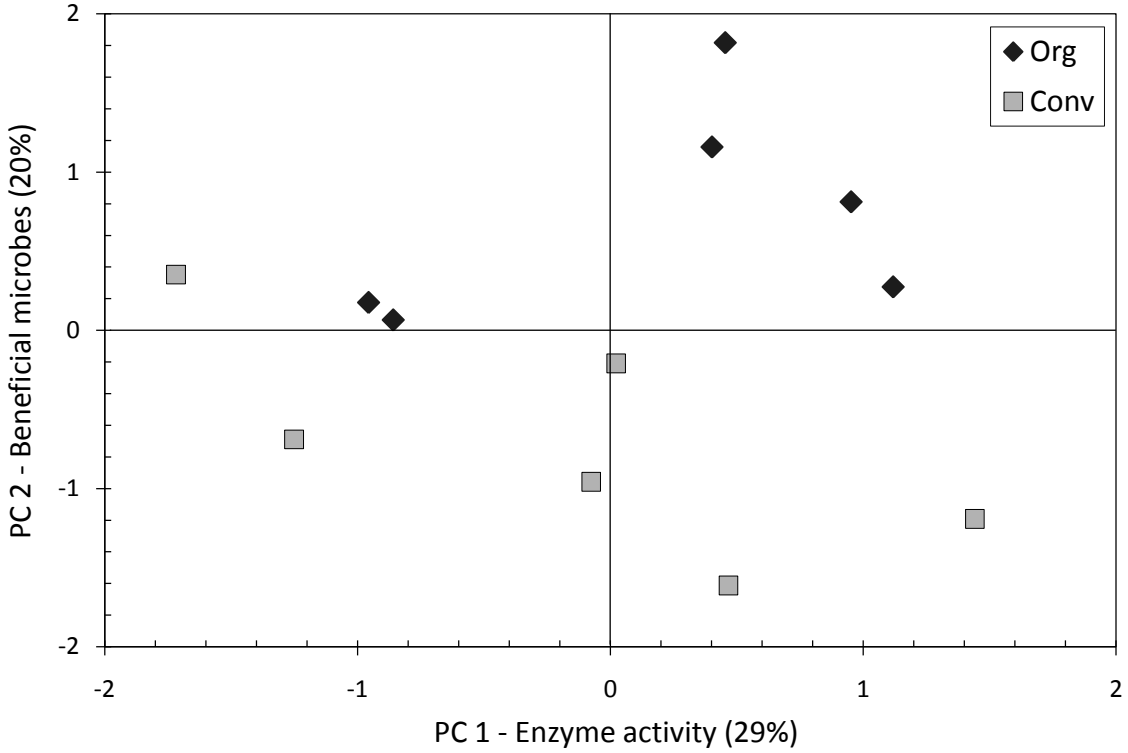


Figure 3.28. The effect of management on populations of plant-parasitic (sum of lesion, dagger, sheath, ring, spiral, and stunt nematodes), non-plant-parasitic (sum of tylenchs, aphelenchs, dorylaims, mononchs, and bacteria-feeding nematodes), bacterial-feeding nematodes, and oligochaetes (<0.2 mm-diameter earthworms) in soil collected at 0−5 cm depth from organic and conventional Michigan blueberry farms on 7-8 June 2010. Error bars represent one standard error of the mean (n=8). Management types are considered significantly different when $P \leq 0.05$ (Mixed-model ANOVA F-test).



Factor score coefficients

	Factor 1	Factor 2
H_2O	-0.05	-0.21
NAG	0.86*	0.17
BG	0.98*	-0.05
CBH	0.95*	-0.03
PHOS	0.51*	-0.67*
POX	-0.04	0.10
PER	0.83*	-0.14
TAP	0.38	-0.67*
CO_2	0.82*	0.09
Bact	-0.06	0.78*
Strep	0.17	0.75*
Pseu	0.20	0.47*
Baci	0.24	0.82*
Fungi	-0.54*	0.21
Trich	0.15	0.15
ERM	-0.35	0.49*
DSE	0.16	0.18

Figure 3.29. Principal component analysis (correlation matrix) of biological characteristics of sandy blueberry soils at 0−30 cm depth (bulk density-weighted average of 0−5 cm and 5−30 cm) collected from organic and conventional Michigan blueberry farms 9-10 Oct 2009. Variable abbreviations are defined in Table 3.2. Asterisks denote coefficients of variables which contribute > 0.4 to a principal component.

Chapter 4.

Evaluation of organic fungicides and cultural methods for control of fruit rots and shoot dieback in highbush blueberries

Abstract

Three on-farm trials were conducted in 2008 and 2009 to evaluate cultural methods and Organic Materials Review Institute (OMRI)-listed fungicides for control of fruit rot and stem diseases in highbush blueberries. In the 2008 trial in a mature 'Jersey' field, the organic fungicide Sporan (composed of rosemary, clove, and thyme oil) provided a similar reduction to anthracnose as the synthetic fungicide. At the second harvest Alternaria rot was reduced by the synthetic fungicide and SP-1 compost tea. In 2009, a slightly different array of treatments was evaluated in a mature 'Jersey' field. At the first harvest, Sporan and Organic Gem (composed of soluble fish byproducts) provided similar anthracnose control as the synthetic fungicide treatment, while anthracnose ranged from 75-100% in organic treatments at the second harvest and was only effectively controlled by the synthetic industry-standard fungicide program. In a 2009 trial in anthracnose-resistant 'Elliott', no treatment (including the synthetic fungicide) reduced anthracnose incidence compared to the untreated control, while Alternaria fruit rot was lowest in the PlantShield (composed of the biocontrol fungus *Trichoderma harzianum* strain T-22) treatment but not significantly different among PlantShield and Tetrasul (lime sulfur), Tetrasul + pruning, pruning + debris removal, and Serenade treatments. In the same trial, Alternaria infection was significantly increased by the SP-1 (compost tea) treatment; seven SP-1 applications during the growing season resulted in four-fold higher Alternaria fruit rot relative to in the unsprayed control. In all trials the synthetic fungicide program was the most effective treatment for control of anthracnose fruit rot. Sporan and Organic Gem were the most promising

organic treatments for control of anthracnose, while PlantShield reduced Alternaria rot. Compost tea reduced Alternaria rot in one trial but increased it in another. Future evaluations of compost tea are needed to determine how the type of compost or manner in which the tea is prepared affects its potential for disease suppression. Dormant copper sprays were ineffective for control of anthracnose and Alternaria fruit rot, while control provided by lime sulfur or pruning was inconsistent and generally modest. Dormant treatments applied in combination with effective seasonally applied organic fungicides may improve control over dormant or seasonal treatments alone and should be evaluated in future trials. These trials provide a knowledge base to guide future studies.

Introduction

In Michigan, blueberries are grown on 575 farms that occupy 19,300 acres (Kleweno and Matthews, 2007). Climatic conditions in the major blueberry-growing regions of Michigan promote fruit diseases, including anthracnose fruit rot, caused by *Colletotrichum acutatum* Simmonds, and Alternaria fruit rot, caused by *Alternaria tenuissima* (Kunze:Fr) Wiltshire. Fruit rots that occur more sporadically are caused by *Phomopsis vaccinii* Shear, *Botrytis cinerea* Pers., and *Pestalotia vaccinii* (Shear) Guba and other fungi (Caruso and Ramsdell, 1995; Schilder et al., 2002). In a two-year survey of organic and conventional fields, a higher percentage of anthracnose infection was recorded for blueberries collected from organic farms, while Alternaria fruit rot occurred at lower levels than anthracnose and was slightly higher on conventionally grown fruit (Chapter 3).

In southern Michigan, twig blight and cane dieback diseases of blueberries are usually caused by *Phomopsis vaccinii* (Caruso and Ramsdell, 1995). Fusicoccum cane dieback, caused by *Fusicoccum putrefaciens* Shear (teleomorph *Godronia cassandrae* Peck) is more problematic

in northern Michigan (Schilder, 2005). *C. acutatum* has also been associated with cane and twig diseases in Michigan, especially in wet years (Schilder, 2005). Phomopsis twig blight and cane dieback can be distinguished by the initial site of pathogen infection. Twig blight infection occurs on flower buds or succulent shoot tips and spreads downward, killing fruiting and vegetative twigs. Late spring frosts can predispose plant tissues to twig blight infection around the time of bloom (Caruso and Ramsdell, 1995). These infections can result in death of part or the entire shoot. Cane dieback, in contrast, can be often be traced to pathogen entry through wounds on lower portions of canes. Wounds occur most often in mechanically harvested fields and by improper use of herbicides, cultivation, or mowing within the plant row (Schilder et al., 2006). Once infection has occurred, *P. vaccinii* remains latent in canes for a lengthy period, potentially longer than one year (Weingartner and Klos, 1975), until high leaf water demand during hot weather overwhelms the vascular tissue, compromised by pathogen obstruction or plant tissue necrosis, and leads to 'flagging' of canes (dead canes with leaves still attached) (Cline and Schilder, 2006).

Less than 0.3% of the total blueberry production area was certified organic in 2007 (personal communication, Registered Organic Certifying Agencies in Michigan; contact information available at http://www.michigan.gov/mda/0,1607,7-125-1569_25516-55175--,00.html). A survey of attendees at the 2007 Great Lakes Fruit and Vegetable Expo, Grand Rapids, MI indicated that a lack of effective disease control options may have contributed to limit the expansion of organic blueberry acreage in the region (Appendix A). Many products are listed for control of diseases in organic production [Organic Materials Review Institute (OMRI)], http://www.omri.org), but most have not been evaluated in independent studies. Cultural practices, such as selective pruning, are recommended for disease control in blueberries, but the

level of disease reduction provided by cultural practices is uncertain. The identification of viable organic disease control options may be more important for growers with older fields, where disease-susceptible cultivars are often grown and pathogen populations have become established.

Trials conducted by Schilder et al. (2001, 2002, 2006a, 2006b, 2007) demonstrated that organic fungicides are less effective for control of fruit rots than synthetic fungicides. For example, Serenade (described as QRD 132), which contains the biocontrol bacterium *Bacillus subtilis* (Ehrenberg) Cohn, reduced anthracnose fruit rot incidence in some trials (Schilder et al., 2005b) but increased anthracnose fruit rot over the unsprayed control treatment in others (Schilder et al., 2001). Furthermore, effective tactics for management of stem and cane diseases in organic blueberries have yet to be identified. Most OMRI-listed fungicides have not been evaluated in Michigan blueberries. Because so few data exist on efficacy of organic fungicides in blueberries, identification of ineffective products can aid growers in selection of disease management inputs. Recent findings confirm that as OMRI-listed products continue to be evaluated, effective materials, such as fish oil for control of leaf spot in southern highbush blueberries, will continue to be identified (Scherm and Krewer, 2008). The objective of this study was to evaluate fungicides and cultural measures for management of post-harvest fruit rots and Phomopsis cane dieback in organic blueberries.

Materials and methods

Experimental design

On-farm trials were established to evaluate OMRI-listed fungicides and cultural methods for control of fruit rot or stem diseases. Sites were selected based on a history of moderate to high disease incidence; all trials were conducted within conventionally managed (i.e., non-certified organic) commercial fields. In each trial different products and cultural methods were

evaluated (Table 4.1). In 2008, we compared treatments for control of post-harvest fruit rots in a mature 'Jersey' field in Berrien County, Michigan. In 2009, fruit rot trials were continued in a 'Jersey' planting adjacent to the 2008 site. A study in an 'Elliott' field in Ottawa County with a history of Phomopsis cane dieback was conducted in 2009 to evaluate cultural methods and organic fungicides for efficacy against cane dieback. Fruit rot incidence was assessed in the Phomopis management trial; however, 'Elliott' is known to be resistant to anthracnose fruit rot. Treatments were chosen based on OMRI status of products at the time of the study, product label descriptions, and in consultation with growers.

In all trials, the dormant treatments (hand-pruning, copper, and lime sulfur) were applied in early April, several weeks prior to bud swell (Wise et al., 2006). Pruning techniques followed those described in Johnson (1959). Older dead, unthrifty, or diseased canes were removed at the base of the crown. Larger crossing or rubbing twigs in the upper portions of the canopy were also taken out, but thin, younger twigs were not removed. Pruning time was limited to four minutes per plot (one minute per bush). Seasonal sprays were applied at 10-14 day intervals beginning at the green tip stage of growth and continuing through the first harvest, except in 2008 when sprays were terminated prior to the first harvest. In the 2009 trial in 'Elliott', "pruning + debris removal" consisted of removal of pruned-out canes from plots after pruning, while in the "pruning + debris" treatment, pruned-out debris was left in place. All trials were conducted with treatments applied to four-bush plots laid out in a randomized complete block design with four (2008 and 2009 trials in 'Jersey') or five (2009 trial in 'Elliott') replications. Blocks were laid out in the outer two to three rows of a field. Sprays were applied at a rate of 40 gallons per acre (GPA) with an R&D Research CO2 cart-styled sprayer equipped with six bottles (0.8 gal each), a twin gauge Norgren pressure regulator set at 55 psi, and a single XR TeeJet 8002VS nozzle on a

5-ft spray boom (2008 and 2009 ‘Jersey’) or an RL Flowmaster 610W manual pump backpack sprayer (2009 ‘Elliott’, except dormant treatments that were applied with the R&D Research sprayer (described above). At fruit harvest, disposable gloves were worn and changed between plots to reduce the potential for cross-contamination between plots. After harvest, fruit were transported in insulated coolers with ice packs and placed in a 4°C cooler within 2 hours of collection.

