ORGANIC AND INORGANIC FERTILIZATION WITH AND WITHOUT MICROBIAL INOCULANTS IN PEAT-BASED SUBSTRATE AND HYDROPONIC CROP PRODUCTION

by

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Abstract

Liquid organic fertilizers and microbial inoculants of beneficial microorganisms are garnering interest from commercial greenhouse growers who seek to produce crops more sustainably, but research about their efficacy is limited and results are conflicting. This research focused on comparing the effect of microbial inoculant addition in two soilless crop production systems under organic versus conventional fertilization. Two experiments were conducted with impatiens (*Impatiens walleriana*) in a peat-based substrate and four experiments were conducted with butterhead lettuce (*Latuca sativa*) in nutrient film technique (NFT) hydroponics.

In the impatiens studies, nitrogen, phosphorus, and potassium were incorporated pre-plant equally across treatments using Osmocote^{TM}, or organic fertilizers Bloodmeal or Feathermeal. An inorganic constant liquid feed (CLF) was also evaluated. Microbial inoculants that contained a variety of beneficial species, including *Bacillus* spp. and *Trichoderma* spp. were drench-applied at the beginning of the cropping cycle. Impatiens growth was comparable between the nutrient regimens in one of the studies. CO_2 respiration was measured on substrate samples. At a 5X application rate, inoculants contributed to subtle increases in plant growth in organic treatments, but microbial activity was unaffected as measured by CO_2 respiration. However, organic nutrient sources contributed to higher CO_2 respiration at day 7 of the production cycle compared to inorganic nutrient sources.

The hydroponic trials consisted of inorganic and organic nutrient regimens, evaluated with and without microbial inoculant addition. Nutrient analyses and CO_2 respiration of the nutrient solutions were collected. Use of inoculants resulted in increased plant growth when used in organic nutrient regimens in some trials. Plant dry weight and CO_2 respiration in the inorganic nutrient regimens were increased in certain instances with inoculant addition. No differences in mycorrhizal root colonization were observed in either nutrient regimen with mycorrhizal inoculant addition. Petiole NO_3-N concentration of lettuce plants grown with inorganic nutrient sources was greater than that of plants in organic regimens.

Organic fertilizers and inoculant products resulted in comparable or positive impacts on plant growth and food crop quality in some treatment scenarios in these studies. The specific circumstances of crop production systems dictate whether plant growth response may occur from inoculant incorporation.

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Chapter 1 - **Literature Review**

Horticultural production firms have a diverse set of options when considering nutrient sources for crop production. A producer's decision begins with a fundamental choice between organic, inorganic or a combination organic and inorganic nutrient management program. Increasing consumer interest in sustainable and organic crop production has resulted in greater demand for organic nutrient sources that are approved by the Organic Materials Review Institute (OMRI). The number of fertilizer products containing organic nutrient sources is increasing. Simultaneously, producer knowledge about how to manage nutrition when these nutrient sources are used is limited. Previous research has compared plant growth from inorganic and organic nutrient sources; and plant growth results varied depending on many factors.

As greenhouse producers have focused on sustainable crop production, a common management decision is the inclusion of microbial inoculants or bio-fungicides in their production regimes. There are two main categories of microbial inoculant products; 1) beneficial bacteria, and 2) *Trichoderma* and/or mycorrhizal fungi. Research results are conflicting regarding the efficacy and observed benefits from inoculant use in both hydroponic and soilless container production systems.

This literature review discusses the comparison of inorganic and organic fertilizers, with an emphasis on beneficial microbial inoculant addition, in both peat-based and hydroponic crop production.

Soilless Container Plant Production

As a common horticultural substrate, sphagnum peat has been a focus of research comparing inorganic and organic fertilizers. Multiple forms of organic nutrient sources have been evaluated with respect to plant growth in soilless substrates. Production firms have an increasing number of organic components that are available for purchase. Processed, dry substrate amendments that are derived from animal by-products such as manure, blood, feather and bone meal are commonly used as organic plant nutrient amendments. Liquid-based products of organic nutrient sources such as fish by-products, sugar beet molasses, or worm castings tea are OMRI listed for certified organic crop production.

Solid Organic Nutrient Sources Used in Container Production

Plant nutrient management systems utilizing solid organic and inorganic nutrient sources have been explored, including pre-plant additions of dry fertilizer components. Kraus and Warren (2000) evaluated turkey litter as a substrate amendment for nursery container production. The litter was described as a viable phosphorous (P) and micronutrient source, while supplemental nitrogen (N) additions were required to produce comparable growth as with the conventional nutrient control. Gaskell (2006) reported the inherent variability in nutrient concentrations from organic sources. Alfalfa meal with relatively low nutrient content (4%N - ≤1%P- ≤1%K) when compared with fish meal (11%N-1.3%P-1%K) would not provide consistent growth responses in the same growing environment. Mikkelsen (2007) reported that many of these common organic nutrient sources from animal by-products are low in available potassium (K