In 2008, disease incidence was assessed by two methods. The first method consisted of 50 fruit equally spaced on a wire rack and incubated for 10 days at room temperature (23°C) and 100% relative humidity. The second method was storage of the remaining berries (50 to 150) at 4°C for 14 days in one-pint plastic clamshell containers stored in; containers were placed in a 0.06 m^3 cooler after fruit were cooled to 4°C to ensure uniform humidity among containers. The room-temperature incubation method allows for optimal recovery of fruit pathogens, while cold storage more closely simulates commercial handling practices. The room-temperature incubation method was used for disease assessment for both trials carried out in 2009. Following storage or incubation, berries were inspected for signs of fruit rot infection. Fruit was collected at two harvests in each trial. Twig blight incidence was determined in the 2008 study in ‘Jersey’ in late June. In 2009 cane dieback incidence was assessed on August 11 in the trial in ‘Elliott’. Stem disease incidence was recorded for the entire 4-bush plot and for the inner two bushes. Only the new (current-year) infections were rated.

Statistical analysis

Treatment means were calculated with the LSMEANS statement and compared with the LINES option in GLIMMIX procedure of SAS computer software (SAS Institute, Cary, NC). Block was specified as a random factor, and appropriate transformations (log, square-root, or

arcsine square root) of the response variables were conducted as needed to meet homogeneity of treatment variance and normality assumptions of the analysis of variance (ANOVA). SAS PROC CORR was used to determine the relationship between measured variables.

Results

2008 trial in 'Jersey'

At the first harvest, room-temperature and cold storage methods both had utility for discerning differences in post-harvest anthracnose control among the treatments (Table 4.2), but berries held in cold storage had an order of magnitude less anthracnose infection than those held at room temperature (Table 4.3). At the first harvest, synthetic and Sporan treatments had the lowest anthracnose infection incidence following room-temperature incubation and refrigerated storage (Table 4.3). At the second harvest there was no significant effect of treatments on anthracnose for berries incubated at room temperature (P=0.10, Table 4.2). However, numerically the standard synthetic program still had the lowest incidence of fruit infection while following cold storage, anthracnose incidence was significantly increased by the Actinovate and Nordox treatments compared to the unsprayed control (Table 4.3).

Alternaria fruit rot originating at the calyx and total Alternaria infection (calyx + stem scar infection) were significantly affected by the treatments only at the second harvest in refrigerated berries (Table 4.4). The synthetic fungicide program significantly reduced Alternaria incidence compared to all treatments except PlantShield and SP-1 compost tea (Table 4.4), which had similar levels of Alternaria fruit rot. Total Alternaria and anthracnose infection were not significantly correlated (r=-0.22, 0.48, -0.14, and 0.14; P=0.17, 0.002, 0.39, and 0.40 at the first and second harvests for room-temperature and cold storage methods, respectively).

The incidence of Botrytis rot, Phomopsis rot, and soft fruit was low and not affected by treatment (Table 4.2). Phomopsis twig blight incidence in late June ranged from 4 to 10 blighted twigs per plot and was not affected by treatment (P=0.29, data not shown).

2009 trial in 'Jersey'

There was a strong negative relationship between the percentage of marketable and anthracnose-infected fruit (r = -0.95, P<0.0001 at both harvests), therefore only anthracnose data are presented. Anthracnose fruit rot incidence was lowest in the synthetic fungicide treatment but was not significantly different from that in the Organic Gem and Sporan treatments (Table 4.5 and 4.6). Anthracnose incidence in the Fungastop treatment was intermediate among the treatments but significantly higher compared to the synthetic treatment. In the remaining treatments, anthracnose infection was not statistically different from the unsprayed control (Table 4.6). At the second harvest, anthracnose incidence was higher and only the synthetic treatment was effective for control, while organic treatment plots were highly infected (Table 4.6). This suggests organic management tactics have little or no efficacy under high anthracnose fruit rot pressure.

A significant treatment effect on Alternaria rot was seen at the second harvest, but this appeared to be an indirect effect of treatment differences in anthracnose suppression rather than direct effects on Alternaria rot (Table 4.5).

The percentage of infection by *Botrytis*, *Phomopsis,* or unidentified fungi, and soft fruit was unaffected by treatments at either harvest (Table 4.2).

2009 trial in 'Elliott'

There were no or only weak treatment effects on anthracnose and Alternaria rot when harvest dates were analyzed separately, due to heteroscedastic, non-normal distributions and

other ANOVA assumption violations, even with transformed data. When harvest dates were analyzed together, there were no significant harvest-date-by treatment interactions for anthracnose (P=0.22) and Alternaria (P=0.33) infection but assumptions for statistical analysis were met. Pooling fruit rot data across harvest dates therefore allowed for a more robust analysis, and ANOVAs for pooled harvest dates had lower P-values than when harvest dates were analyzed separately (Table 4.7).

Anthracnose incidence was reduced to 1% in the synthetic fungicide treatment, while anthracnose incidence in the PlantShield, SP-1 compost tea, and ProPhyt treatments was numerically higher but not statistically different from the synthetic fungicide treatment (Table 4.8). Dormant treatments did not improve anthracnose fruit rot control relative to the unsprayed treatment (Table 4.8).

Alternaria fruit rot was lowest in the PlantShield treatment (2%) but not statically different among PlantShield, Tetrasul, Tetrasul + pruning, pruning + debris removal, Serenade Max, or synthetic fungicide treatments (Table 4.8).

Soft and marketable fruit did not vary according to treatment (P= 0.56 and 0.32, respectively). Incidence of Phomopsis and Botrytis fruit rot was too low to proceed with ANOVA. Phomopsis cane dieback incidence was much lower than anticipated and no significant treatment effects were observed (Table 4.7, 4.8).

Discussion

In the 2008 and 2009 trials in the blueberry cultivar Jersey, Sporan reduced anthracnose fruit rot infection to a similar degree as the standard synthetic fungicide program except at the second harvest in 2009 when anthracnose pressure was very high. Organic Gem reduced anthracnose to a similar level as the synthetic fungicide treatment at the first harvest in ‘Jersey’

in 2009 as well. Serenade Max and Oxidate fungicides are well-known among organic blueberry growers and have been OMRI-listed longer than most organic fungicides, but mixed efficacy of Serenade Max and a lack of efficacy of Oxidate indicates that these treatments cannot be recommended for anthracnose control in blueberries. Other seasonal treatments provided inconsistent control or were not included in more than one trial, which limits conclusions that can be drawn regarding their performance.

The fact that the unsprayed treatment ranked favorably compared to other treatments for anthracnose control indicates that many of the treatments evaluated have a low potential for anthracnose reduction. Increased anthracnose fruit rot relative to the control may result from periods of wetness following spray applications, and possible detrimental effects of some organic fungicides on naturally occurring disease-suppressive microbes. In agreement with other studies (Schilder et al., 2006), dormant copper (Nordox) was among the least effective treatments for anthracnose control in all trials. In addition, dormant copper did not significantly reduce Alternaria rot in most cases, and therefore is not recommended for control of post-harvest diseases in Michigan blueberries. Lime-sulfur and selective pruning treatments had inconsistent effects but generally showed modest anthracnose reduction.

In the 2008 trial in 'Jersey', the absence of an effective treatment against anthracnose at the second harvest may be due to the lengthy period (3 weeks) between the last seasonal spray and the date of the second fruit harvest. Also, fruit rot levels typically increase at later harvests (Schilder et al., 2002). Residual activity of the synthetic treatment against anthracnose following harvest was apparent at the second harvest in this trial, where anthracnose was reduced by 40-85% compared to all other treatments following room temperature incubation. Natural fungicides generally break down (i.e., lose activity) rapidly, are easily washed from fruit surfaces, and lack

a systemic mode of action, meaning disease control potential for these products is attained only if they are applied on a preventative basis (Ellis and Nita, 2004). In contrast, locally systemic synthetic fungicides such as Pristine, which was applied at the two spray dates leading up to harvest in the 2008 trial, are assimilated by plant tissues and maintain fungicidal activity even after harvest (http://www.agproducts.basf.com/products/pristine-fungicide.pdf), which likely explains the superior efficacy of synthetic fungicides when anthracnose pressure was high. This reiterates the fact that organic fruit rot management tactics, if needed, must be applied before infection occurs.

A statistically non-significant relationship between anthracnose and Alternaria rot in the 2008 trial in 'Jersey' was unusual considering the competitive relationship often observed between *C. acutatum* and *Alternaria* spp. on blueberries fruit (Schilder et al., 2002). In the 2009 trial in 'Jersey', Alternaria rot appeared to be inversely related to anthracnose (e.g., the Nordox treatment had the lowest incidence of Alternaria rot but anthracnose infection was 100%), such that a high percentage of anthracnose incidence was likely to have obscured co-occurring Alternaria infections when rot levels were assessed. Likewise, the higher Alternaria infection in the synthetic fungicide treatment was probably related to suppression of anthracnose infection, which resulted in unimpeded access for post-harvest fruit infection by *Alternaria* sp., an opportunistic pathogen that often increases in response to treatment with fungicides with specific activity against anthracnose. This indicates that combined treatment effects against multiple genera of fruit rotting pathogens must be considered in disease management trials.

Anthracnose was much lower in 'Elliott' than in 'Jersey'. Cultivar resistance to anthracnose is therefore a promising option for management of anthracnose in organic blueberries. Conversely, anthracnose losses reached unacceptably high levels in the susceptible

cultivar Jersey, especially at later harvests (Schilder et al., 2002), which suggests that 'Jersey' is a poor choice for organic production as it may suffer from high levels of anthracnose.

An unexpected finding in the 2009 trial in 'Elliott' was that Alternaria rot was significantly increased by the SP-1 compost tea treatment. ThermX-70, the spreader-sticker recommended by the distributor for the SP-1 compost tea treatment in 2009, cannot be discounted as the cause of increased Alternaria rot. The product is composed of yucca extract, which contains sugars that could promote the growth of *Alternaria* spp. However, only 3.2 ml per acre (7.91 ml per ha) of ThermX-70 was applied at each spray. Assuming sugar content of 50% (w/v) in ThermX-70, only a negligible amount of sugar (1.6 g/acre, or about 1.8 mg/plant) would have been applied at each spray. Higher Alternaria fruit rot incidence in the SP-1 compost tea treatment was therefore likely to be related to the compost tea rather than sugars contained in the ThermX-70 spreader sticker.

Leaving debris in the planting row after pruning appeared to increase anthracnose fruit infection in 'Elliott' compared to the unpruned control treatment, meaning accumulation of pruning debris may influence the microclimate in the blueberry row, possibly through wetness, humidity, or increased inoculum levels, leading to higher fruit rot incidence in the treatment where debris was left in the field. These effects may be more important in organic fields where anthracnose-susceptible cultivars are grown and incremental effects of several management practices may be needed to reduce anthracnose rot to acceptable levels.

Unfortunately, in the 2009 trial in 'Elliott' no significant treatment effects on cane dieback incidence were observed. At this site, Phomopsis infection appeared to originate from the crown of the bushes. Poor management may have also played a role in development of dead

canes. Parker and Ramsdell (1977) noted that fungicides are likely to have poor efficacy where below-ground crown tissues are infected by *Phomopsis vaccinii*.

Conclusion

Promising organic fungicides were identified but their efficacy needs to be confirmed in additional trials. The observation of low efficacy in certain treatments over multiple seasons allows the reasonable conclusion that these have limited disease management potential in commercial blueberries in Michigan. Growers can instead be steered towards disease management options with known efficacy, which should increase efficiency of organic blueberry production in Michigan. Likewise, these findings free up resources for use in researching more promising treatments, such as combinations of dormant pruning and seasonal sprays, untested materials and effects of cultural practices on inoculum levels, microclimates within bush canopies, and how each of these contribute to disease occurrence in blueberries. Continued research into organic disease management tactics will provide needed information to aid in growth of organic blueberry production in Michigan.

Table 4.1. Treatments evaluated in disease management trials in blueberries in 2008 and 2009. Application rates are noted in tables for each trial (below).

Dormant treatments	**Manufacturer**	**Active ingredient/Description**
Nordox 75WG	Monterey AgResources	Copper oxychloride (Copper (I) oxide)
Lime sulfur/ Tetrasul 4s5	OR-Cal, Inc.	Calcium polysulfide (Lime sulfur)
Pruning		One minute per bush, pruning debris (stems and canes) left in place
Pruning + debris removal		One minute per bush, debris removed
Tetrasul 4s5 + pruning		Lime sulfur + one minute pruning per bush, debris left in place
In-season protective treatments		
Actinovate AG	Natural Industries, Inc.	*Streptomyces lydicus* WYEC 108
Basic Copper 53	Albaugh, Inc.	Copper sulfate
Fungastop L&G	Soil Technologies	Citric acid, mint oil, fish oil
Organic Gem	Advanced Marine Technologies	Soluble fish hydrolysate
ProPhyt	Helena Chemical Company	Potassium phosphite
PlantShield HC	BioWorks, Inc.	*Trichoderma harzianum* Rifai strain KRL-AG2
Serenade Max	AgraQuest, Inc.	*Bacillus subtilis* strain QST 713
SP-1 compost tea	AgriEnergy Resources, L.L.C.	Plant-derived aerobic compost extract
Sporan EC	EcoSMART Technologies, Inc.	Rosemary, clove and thyme oil
Synthetic	Various	Conventional standard fungicide program
Spreader-stickers		
Nu-Film P	Miller Chemical and Fertilizer Corp.	Poly-1-p-menthene
ThermX 70	American Extracts	Yucca extract saponins

Table 4.2. Mixed model analysis of variance of disease incidence in 'Jersey' blueberries in an organic fruit rot management trial in Berrien County, Michigan, 2008. Significant (P<0.05) P-values are shown in bold.

	Harvest one		Harvest two	
	Room temp.[z]	Cold storage[z]	Room temp.	Cold Storage
	$P > F$			
Anthracnose	**0.03**	**0.03**	0.10	**0.02**
Alternaria (calyx)	0.16	0.21	0.61	**0.02**
Alternaria (total)	0.20	0.64	0.93	**<0.01**
Botrytis	0.18	0.48	0.18	-
Phomopsis	-[y]	0.59	-	-
Soft fruit	-	0.96	-	0.37
Marketable fruit[x]	**<0.01**	0.98	0.30	0.10

[z]Fruit stored in one-pint clamshells at 4°C for 14 days to simulate standard commercial practices or incubated for 10 days at 23°C and 100% relative humidity to maximize recovery of fruit rot pathogens.

[y]Indicates insufficient number of non-zero observations for ANOVA.

[x]Disease-free, firm fruit.

Table 4.3. Anthracnose fruit rot incidence in 'Jersey' blueberries at the first and second harvest after incubation at room-temperature (23°C) and cold storage (4°C) in an organic fruit rot management trial in Berrien County, Michigan, 2008.

		Anthracnose fruit rot incidence (%)			
		Harvest one		Harvest two[y]	
Treatment, rate/A	Application timing[z]	Room temp.	Cold storage	Room temp.	Cold storage
Untreated		40 bcd[x]	8 d	54 ns[w]	1 a
Nordox 5 lb	1	61 d	9 d	44	3 b
Selective pruning[v]	1	40 abcd	4 ab	43	1 a
Lime sulfur 5 gal	1	45 bcd	4 abcd	38	2 a
Serenade Max 3 lb + Nu-Film-17 1.0 pt	2, 3, 4, 5	59 cd	8 bcd	62	2 ab
Actinovate 12 oz + Nu-Film-17 1.0 pt	2, 3, 4, 5	40 abcd	5 bcd	70	4 b
PlantShield 20 oz + Nu-Film-17 1.0 pt	2, 3, 4, 5	33 ab	5 abcd	26	2 ab
SP-1 Compost tea 2 gal + Nu-Film-17 1.0 pt	2, 3, 4, 5	36 abc	8 cd	46	2 ab
Sporan 3 pt	2, 3, 4, 5	28 ab	1 ab	42	2 ab
Captec 4L 2 qt + Topsin M 1 lb + Nu-Film-17 1.0 pt	2				
Indar 2F 6 fl oz + Nu-Film-17 1.0 pt	3				
Pristine 23 oz + Nu-Film-17 1.0 pt	4, 5	17 a	3 abc	16	1 a

[z]Spray dates: 1 = 14 Apr (dormant), 2 = 12 May (pink bud), 3 = 21 May (bloom), 4 = 11 Jun (green fruit), and 5 = 8 Jul (10% blue fruit).

[y]Harvest dates: one=22 Jul and two=30 Jul.

[x]Column means followed by the same letter are not significantly different according to Fisher's Protected LSD test ($P \leq 0.05$). The dependent variable was arcsine-square root transformed for analysis. Untransformed means are shown.

[w]ANOVA not significant at $P<0.05$.

[v]Diseased and crossing twigs and canes removed.

Table 4.4. Alternaria fruit rot incidence in 'Jersey' blueberries at the first and second harvest after incubation at room-temperature (23°C) and cold storage (4°C) in 'Jersey' blueberries in an organic fruit rot management trial in Berrien County, Michigan, 2008.

		Alternaria fruit rot incidence (%)			
Treatment, rate/A		Harvest one		Harvest two	
	Application timing[z]	Room temp.	Cold storage	Room temp.	Cold storage
Untreated		21 ns	12 ns	31 ns	12 cd[x]
Nordox 5 lb.....	1	24	17	37	10 d
Lime sulfur 5 gal	1	34	14	38	15 d
Selective pruning[v]	1	29	12	38	10 cd
Serenade Max 3 lb + Nu-Film-17 1.0 pt.....	2, 3, 4, 5	27	20	35	12 d
Sporan 3 pt + Nu-Film-17 1.0 pt.....	2, 3, 4, 5	26	17	38	8 bcd
Actinovate 12 oz + Nu-Film-17 1.0 pt.....	2, 3, 4, 5	28	12	26	13 d
PlantShield 20 oz + Nu-Film-17 1.0 pt.....	2, 3, 4, 5	31	13	30	5 abc
SP-1 Compost tea 2 gal + Nu-Film-17 1.0 pt.....	2, 3, 4, 5	19	11	34	5 ab
Captec 4L 2 qt + Topsin M 1 lb + Nu-Film-17 1.0 pt.....	2				
Indar 2F 6 fl oz + Nu-Film-17 1.0 pt.....	3				
Pristine 23 oz + Nu-Film-17 1.0 pt.....	4, 5	31	16	31	5 a

[z]Spray dates: 1 = 14 Apr (dormant), 2 = 12 May (pink bud), 3 = 21 May (bloom), 4 = 11 Jun (green fruit), and 5 = 8 Jul (10% blue fruit).

[y]Harvest dates: 1=22 Jul and 2=30 Jul.

[x]Column means followed by the same letter are not significantly different according to Fisher's Protected LSD test ($P \leq 0.05$). Alternaria data were log-transformed for analysis; untransformed means are shown.

[w]ANOVA not significant at $P<0.05$.

[v]Diseased and crossing twigs and canes removed.

Table 4.5. Mixed model analysis of variance of disease incidence in 'Jersey' blueberries in an organic fruit rot management trial in Berrien County, Michigan, 2009. Significant ($P<0.05$) *P*-values are shown in bold.

	Harvest one[z]	Harvest two
	P > F	
Anthracnose	**<0.01**	**<0.01**
Alternaria (calyx)	0.09	0.18
Alternaria (total)	0.37	**0.02**
Botrytis	0.11	0.09
Phomopsis	-[y]	-
Soft fruit	0.64	0.32
Marketable fruit[x]	**<0.01**	**<0.01**

[z]Fruit incubated at 23°C and 100% relative humidity for 10 days.

[y]Indicates insufficient number of non-zero observations for ANOVA.

[x]Disease-free, firm fruit.

Table 4.6. Anthracnose and Alternaria fruit rot incidence in 'Jersey' blueberries at the first and second harvest after incubation at room-temperature (23°C) for 10 days in an organic fruit rot management trial in Berrien County, Michigan, 2009.

Treatment, rate/A	Application timing[z]	Anthracnose (% fruit infection) Harvest one[y]	Alternaria (% fruit infection) Harvest two	Anthracnose (% fruit infection) Harvest one	Alternaria (% fruit infection) Harvest two
Untreated		41 cde	6 ns	93 bc	12 ab
Nordox 5 lb	1	56 e	6	100 d	6 a
Tetrasul 2.5 gal	1	34 bcde	6	80 bc	14 bc
Selective pruning[w]	1	53 e	7	92 bc	11 bc
Oxidate (variable rate[v]) + Nu-Film-P 0.5 pt	2, 3, 4, 5, 6	50 de	6	83 bc	16 bc
Basic Copper 53 5 lbs + Nu-Film-P 0.5 pt	2, 3, 4, 5, 6	40 cde	8	87 bc	19 bc
SP-1 Compost tea 4 gal + ThermX 70 3.2 fl oz.	2, 3, 4, 5, 6	38 bcde	7	91 bc	18 bc
Fungastop 6 fl oz + Nu-Film-P 0.5 pt	2, 3, 4, 5, 6	25 bcd	6	75 b	18 bc
Serenade Max 3 lb + Nu-Film-P 0.5 pt	2, 3, 4, 5, 6	23 bc	6	94 cd	14 bc
Sporan 6 pt + Nu-Film-P 0.5 pt	2, 3, 4, 5, 6	21 abc	8	79 bc	13 bc
Organic Gem 0.8 gal	2, 3, 4, 5, 6	13 ab	7	75 b	27 bc
Captec 4L 2 qt + Topsin M 1 lb + Nu-Film-P 0.5 pt	2 4				
Indar 2F 6 fl oz + Nu-Film-P 0.5 pt	3				
Pristine 23 oz + Nu-Film-P 0.5 pt	5, 6	4 a	6	22 a	21 c

[z]Spray dates: 1 = 1 Apr (dormant), 2 = 1 May (late green tip/tight cluster), 3 = 8 May (pink bud), 4 = 14 May (bloom/petal fall), 5 = 21 May (bloom), 6 = 28 May (bloom/petal fall), 7 = 15 Jun (green fruit), 8 = 1 Jul (10% blue), 9 = 24 Jul (blue).

[y]Harvest dates: 1=22 Jul and 2=30 Jul.

[x]Column means followed by the same letter are not significantly different according to Fisher's Protected LSD test ($P \leq 0.05$). The dependent variables were arcsine-square root transformed for analysis; untransformed means are shown.

Table 4.6 (cont'd).

[w]Diseased and crossing twigs and canes removed.

[v]0.4 gal per acre through bloom, then 0.12 gal per acre.

Table 4.7. Mixed model analysis of variance of disease incidence in 'Elliott' blueberries in an organic fruit rot and Phomopsis cane dieback management trial in Ottawa County, Michigan, 2009. Significant (P<0.05) P-values are shown in bold.

	Harvest one[z]	Harvest two	Both harvests
		$P > F$	
Anthracnose	**<0.05**	0.13	**<0.01**
Alternaria (calyx)	0.67	0.16	0.12
Alternaria (total)	0.14	**0.04**	**0.02**
Botrytis	-[y]	-	-
Phomopsis	-	-	-
Soft fruit	0.11	0.22	0.56
Marketable fruit[x]	0.49	0.53	0.32
Phomopsis cane dieback[w]	0.94		

[z]Fruit incubated at 23° C and 100% relative humidity for 10 days.

[y]Indicates insufficient number of non-zero observations for ANOVA.

[x]Disease-free, firm fruit.

[w]Assessed on 11 Aug 2009.

Table 4.8. Anthracnose and Alternaria fruit rot and Phomopsis cane dieback incidence in 'Elliott' blueberries after incubation at room-temperature (23°C) for 10 days in an organic disease management trial in Ottawa County, MI, 2009.

Treatment, rate/A	Application timing[z]	Anthracnose (% fruit infection)[y]	Alternaria (% fruit infection)	Phomopsis dieback (number of canes per bush)[x]
Untreated		5.8 ab	2.8 ab	0.4 ns
Nordox 10 lb	1	8.6 bcd	7.4 bc	0.2
Tetrasul 2.5 gal	1	3.5 bcd	3.2 ab	0.4
Tetrasul 2.5 gal + pruning	1	16.6 abc	4.4 ab	0.5
Pruning + removal	1	17.2 bcd	5.4 ab	0.7
Pruning + debris	1	10.8 d	6.4 bc	0.5
Serenade Max 3 lb + Nu-Film-P 0.5 pt	2, 3, 4, 5, 6, 7, 8	13.4 cd	5.0 ab	0.4
SP-1 compost tea 4 gal + ThermX 70 3.2 fl oz	2, 3, 4, 5, 6, 7, 8	3.5 abc	11.1 c	0.4
ProPhyt 4 pt + Nu-Film-P 0.5 pt	2, 3, 4, 5, 6, 7, 8	1.4 abc	7.2 bc	0.6
PlantShield 20 oz + Nu-Film-P 0.5 pt	2, 3, 4, 5, 6, 7, 8	1.2 ab	2.0 a	0.5
Indar 2F 6 fl oz + Nu-Film-P 0.5 pt	2, 3			
Ziram 76DF 4 lb + Nu-Film-P 0.5 pt	4 7			
Pristine 23 oz + Nu-Film-P 0.5 pt	5, 6 8	1.0 a	6.2 ab	0.7

[z]Spray dates: 1 = 17 Apr (dormant), 2 = 4 May (green tip), 3 = 16 May (pink bud), 4 = 12 Jun (late petal fall), 5 = 26 Jun (green fruit), 6 = 14 Jul (<10% blue), 7 = 29 Jul

[y]Column means followed by the same letter are not significantly different according to Fisher's protected LSD test ($P \leq 0.05$). Anthracnose and Alternaria incidence data were log-transformed and square-root-transformed, respectively, for analysis; untransformed means are shown. Ns = not significant.

[x]Mean of two intraplot bushes.

APPENDICES

Appendix A.

Results of a survey questionnaire distributed at the Great Lakes Fruit and Vegetable Expo in 2007

A 4-question survey was distributed to audience members during the "Organic Blueberry Production: What We Know and Need to Learn" presentation at the 2007 Great Lakes Fruit, Vegetable, and Farm Market Expo in Grand Rapids, MI. Thirty-three completed surveys were collected. Eleven of fifteen respondents who included contact information were from Michigan, with the remaining respondents hailing from Wisconsin, Iowa, Illinois, or Ohio. This report summarizes the data and comments collected in this survey.

Of the 33 responses to question one, "What is your interest level in organic blueberry production?", 25 respondents indicated they were considering organic blueberry production, two respondents indicated no interest, and one respondent indicated current organic-certified blueberry acreage in production. Of the 25 respondents considering organic blueberry production, 17 indicated an interest in establishing new blueberry plantings, three had an interest in both new and established plantings, and six were interested in transitioning mature plantings to organic production.

Approximately three-quarters of those surveyed regarded aspects of cultural management as major concerns. Responses included insect pest management (27 responses, 82%), nutrition (26 responses, 79%), weed control (26 responses, 79%), and disease management (25 responses, 76%). Slightly less than half of respondents indicated marketing (13 responses, 39%) or labor (13 responses, 39%) as major concerns.

Six respondents indicated that they were willing to serve as research grower cooperators in 2008; five of these resided in Michigan. Four potential grower cooperators reported that they

were most interested in establishment of new plantings, while the other two potential grower-cooperators had an interest in research in both established plantings and new plantings.

Many respondents included additional comments at the end of the survey. Four respondents offered concerns regarding cost of production and marketing. Four other respondents questioned the reliability of organic production inputs. Concerns about management of pests were noted by four respondents. Three respondents commented on nutrient management. Finally, two respondents voiced concerns about *E. coli* contamination stemming from the use of animal manures.

Overall, the results of this survey indicated a significant interest in organic production of blueberries in Michigan and other Midwest states. However, results of this survey may not be an accurate representation of the opinions of all blueberry producers in Michigan, as indicated by the relatively small number of surveys collected. This survey nonetheless enhanced our awareness of concerns of prospective and current organic blueberry growers. While production-related issues ranked highest among respondents, economics (labor cost and marketing) were also a significant concern. Both of these aspects of organic blueberry production need to be addressed in future research at Michigan State University.

Organic Blueberry Production: What We Know and What We Need to Learn

2007 Great Lakes Fruit, Vegetable, and Farm Market Expo
Research Questionnaire

1) Which areas of blueberry production do you anticipate being most challenging using organic methods?

2) Are you aware of any potential approaches to these production problems that we could evaluate in this research project?

3) Are you willing to serve as a grower cooperator for the MSU organic blueberry research project for at least one growing season (if so, please include contact information, amount of land available, and if the land is certified / has history of organic management)?

4) What was the most useful information included in this presentation today?

5) Please share any other thoughts you have regarding this research on organic blueberries.

Appendix B.

Evaluation of Sanidate 5.0 for post-harvest control of anthracnose fruit rot in 'Jersey' blueberries, 2009

We designed an experiment to compare the treatment method (spray and dip) and concentration (0.1% and 0.15%) of Sanidate 5.0 (23% hydrogen peroxide and 5.3% peroxyacetic acid) for post-harvest control of anthracnose fruit rot caused by *Colletotrichum acutatum* in blueberries. Water spray and dip treatments served as controls for each treatment method, and a no-water spray or dip treatment was included to determine whether spray or dip methods affected the efficacy of the product. We included four replications per treatment by concentration combination and evaluated 50 berries per replication. We applied the spray treatment with a fine-mist atomizer to the point of runoff. The dip treatment was performed by completely submerging berries for 90 seconds in the solution. Berries were then placed equidistantly on mesh screens and incubated at room temperature (23°C) and 100% relative humidity for 14 days.

After incubation, we visually assessed berries for incidence of fruit rot and presence of fruit rot pathogens. Data were subjected to one-way ANOVA and log-transformed to meet the heterogeneity of variance assumption in ANOVA as needed; untransformed values are presented. SAS 9.2 (SAS Institute, Cary, NC) was used for statistical analysis.

Anthracnose incidence was very high; the mean infection incidence was greater than 90% for all treatments. The ANOVA was not statistically significant (p=0.18). The, the 0.15% spray and 0.1% dip treatments tended to reduce anthracnose incidence relative to the untreated control (Table B.1). Despite the modest reduction in anthracnose, our results indicate that Sanidate 5.0 has some activity against *C. acutatum*, and may be most effective when applied at 0.1% as a dip and 0.15% as a spray. Further experiments should evaluate Sanidate 5.0 on a wider range of cultivars and concentrations.

Table B.1. Anthracnose fruit rot incidence in 'Jersey' blueberries after incubation at room-temperature (23°C) for 14 days in an evaluation of Sanidate 5.0 concentrations and methods of application for control of post-harvest fruit rot.

Method of application (concentration Sanidate 5.0)	Anthracnose fruit rot incidence (%) ± SE[z]
Untreated	98 ± 1.5
Spray (0%)	95 ± 1.0
Spray (0.10%)	95 ± 1.5
Spray (0.15%)	92 ± 1.3
Dip (0%)	95 ± 1.5
Dip (0.10%)	95 ± 2.8
Dip (0.15%)	95 ± 2.1

[z]Standard error of the mean.

Appendix C.

Crown rot caused by *Calonectria colhounii (*anamorph: *Cylindrocladium colhounii)* on *Vaccinium corymbosum* L.

Blighted stems and leaves and necrotic crowns were observed on 1-year-old blueberry plants (*Vaccinium corymbosum* L. 'Polaris' and 'Liberty') grown in trays in a greenhouse in Michigan in 2008. A fungus was isolated from symptomatic stems. On PDA, colonies were at first creamy orange, then rusty brown and zonate with an irregular margin. Conidia were abundant and produced in parallel clusters held together with colorless slime. Conidia were hyaline, cylindrical with rounded ends, 3-septate, and 60 ± 2 × 5.5 ± 0.2 µm (range 48 to 73 × 5 to 7 µm) in size. Conidiophores were extensively branched and had a stipe extension with a 3.5- to 4.5-µm-wide clavate vesicle. Perithecia produced on water agar were globose to subglobose, bright to dark yellow, with a height of 288 to 322 µm and diameter of 255 to 294 µm. Ascospores were hyaline, 2 to 3-septate, fusoid to crescent-shaped and 57 ± 3 × 5.3 ± 0.2 µm (range 30 to 88 × 5 to 7.5 µm). Based on morphology, the fungus was identified as *Calonectria colhounii* Peerally (anamorph: *Cylindrocladium colhounii* Peerally) (Crous, 2002).

To confirm pathogenicity, 5 ml of a conidial suspension (1 x 10^5 conidia/ml) was applied as a foliar spray or soil drench to healthy 10- to 15-cm tall blueberry plants ('Bluecrop') in 10-cm plastic pots in a greenhouse. Four plants were used per treatment, and two water-sprayed or drenched plants served as controls. Plants were misted intermittently for 2 days after inoculation. After 7 days at 25 ± 3°C, drench-inoculated plants developed necrotic, sporulating stem lesions at the soil line, while spray-inoculated plants developed reddish brown leaf lesions (up to 5 mm in diameter) with sporulation on the upper and lower leaf surfaces. Signs of root rot were not observed. At 28 days, three out of four drench-inoculated plants and one out of four spray-

inoculated plants had died, and stem necrosis and wilt were observed on the remaining inoculated plants. No symptoms were observed on water-sprayed control plants. Fungal colonies re-isolated from surface-disinfested symptomatic stem, leaf, and root segments appeared similar to the original isolates.

Cylindrocladium colhounii was first reported as causing a leaf spot on blueberries in nurseries in China (Luan et al., 2006) while *Calonectria crotalariae* (Loos) D.K. Bell & Sobers (=*Calonectria ilicicola* Boedijn & Reitsma) causes a stem and root rot of blueberries in North Carolina (Milholland, 1974). To our knowledge, this is the first report of *Cylindrocladium colhounii* causing a disease of blueberry in Michigan or in the United States. Due to its destructive potential, this pathogen poses a significant threat to blueberry nurseries. The fungicide fludioxonil appeared to be effective at curtailing disease development under experimental conditions and should be further evaluated. In addition, modifications of the environment may help reduce infection incidence and severity.

Appendix D.

The effects of mycorrhizal inoculation and fertilizer type on plant growth and mycorrhizal colonization of *Vaccinium corymbosum* 'Bluecrop'

Abstract

The effects of different fertilizers and mycorrhizal inoculation on growth and mycorrhizal colonization in container-grown rooted cuttings of *V. corymbosum* 'Bluecrop' were evaluated in a greenhouse experiment. Fertilization with ammonium sulfate resulted in plant mortality shortly after it was applied. In the feather meal fertilization treatment, the total shoot length of plants inoculated with *Rhizoscyphus ericae* Zhuang and Korf (UAMH 9270) was significantly greater than plants inoculated with *Oidiodendron maius* Barron (UAMH 9263), an unidentified ericaceous root-associated fungus (UAMH 9264), or a mixed species inoculum of all three fungi, the total shoot length of plants inoculated with mycorrhizal fungi was greater than uninoculated plants. Mycorrhizal inoculation did not affect the shoot length of plants fertilized with compost. At the end of the experiment, 180 days after mycorrhizal inoculation, mycorrhizal colonization of hair-root epidermal cells averaged 8% in the compost fertilizer treatment but was nearly absent in plants fertilized with feather meal. The pH of the container substrate increased temporarily two weeks after fertilization with feather meal, but not compost amended with elemental sulfur. After 180 days, the concentration of inorganic nitrogen in leachate collected from the container substrate was 25 ppm in the feather meal treatment and less than 1 ppm in the compost treatment, suggesting more prolonged nitrogen release from feather meal than compost. This study provides information on interactions between fertilizer type and different species of ericoid mycorrhizal fungi, and may advise further investigation into the role of mycorrhizae in the nutrition and growth of blueberries.

Introduction

Nitrogen fertilizers used in organic crop production are limited to materials derived from natural sources (http://www.ams.usda.gov/AMSv1.0/nop) such as compost and protein meals. Conventional growers fertilize crops with inorganic nitrogen or urea because synthetic nitrogen sources are less costly than organic fertilizers. Ericaceous plants such as highbush blueberry (*Vaccinium corymbosum* L.) are better able to utilize organic nitrogen when colonized by ericoid mycorrhizal (ERM) fungi (Stribley and Read, 1980; Bawja and Read, 1986; Sokolovski et al., 2002). Scagel (2005) reported that nursery-grown blueberries supplied with organic fertilizers grew more vigorously when inoculated with ERM fungi, but the growth of plants fertilized with synthetic nutrients was superior to those grown with organic fertilizer and less affected by ERM inoculation. However, Scagel (2005) applied synthetic and organic fertilizers at the same rate of total N and did not consider the possibility for different nitrogen release patterns over time between organic and synthetic fertilizers. High levels of inorganic nitrogen may inhibit ERM colonization of ericaceous plants (Stribley and Read, 1976), but the question of whether high availability of organic nitrogen inhibits ERM colonization has not been tested. Organic forms of nitrogen such as amino acids stimulate fungal growth when added to soil (Lucas et al., 2007). In Michigan, soil inorganic nitrogen levels are higher in conventional compared to organic blueberry fields when plant nitrogen demand is highest (Chapter 3). However, leaf tissue nitrogen content is not affected by management type (unpublished data). In addition, ERM colonization in Michigan is, on average, higher in fields under organic management (Chapter 3). Taken together, these data suggest ERM may be particularly important in plant uptake of nutrients from organic fertilizers. The objective of this study was to determine the effect of

nitrogen fertilizer type on growth and ERM colonization of container-grown blueberries inoculated with three species of ERM fungi.

Materials and methods

Shoot tip cuttings of *Vaccinium corymbosum* 'Bluecrop' were propagated in a coir substrate (Crop Circles, Lansing, Michigan) that was autoclaved for two hours to eliminate ERM fungi (Vohnik et al., 2003). The coir substrate pH was 5.8 and electrical conductivity (EC) was 0.94 mmhos cm^{-1}, as determined by a commercial soil testing service (A & L Great Lakes Laboratories, Inc.). Rooting of cuttings occurred after 12 to 14 weeks, longer than 6 to 8 weeks normally required for rooting of shoot tip cuttings in peat moss (personal observation). After rooting, plants were transferred to a fine-textured coir substrate (Cocogro, American Agritech, Chandler, AZ) that was mixed with perlite at a ratio of three parts coir to one part perlite by volume. The Cocogro coir had a pH of 6.1, EC of 0.77 mmhos cm^{-1}, and less than 1 ppm inorganic nitrogen. Plants were grown under cool fluorescent bulbs for 28 days and watered with deionized water titrated to a pH of 4.5 with sulfuric acid to maintain the substrate pH in the appropriate pH range for blueberries.

Slant cultures of *Oidiodendron maius* Barron (UAMH 9263), *Rhizoscyphus ericae* Zhuang and Korf (UAMH 9270), and an unidentified root-associated fungus of Ericaceae (UAMH 9264) were obtained from the University of Alberta Microfungus Collection and Herbarium (Edmonton, Alberta, Canada). These fungal species were isolated from roots of native *Vaccinium angustifolium* Ait. or *V. corymbosum* in Rothrock State Forest, Pennsylvania, USA (Stevens et al., 1997; Yang, 1999). The strains of fungi increased plant growth or nutrient uptake when inoculated onto roots of *V. corymbosum* in previous greenhouse and field studies

(Yang, 1999; Yang et al., 2002). A scalpel blade was used to cut 1 × 1 mm squares of mycelium from the margins of 2-week-old cultures grown on modified Melin Norkrans (MMN) agar (Hutchison, 1981). Two mycelial blocks were transferred to sterile plastic tubes containing 40 ml of liquid MMN medium. Liquid cultures were placed on an orbital shaker and incubated at 23°C under ambient fluorescent light. After 28 days, mycelium was collected on Whatman No. 1 filter paper under partial vacuum pressure and rinsed three times with deionized water. A mycelial suspension was prepared by macerating fungal mycelium in 200 ml deionized water with a Waring 33BL79 blender (Dynamics Corporation, New Hartford, CT) for 90 sec. The final inoculum concentrations were 10 mg ml^{-1} for *O. maius*, 13 mg ml^{-1} for *R. ericae*, and 17 mg ml^{-1} for UAMH 9264 on a fresh-weight basis. Slightly different inoculum concentrations were due to different grown rates of fungal species in liquid culture; all mycelium was harvested and used for inoculation. The mixed inoculum treatment consisted of equal volumes of mycelial slurries prepared from each fungal species.

Rooted cuttings were removed from pots, washed of adherent coir under tap water, and roots were placed in 300 ml of mycelial slurry or deionized water (uninoculated control) for 12 hours (Figure D.1). A root-dip inoculation method was attempted rather than pipetting mycelial slurries over the growth substrate (Yang, 1999) or beneath plant roots (Jansa and Vosátka, 2000) because the latter two methods resulted in sparse ERM colonization in our preliminary experiments. The viability of the fungal inocula was confirmed by plating 0.1 ml onto potato dextrose agar amended with 10 ppm chloramphenicol on the same day plants were inoculated. The number of colony-forming units (CFU) 10 days after plating was 1, 4, and 2 CFU ml^{-1} for *O. maius*, *R. ericae*, and UAMH 9264, respectively. Following inoculation, plants were

transplanted singly into 8.5 × 8.5 × 8.5 cm green plastic containers filled with three parts Cocogro-brand coir to one part perlite by volume.

Five days after inoculation, feather meal and compost fertilizer were incorporated into the upper 2-cm surface of the coir substrate (Figure D.2). Separate utensils were used to incorporate fertilizer between inoculation treatments to prevent cross-contamination. The feather meal contained 14.8% nitrogen according to a certificate of analysis provided by the supplier (Morgan's Composting, Evart, MI). Feather meal was applied at 1.4 g per pot to provide the equivalent of 40 lb of available nitrogen per acre on an aerial basis assuming 65% nitrogen mineralization over the course of the experiment (Hadas and Kautsky, 1994; Gaskell and Smith, 2007). Dairy manure compost (Farm peat, Green Valley Agricultural, Inc., Caledonia, MI) specifically designed for container production (personal communication, Goris Passchier, Green Valley Agricultural, Inc., December 2009) was used in the compost treatment. The dairy compost had a pH of 7.7, EC of 1.75 mmhos cm^{-1}, NO_3^--N concentration of 79 ppm, nitrogen content of 0.97% and a carbon to nitrogen ratio of 14:1 on a fresh-weight basis (NH_4^+-N concentration was not determined). The compost was applied at a rate of 30 g per pot assuming 15% nitrogen mineralization (Gaskell and Smith, 2007) to provide a field-rate equivalent of 40 lbs of available nitrogen per acre. Prior to adding the compost, a subsample was titrated with dilute sulfuric acid to estimate the amount of elemental sulfur needed to lower the pH of the compost to 5.0. 1.8 g of powdered sulfur was added to moist compost one month prior use in the experiment.

For the synthetic fertilizer treatment, a solution of 0.3 g ammonium sulfate in 10 ml deionized water was applied and was to be added at the same rate 6 weeks later to provide a split-application to simulate standard fertilization practices for field-grown blueberries (Hanson

and Hancock, 1996). However, 21 days after application, the initial ammonium sulfate application was lethal to 38 of 40 plants. In preliminary trials, we applied the same concentration of ammonium sulfate to plants of similar age and stature with no obvious detrimental effects. However, in the preliminary trials, plant roots were not disturbed prior to addition of ammonium sulfate. In the current experiment, adherent coir was rinsed from roots prior to inoculation to facilitate direct contact between fungal inoculum and root tissue. Root disturbance may have increased root sensitivity to high ammonium concentrations. We continued the experiment with the compost and feather meal treatments.

Plastic trays were used for capture of excess leachate from containers, as stipulated by a USDA-APHIS permit that was required for importation of ERM fungi from the UAMH. A nutrient solution without nitrogen (Stribley and Read, 1976) was added to saturate the media 4 weeks after transplanting and every 2 weeks thereafter in both feather meal and compost fertilizer treatments. The nutrient solution was applied because a preliminary experiment showed that plants were deficient in phosphorus after being grown in the Cocogro coir for two months with no supplemental fertilizer. In the first 90 days of the experiment, plants were watered daily with deionized water adjusted to a pH of 4.5 with sulfuric acid. In the latter 90 days, deionized water without added sulfuric acid was used because the pH of the substrate stabilized below 5.5 (Figure D.10) in both the compost and feather meal treatments. Daily watering was needed to keep the container substrate moist but not waterlogged. The container substrate was leached with at least 75 ml of water on four occasions over the course of the experiment, the first two instances due to accidental overwatering and intentionally in the latter two instances because the EC of the substrate had reached 2 mmhos cm^{-1}.

Each fertilizer by ERM fungus species combination was replicated eight times in a randomized complete block design. Plant containers in each block were grouped on 40 × 60 cm plastic trays. Over the first 90 days of the experiment, plants were grown in a controlled-environment chamber (Figure D.3) at a temperature of 23°C (s.d. ± 1°C) with a 16-hour photoperiod and 95 µmol m^{-2} s^{-1} photosynthetically active radiation provided by six 61-cm fluorescent bulbs. Trays were positioned on each of two levels of two growth chambers. The position of trays was rotated within chambers in a systematic pattern every 2 to 3 weeks. After 90 days in the growth chamber, plants were transferred to a greenhouse as the growth chambers were needed for other research projects. The daily minimum and maximum temperatures were monitored on an hourly basis over 5 days. The temperature minima and maxima in the greenhouse were 21°C (± 0.4°C) and 33°C (s.d. ± 4°C). The temperature in root zone of the coir substrate was similar to the ambient air temperature. Plants remained in the greenhouse for the latter 90 days of the experiment.

Shoot growth was measured six times, at the start of the experiment and after new flushes of shoot growth were observed. Shoot length was recorded as the length of plant stems with green leaves. Root length and mass were not determined at destructive plant harvest because it was physically impossible to separate the coir from the fine roots.

The substrate pH and EC were measured five times over the first 50 days of the experiment and 2 weeks prior to the end of the experiment by collecting 10 ml of leachate from 2 randomly sampled pots per fertilizer treatment after flushing with deionized water. The nitrate and ammonium concentration of the leachate was determined at the end of the experiment.

Roots samples were collected for assessment of ERM colonization 50 days after inoculation and at the end of the experiment and stored at 4°C in 50%. Using a scalpel blade,

root systems harvested at the end of the experiment were divided in half longitudinally. Each half was sectioned into six clumps; six of the twelve clumps per plant were assessed for colonization by ERM and dark septate endophyte (DSE) microsclerotia. After > 2 mm-diameter roots were removed, roots were placed in histology capsules and cleared in 5% KOH at room temperature for 12 hours. Following the clearing step, roots were soaked in several changes of deionized water until no brown solute leaked from the capsules, soaked in 1% HCl for 2 hours, and then stained at room temperature for 24 hours in 0.05% methyl blue dissolved in a 1:1 mixture of glycerol and water acidified with 1% HCl (modified from Yang, 1999 and Brundrett et al., 1996). After roots were destained in an acidified glycerol-water solution, fine hair roots were excised from large root segments using a scalpel blade, mounted on glass slides, and covered with glass cover slips with slight pressure to flatten cylindrical roots onto a horizontal plane for detailed microscopic examination. ERM or DSE colonization is reported as the number of intracellular hyphal coils (Figure D.4) or microsclerotia (Figures D.5c and D.5d) observed per 50 epidermal cells over an approximate root length of 6 mm (modified from McGonigle et al., 1990). Fifty randomly selected, less than 125 µm-diameter hair roots were examined per plant, corresponding to a total hair root length of 30 cm per plant.

Statistical analysis was performed with SAS 9.2 (SAS Institute, Cary, NC). ERM inoculation and fertilizer effects on ERM and DSE colonization, and fungal inoculum, fertilizer, and date effects on shoot length were evaluated by two- or three-way ANOVA, respectively. Least square mean differences for main and interaction effects were determined using the GLIMMIX procedure with sampling date specified as a repeated factor. The ammonium sulfate fertilizer treatment was omitted from the analysis due to greater than 95% plant mortality.

Fifteen percent of plants in the compost and feather meal treatments died over the course of the experiment. Plant mortality did not differ among ERM inoculation or fertilizer treatments.

Results

Ericoid mycorrhizal and dark septate endophyte colonization

A qualitative assessment of ERM colonization of roots collected from two to three plants from each inoculation treatment and composited by fertilizer treatment on day 50 of the experiment indicated that inoculated plants were colonized by ERM fungi (Figure D.6), while uninoculated plants were not colonized. By the end of the experiment, plants treated with compost had higher ERM colonization than plants treated with feather meal (Figure D.7a), but colonization did not differ significantly among ERM inoculation treatments (Table D.1). Intracellular colonization by DSE microsclerotia was higher in plants fertilized with feather meal than compost (Figure D.7B) but was not significantly different among ERM inoculation treatments (Table D.1). Two distinct intracellular ERM morphologies were observed: tightly wound, fine-diameter hyphae and more diffuse, thicker-diameter hyphae (Figure D.4).

Shoot length

There were significant effects of fertilizer type, inoculum type, and date on plant shoot length (Table 1). Fertilizer × inoculum and fertilizer × date interactions were significant (Table 1) indicating inconsistent effects of ERM inoculum between fertilizers and fertilizers over time. Overall, shoots of plants fertilized with compost grew faster than those of plants fertilized with feather meal (Figure D.9). The shoot length of plants fertilized with compost was not affected by ERM inoculation (Figure D.8). Within the feather meal treatment, plants inoculated with *R. ericae* had significantly more shoot length the those plants inoculated with *O. maius* or UAMH 9264, while shoot length of uninoculated plants was less than plants inoculated with ERM fungi

(Figure D.8). A decrease in shoot length in the latter 90 days of the experiment was likely due to a harsher environment in the greenhouse, which included temperatures above 35°C and severe whitefly infestation.

Container substrate pH and inorganic nitrogen concentration

The container substrate in the feather meal treatment had an initial pH of 3.6, increased to a pH of 6.4 by day 20, and then stabilized at a pH of 4.5 on day 50 (Figure D.10). In the compost treatment, the substrate pH remained between 4.0 and 4.4 on all measurement dates (Figure D.10). The pH in the ammonium sulfate treatment was near 3.0 on all measurement dates (Figure D.10). The substrate pH was monitored as a diagnostic measure to determine whether acidification of water supplied to plants was necessary and therefore was not subject to statistical analysis. At 180 days after fertilizers were added, the concentration of inorganic nitrogen in the leachate of the container substrate fertilized with feather meal was nearly 30 ppm while the leachate of containers fertilized with compost had an inorganic nitrogen concentration of less than 1 ppm (Figure D.11).

Discussion

This was the first study we are aware of which investigated the effect of different organic fertilizers on ERM colonization and growth of blueberries. Plants fertilized with feather meal had increased shoot length in response to inoculation with ERM fungi (Figure D.8), which agrees with previous reports of increased shoot growth of ERM compared to non-ERM plants provided with protein or amino acids as a source of nitrogen (Stribley and Read, 1980; Bajwa and Read, 1986; Yang, 1999; Scagel et al., 2005). Yang (1999) found that, compared to uninoculated plants, those plants inoculated with the some of the strains of ERM fungi used in the current study and grown in a sand substrate obtained more nitrogen from bovine serum albumin (a

complex protein) as the sole nitrogen source, but the effect of inoculation on shoot growth was less apparent. Diverse carbon sources in the coir substrate utilized in our study may have supported a more complex microbial community compared to the sand culture system used by Yang (1999), and intensified plant-microbial competition for N. Results of a recent study conducted by Walker et al. (2010) suggest the primary benefit conferred to plants by ERM is increased access to organic N because a high activity of saprotrophic microbiota rapidly immobilizes mineral N in the soil solution.

Enhanced shoot growth of plants inoculated with ERM fungi in the feather meal treatment indicates that ERM fungi may improve plant uptake of protein-based fertilizers in blueberry production fields. *R. ericae* proved to be superior to the other ERM fungi in terms of plant shoot growth enhancement under the conditions of our experiment. Variation in amino acid and protein utilization among species of ERM fungi has been reported previously (Xiao and Berch, 1999) and may account to some extent for the differences observed among ERM inoculation treatments.

Compost promoted shoot growth relative to feather meal on all measurement dates except the last, on which plants grown with feather meal had significantly more shoot growth than those grown with compost (Figure D.9). This may be due to differences in temporal nitrogen release patterns between compost and feather meal. Nitrogen availability in the compost and feather meal treatments was not monitored until two weeks prior to the last date that shoots were measured, 165 days after fertilizer was added. At this time, the inorganic nitrogen concentration in the feather meal treatment was at least 30 times higher than in the compost treatment. A near-absence of ERM colonization of plants in the feather meal treatment at the end of the experiment may have resulted from higher levels of available nitrogen in the feather meal compared to the

compost treatment. Stribley and Read (1976) observed that cranberries (*Vaccinium macrocarpon* Ait.) grown in sand had reduced levels of ERM colonization when nitrogen was supplied as ammonium at concentrations greater than 20.5 ppm, which is near the concentration of ammonium-N (26 ppm) observed in leachate collected from the feather meal treatment containers two weeks prior to the end of our study. However, in the same study (Stribley and Read, 1976), ERM colonization averaged 15% for plants supplied with 56 ppm ammonium-N, which is much higher than observed in the feather meal treatment at the end of the current study, which suggests factors other than high nitrogen availability may have limited ERM colonization in both compost and feather meal fertilizer treatments.

In the compost treatment, ERM colonization levels were similar between inoculated and uninoculated plants at the end of the experiment (discussed below). It is possible that nitrogen supplied in the compost treatment was initially high but declined more rapidly than in the feather meal treatment. A significant interaction between the effects of fertilizer type and measurement date on shoot growth, with more shoot growth in the compost treatment on earlier dates but more shoot growth in the feather meal treatment on the last measurement date, supports this possibility. It is important to note that the containers were accidentally or intentionally flushed with water at several points over the course of the experiment, which probably resulted in losses of soluble nitrogen at each leaching event. Hadas and Kautsky (1994) reported that more than 99% of nitrogen contained in feather meal is insoluble. Nitrogen release from feather meal occurs after insoluble proteins are broken down by saprotrophic microbes, including ERM fungi. Gaskell and Smith (2007) and Hadas and Kautsky (1994) reported that 60 to 65% of nitrogen in feather meal was mineralized 8 weeks after its addition to soil. A difference between feather meal and compost fertilizers in nitrogen release over time has implications for organic blueberry

growers. Maintenance of soil nitrogen in less soluble forms may reduce leaching of nitrogen below the plant root zone. However, delayed release of nitrogen and prolonged nitrogen availability into fall discourages plant hardening and may increase the risk of winter injury to late-season shoot growth that is stimulated by high availability of nitrogen (Hanson and Hancock, 1996).

Inoculated plants were colonized by ERM, however uninoculated plants fertilized with compost also eventually became colonized by ERM fungi. At 55 days after ERM inoculation, we confirmed that inoculated plants were colonized, while roots of uninoculated plants showed no signs of ERM colonization. Therefore, at the end of the experiment we were surprised to find that ERM colonization was 8% in the compost treatment, near zero in the feather meal treatment, and not significantly increased by inoculation with ERM fungi.

Disagreement in ERM colonization levels observed at different time points in the experiment may be related to sampling rigor. At the end of the experiment, entire root systems were harvested and colonization was assessed on 50 hair roots from each plant. Earlier in the experiment, only small subsamples of roots were taken from each inoculation treatment and pooled by fertilizer treatment to qualitatively assess colonization levels, which appeared much higher than was observed at the final measurement.

Unfortunately, when destructive samples were taken for quantitative assessment of colonization at the end of the experiment, plants stress caused by 90 days under excessive heat and insect pest pressure in the greenhouse may have detrimentally impacted both plant vigor and ERM colonization. Therefore is likely that ERM colonization levels at the end of the experiment did not reflect those at earlier time points. ERM fungi may have also been introduced to the uninoculated treatment containers by airborne contamination. Peat moss contains ERM fungi

(Rich and Currah, 2006; Gorman and Starrett, 2003) and was used as a container substrate in the greenhouses adjacent to our trial. Propagules of ERM fungi in peat moss may have been introduced to uninoculated plant containers via airborne contamination. Cross-contamination between inoculated and uninoculated treatments also may have also occurred shortly after plants were transferred to the greenhouse when plants were watered from above with a handheld sprinkler, possibly splash-dispersing hyphae of ERM fungi between containers. Additionally, the compost used in this experiment may have contained fungi capable of forming ERM even though it was autoclaved prior to use. Yang et al. (1996) reported that autoclaving did not completely eradicate ERM inoculum from soil. In future trials greater care should be taken to exclude all possible sources of ERM fungi from uninoculated plants and utilize sterilization methods other than autoclaving, such as irradiation, if non-ERM control plants are desired.

Compared to the controlled conditions in the greenhouse, the environment was noticeably hotter, less humid, and generally more variable in the greenhouse. High temperatures in the greenhouse may have caused heat or moisture stress in plants even though the moisture supply was maintained by daily watering. Northern highbush blueberries grow best at temperatures below 26°C (Trehane, 2004). Heat stress reduces CO_2 assimilation (Hancock et al., 1992) and may increase plant mortality under greenhouse and field conditions (Kender and Brightwell, 1966). Leaf drop that occurred in the current study may have resulted from direct damage by whiteflies and plant sensitivity to foliar insecticides applied for whitefly control. A negative impact of the hostile greenhouse environment on plant shoot longevity is apparent because the length of green shoots did not increase under greenhouse conditions.

When present as plant symbionts, ERM fungi depend on carbohydrates supplied by the plant host (Stribley and Read, 1974). Plant defoliation may have halted photosynthate supplied to

ERM in favor of shoot regeneration. Scagel and Yang (2005) and Korcak (1988) suggested that ERM colonization parallels carbohydrate levels in the plant host. Scagel et al. (2005) reported a change in environment from a sheltered indoor nursery to outdoor conditions temporarily reduced the percentage of blueberry plants colonized by ERM. Additionally, a 4-fold decrease in ERM colonization resulted from partial defoliation of the ericaceous plant *Calluna vulgaris* (L.) Hull (Hartley and Amos, 1999). A reduction in the plant canopy increases the relative carbon cost of mycorrhizal associations to plants (Johnson et al., 1997). In our experiment, ERM colonization may have been substantially higher in the first 90 days of our experiment than at the end when ERM colonization levels were assessed. Our results provide further evidence that plant allocation of reduced carbon to aboveground growth is prioritized over the needs of ERM fungi when plant carbohydrates are in short supply, e.g., following defoliation. A reduction in shoot longevity caused by excessive heat and insect pest damage in the may have been severe enough to induce carbon starvation of ERM fungi. Isolation from insect pests and maintenance of greenhouse temperatures below 30°C are necessary in future experiments.

Under conditions of limited carbohydrate supply from plant hosts, root symbionts would need to survive as saprotrophs or enter a stage of dormancy. In forest soils, ERM fungi may survive in soil for years in the absence of a suitable host (Bergero et al., 2003). The ERM fungi *R. ericae* and *O. maius* produce an array of enzymes to degrade simple and complex sources of carbon including cellulose, hemicellulose, and pectin but are only able to partially decompose lignin (Cairney and Burke, 1998). The saprophytic abilities of UAMH 9264 have not been determined. Coir is composed of ground fibers of coconut husks and contains approximately 35% cellulose, 55% lignin, and 10% hemicellulose (Abad et al., 2002). The container substrate used in this experiment was therefore not entirely utilizable as a carbon source by ERM fungi

because of its lignin-rich composition, and more labile constituents may have been utilized by saprotrophic microbes early in the experiment. Hydrolyzed feather meal has a C:N ratio of less than 5:1 and thus provided about 1 g of carbon per container. Most carbon in feather meal is utilized by microbes shortly after it is applied, with a secondary stimulation of microbial activity after 14 days (Hadas and Kautsky, 1994). In contrast, addition of compost can increase biological activity in soil even 12 months after application (Darby et al., 2003). The compost in this study contained 80% organic matter on a dry weight basis and had a moisture content of 55% at application, thus providing greater than 10-fold more organic matter to each container substrate than feather meal. The amount of carbon available in the compost-fertilized containers to meet the needs of ERM fungi when plants were partially defoliated is not known. However, because compost provides a sustained release of carbon (Darby et al., 2003), it may have been sufficient to provide for the carbon needs of ERM fungi when plant carbohydrate supply was low. As new shoots formed in the last 30 to 40 days of the experiment, ERM fungi may have recolonized roots as photosynthate supplied to roots returned to normal levels. Plants fertilized with feather meal were colonized by ERM prior to but not after the occurrence of partial plant defoliation. Therefore, additional carbon supplied in compost-amended pots may explain why plants fertilized with compost but not feather meal were colonized by ERM at the end of the experiment. Under field conditions, compost may provide a carbon source for ERM fungi when plants do not allocate energy to root formation, which is a prerequisite to ERM colonization (Valenzuela-Estrada et al., 2008). In this manner, ERM fungal inoculum residing in compost and other soil organic matter may facilitate rapid colonization of new hair roots as they are formed. The influence of the biochemical composition of soil organic matter on the persistence of ERM

fungi may provide insights into the wide range of ERM colonization levels observed in blueberry production fields.

We observed two distinct ERM morphologies: tightly wound, fine-diameter hyphal coils and more diffuse, thicker-diameter hyphal coils (Figure D.4). Variation in the colonization morphology of ERM may be widespread but has not been studied in detail (Brundrett, 2004). It is not known whether hyphal morphology in ERM varies according to fungal species or is under the control their plant hosts. The physical appearance of ERM was not related to the species of fungi inoculated onto plant roots, as both loose and tightly wound hyphal coils were observed in all inoculation treatments. Morphological differences in ERM may warrant further investigation in light of our and others' (e.g., McLean and Lawrie, 1996) observations. Blue-stained, spore-like structures similar to those reported in previous studies (Reich et al., 1982) were observed and sometimes associated with ERM colonization (Figure D.5a). It is interesting to note that DSE microsclerotia were more abundant in roots of feather meal vs. compost-fertilized plants. Perhaps the formation of intracellular resting structures in plant roots, the microsclerotia, was a response of resident fungi to carbon starvation that may have occurred in the feather meal treatment in the greenhouse environment, as discussed above.

The pH of the container substrate increased 2 to 3 weeks after feather meal was added, while a similar effect was not observed in the compost fertilizer treatment. At the start of the experiment compost was amended with sulfur to neutralize its slightly alkaline pH, but we assumed feather meal would have a negligible impact on the pH of the container substrate. The amino acid profile of hydrolyzed feather meal consists of mainly acidic and neutral amino acids (http://www.americanproteins.com/hydrolyzed-meal.htm?zoom_highlight=feather+meal). Bajwa and Read (1986) observed that glutamic or aspartic acid (acidic amino acids) provided as a

nitrogen source to *Hymenoscyphus ericae* (=*R. ericae*) in liquid culture caused a 1.5- to 2-point increase in the pH of the medium after 20 days, while basic or neutral amino acids either acidified or had a modest effect on the medium pH, respectively. At low carbon availability, increasing pH with utilization of glutamate by ERM fungi was also reported by Grelet et al. (2005). An increase in the substrate pH with utilization of organic nitrogen was likely related to microbial breakdown of low C:N ratio feather meal and resultant short-term accumulation of ammonium-nitrogen in excess of microbial and plant demand (Bardgett, 2005; Summerbell, 2005). Increased pH resulting from plant and microbial utilization of acidic amino acids likely reflects the low buffering capacity of the coir substrate. Under field conditions, increases in pH related to uptake of amino acids in feather meal would likely be more modest because substantial quantities of lime or sulfur are needed to raise or lower the pH of most soils (Hanson and Hancock, 1996).

Conclusion

ERM inoculation increased shoot length of plants grown with feather meal but not compost. Inoculation with *R. ericae* had the greatest effect on the total length of shoots of blueberries grown with feather meal under our experimental conditions. Contrasting results in the effect of ERM inoculation on plants fertilized with compost and feather meal suggest two alternative explanations: either ERM fungi indigenous to the compost provided similar benefit to plants as the ERM inoculum or inoculation with ERM fungi alleviated plant nutrient deficiency in plants fertilized with feather meal but not compost. Because we did not conduct a rigorous assessment of ERM colonization or nitrogen availability at multiple time points or determine the identity of fungal species that colonized roots of plants grown with compost, a follow up experiment is needed to substantiate or refute these hypotheses. Assessment of ERM

colonization and nitrogen availability at several time points would provide a more thorough understanding the relationship between ERM colonization and growth of blueberries resulting from variation in fertilization practices. Observations after plants were moved to the more stressful greenhouse environment suggest that there is competition between shoot growth and ERM fungi for photosynthate, such that mature leaves are able to sustain carbohydrate needs of ERM fungi but growth of new shoots is prioritized over the needs of ERM fungi when plant photosynthate is low. The limited scope of parameters we measured and the unexpected demise of plants treated with synthetic fertilizer limit our conclusions regarding the effects of different types of fertilizer nitrogen on ERM colonization of blueberries. Nonetheless, results of this experiment provide useful information about potential interactions between ERM and fertilization type in organically grown blueberries and set the stage for additional experiments.

Table D.1. Repeated measures analysis of variance of the effects of fertilizer type, species of mycorrhizal inoculum, and sampling date on shoot length, root colonization by ericoid mycorrhizae (ERM) and dark septate endophytes (DSE), and container-leachate inorganic N concentration in container-grown plants of *V. corymbosum* 'Bluecrop'.

	Num df[z]	Den df	Shoot length	ERM	DSE	Leachate inorganic N[y]
				P < *F*		
Fertilizer	1	29	0.0003	<0.0001	<0.0001	<0.0001[x]
Inoculum	4	27	0.03	0.62	0.43	-
Fertilizer*inoculum	4	29	0.004	0.88	0.86	-
Date	4	237	<0.0001	-[x]	-	-
Fertilizer*date	4	237	<0.0001	-	-	-
Inoculum*date	16	237	0.78	-	-	-
Fertilizer*inoculum*date	16	237	0.75	-	-	-

[z]Numerator or denominator degrees of freedom

[y]Determined two weeks prior to the end of the experiment.

[x]Denominator degrees of freedom = 8.

[w]Not determined. ERM colonization assessed at termination of the experiment.

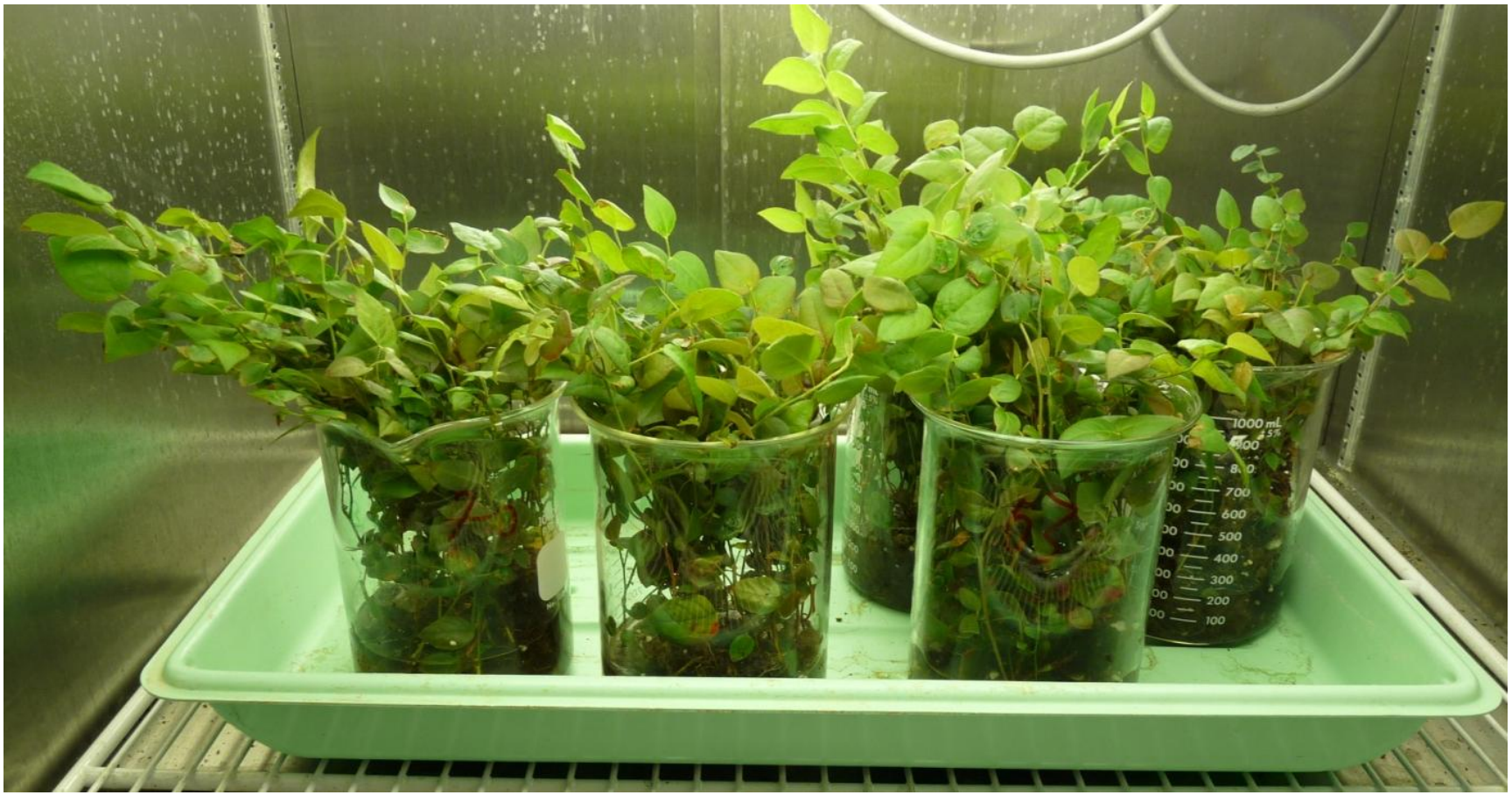


Figure D.1. Rooted cuttings of *Vaccinium corymbosum* ‘Bluecrop’ inoculated with mycelial slurries of ericoid mycorrhizal fungi.

Figure D.2. Surface of coir substrate of container-grown blueberry plants amended with feather meal (top) and compost (bottom).

Figure D.3. Twelve-week-old rooted cuttings of *Vaccinium corymbosum* ‘Bluecrop’ grown under fluorescent lights in controlled-environment growth chamber used for an experiment.

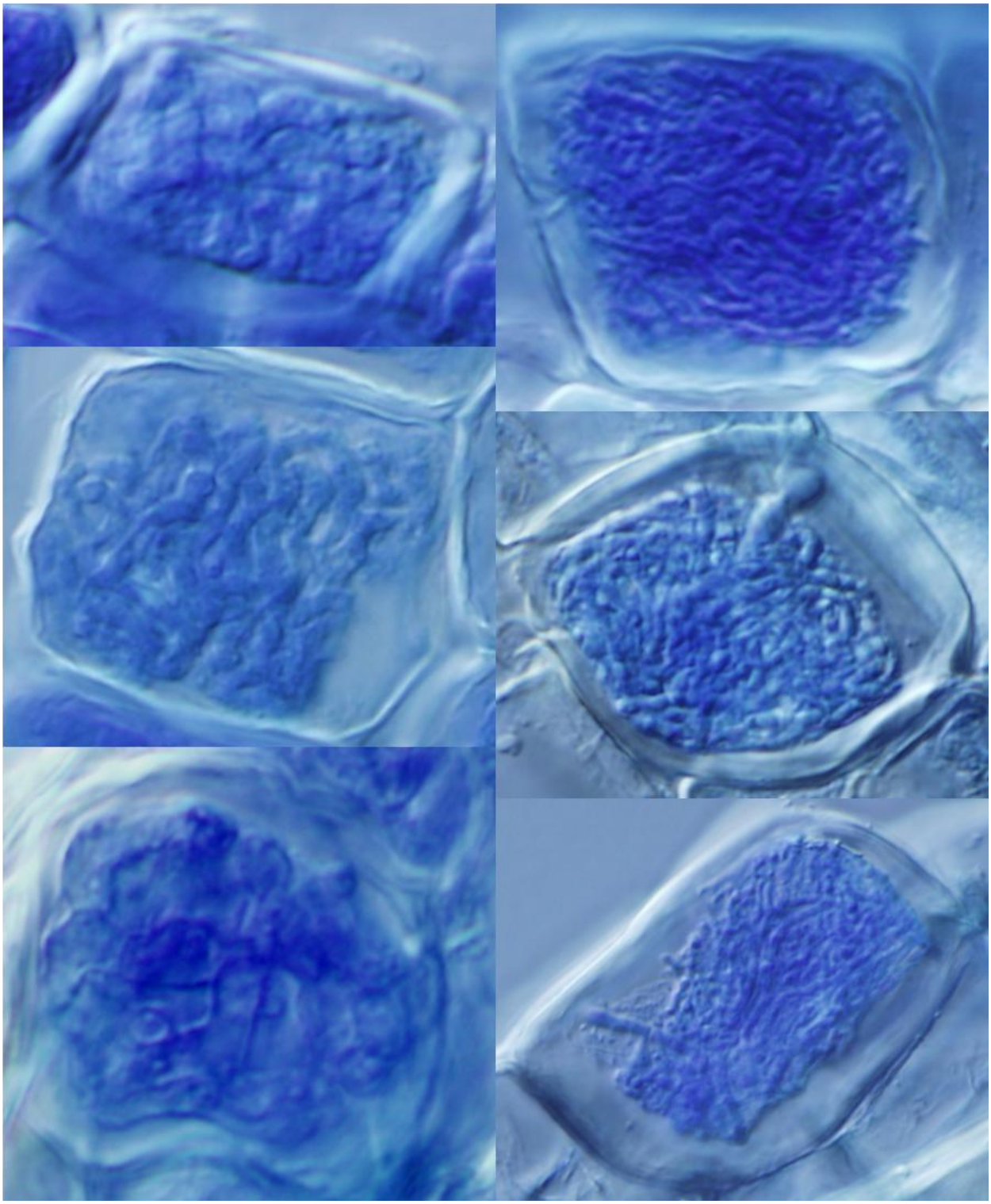


Figure D.4. Morphological variation in ericoid mycorrhizal colonization of hair root epidermal cells of 180-day-old rooted cuttings of *Vaccinium corymbosum* ‘Bluecrop’ grown in a coir substrate amended with dairy compost as observed by differential interference contrast (DIC) microscopy at 960X magnification.

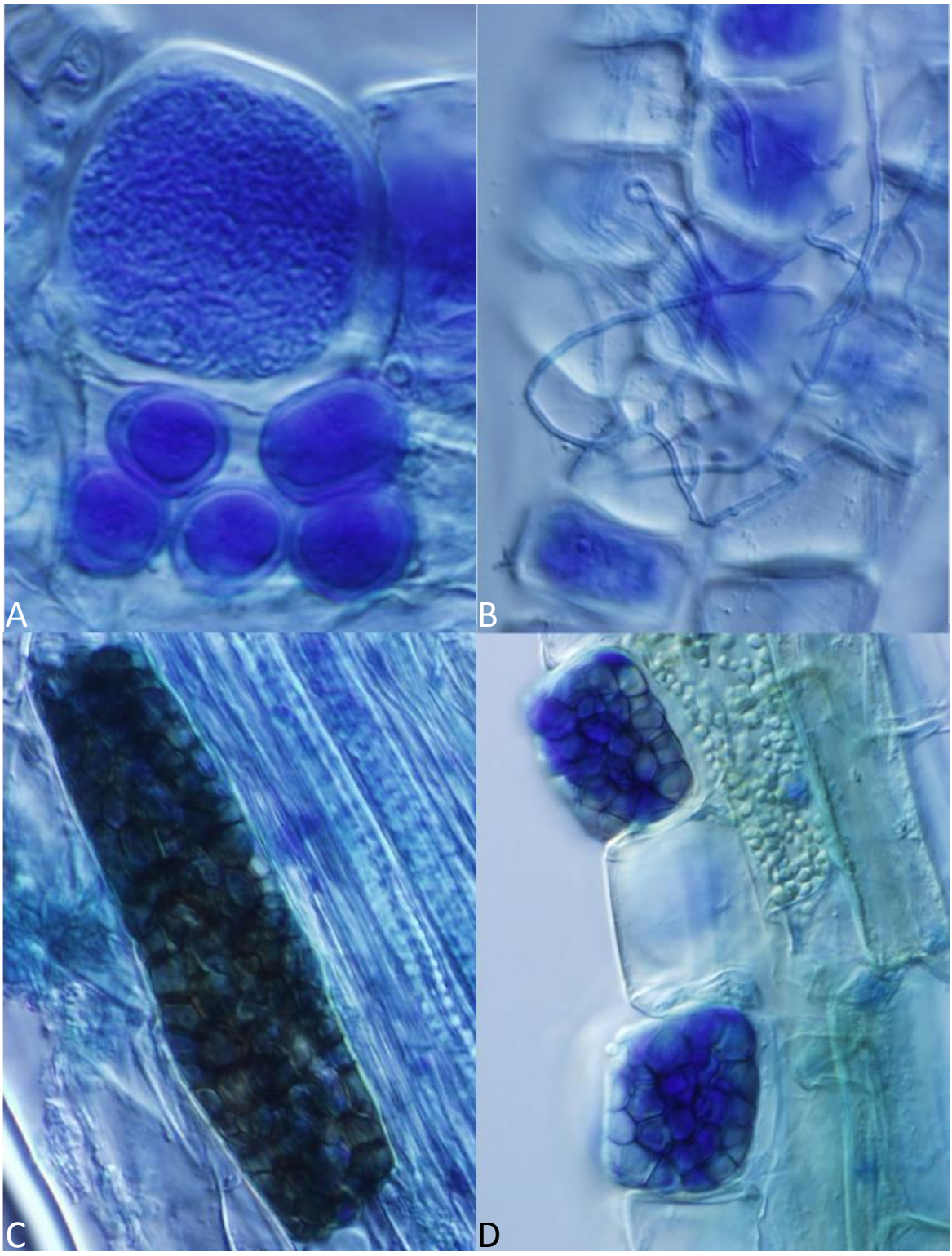


Figure D.5. Fungal colonization of hair roots cells of rooted cuttings of *Vaccinium corymbosum* 'Bluecrop' plants grown in coir and provided with compost or feather meal as a nitrogen source as observed by DIC microscopy at 960X magnification. A) Ericoid mycorrhiza adjacent to blue-staining spore-like structures. B) Surface hyphae on mycorrhizal root. C) and D) Dark septate endophyte microsclerotia in root cells.

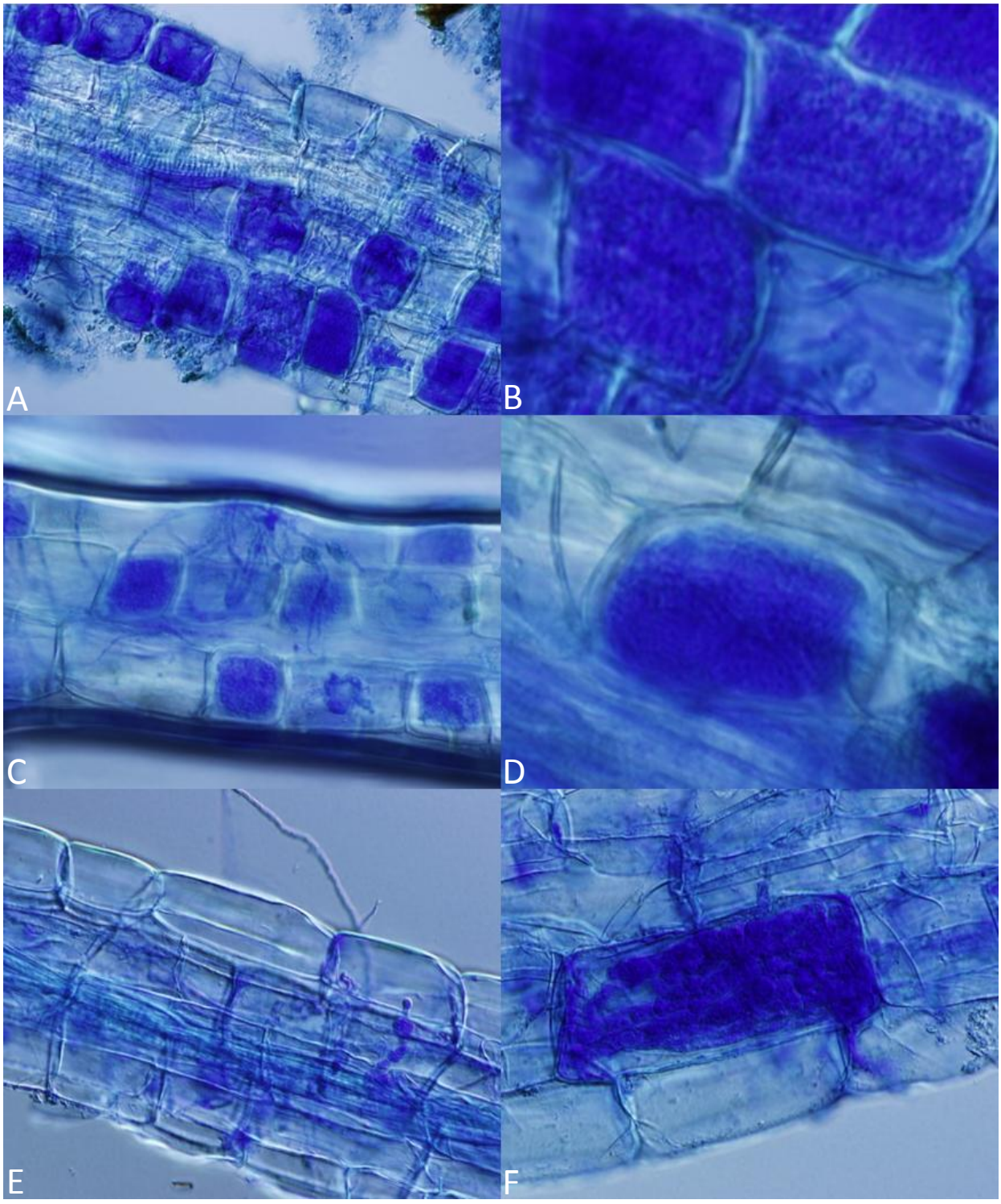


Figure D.6. Squash-mounts of blueberry hair root epidermal cells 50 days after inoculation with ericoid mycorrhizal fungi as observed by DIC microscopy at 600 to 960X magnification. A, B) Ericoid mycorrhizae in roots inoculated with *Oidiodendron maius* (UAMH 9263). C, D) Ericoid mycorrhizae in roots inoculated with *Rhizoscyphus ericae* (UAMH 9270). Surface hyphae (E) and intracellular hyphae (F) in roots inoculated with a root-associated fungus of the Ericaceae (UAMH 9264).

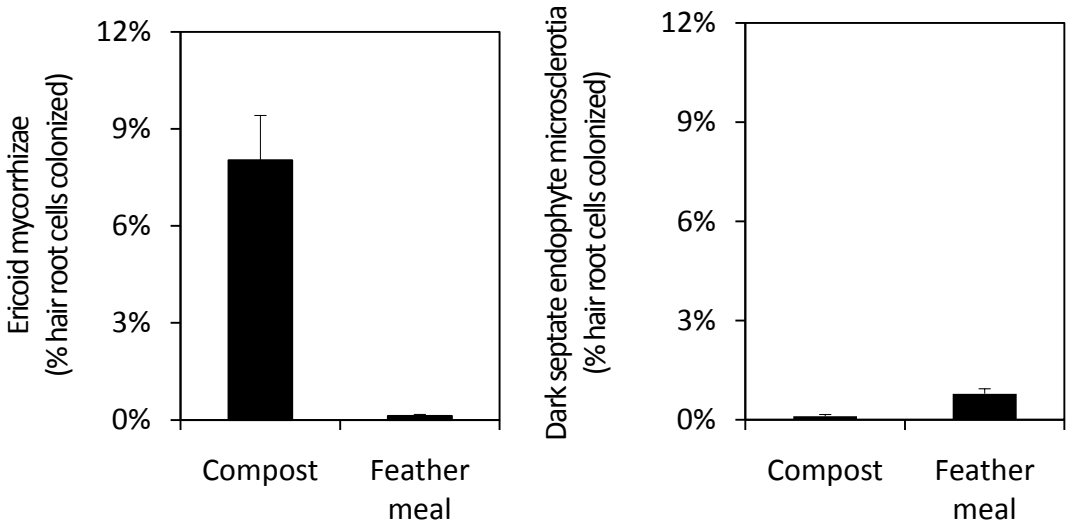


Figure D.7. Ericoid mycorrhizae and dark septate endophyte colonization of container-grown rooted cuttings of *Vaccinium corymbosum* 'Bluecrop' grown in coir and fertilized with compost and feather meal 180 days after fertilizers were applied. Error bars represent one standard error of the mean.

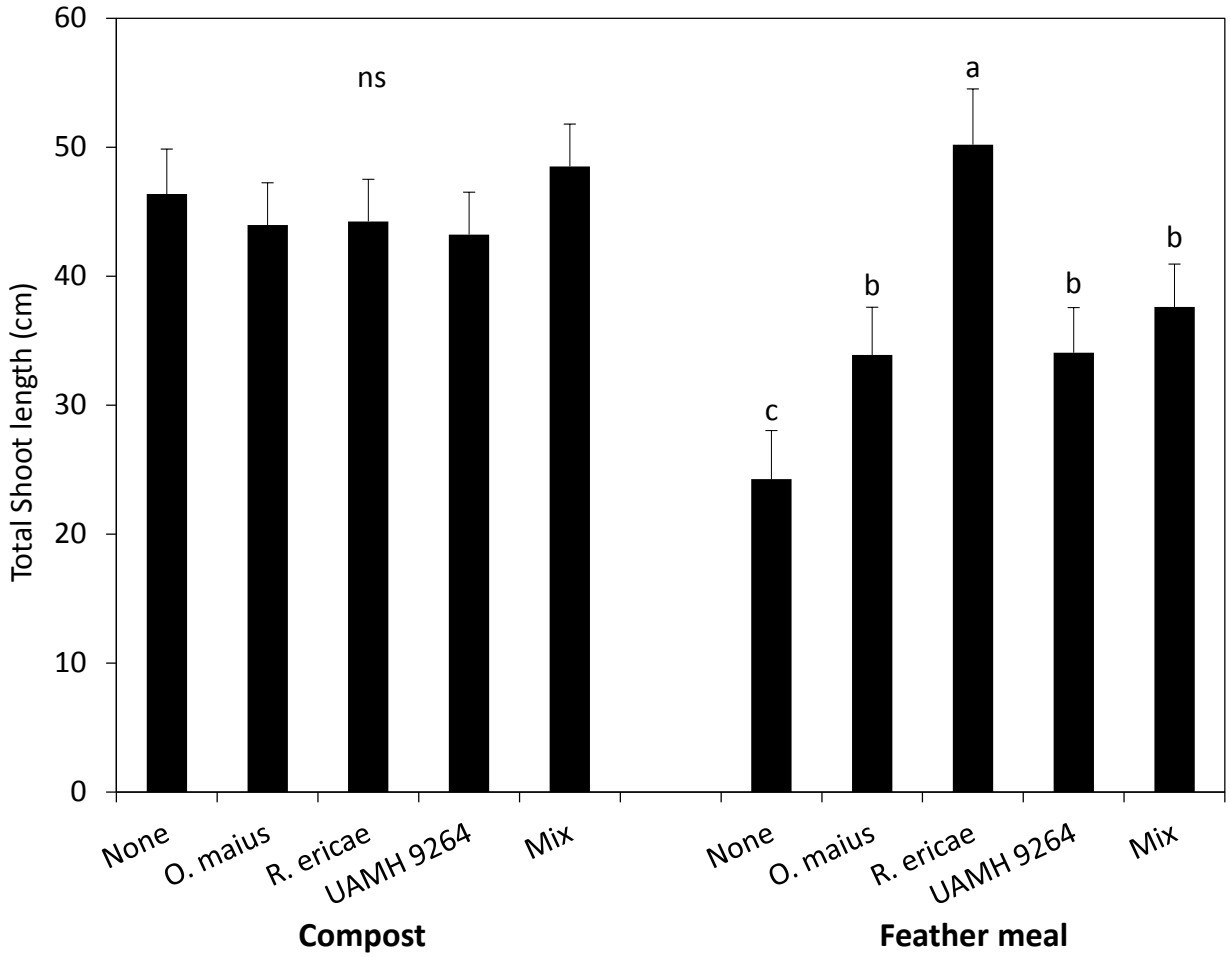


Figure D.8. The effect of inoculation with mycorrhizal fungi *Oidiodendron maius* [University of Alberta Microfungus Collection and Herbarium (UAMH) 9263], *Rhizoscyphus ericae* (UAMH 9270), an unidentified root-associated fungus (UAMH 9264), or a mixture of all three fungi (*O. maius*, *R. ericae*, and UAMH 9264) on total shoot length per plant of container-grown rooted cuttings of *Vaccinium corymbosum* 'Bluecrop' grown in coir and fertilized with compost or feather meal. Data were averaged across measurement dates. Error bars represent one standard error of the mean of eight replicates per fertilizer × inoculum treatment combination. Same letters above bars denote no significant difference within each fertilizer treatment at $P<0.05$; "ns" indicates no significant difference.

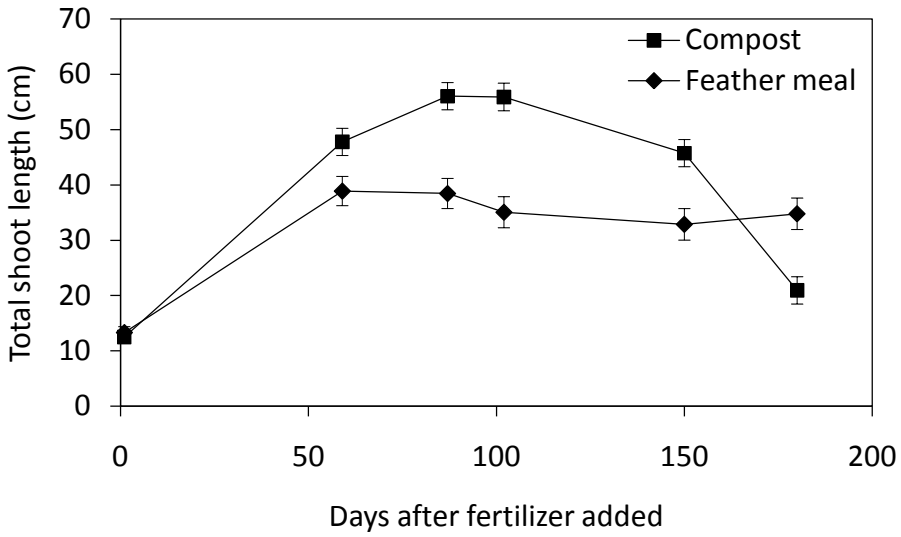


Figure D.9. Total shoot length of container-grown rooted cuttings of *V. corymbosum* 'Bluecrop' grown in coir and fertilized with compost or feather meal over 180 days. Shoot length was significantly different between compost and feather meal treatments ($P<0.05$) on each measurement date except on the first measurement date. Error bars represent one standard error of the mean (n=40).

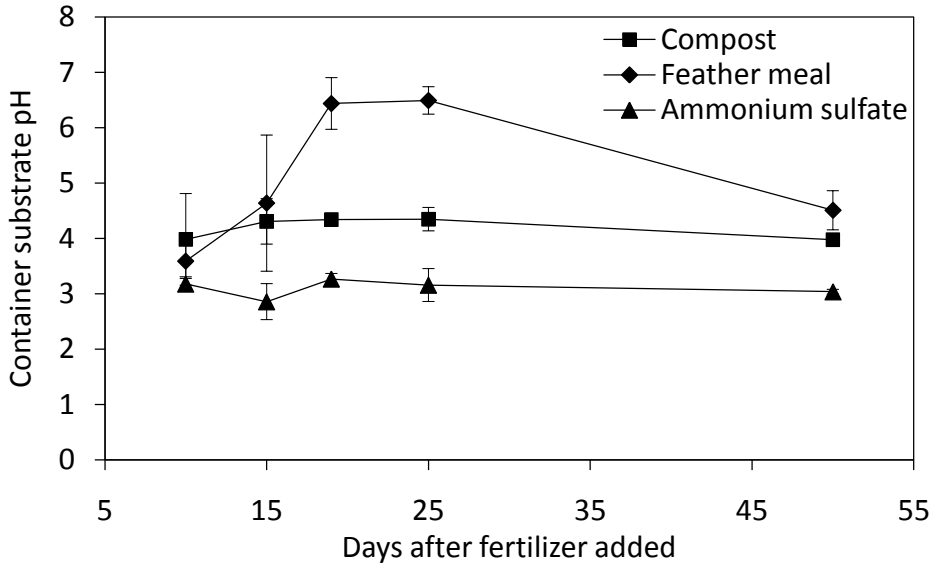


Figure D.10. pH of leachate from container-grown rooted cuttings of *V. corymbosum* 'Bluecrop' plants grown in coir and fertilized with compost, feather meal, or ammonium sulfate as sources of nitrogen fertilizer in the first 50 days after fertilizers were added. Error bars represent one standard error of the mean (n=2).

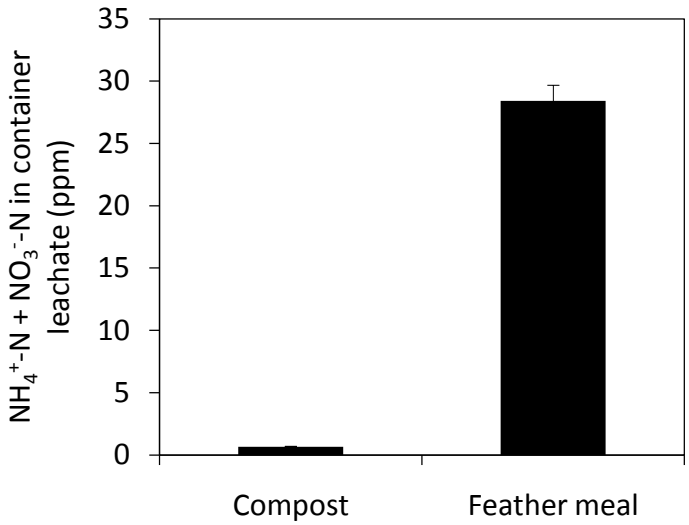


Figure D.11. Ammonium- and nitrate-nitrogen in leachate collected from the container substrate of blueberry ‘Bluecrop’ plants grown in coir 165 days after compost and feather meal were applied. Error bars represent one standard error of the mean.

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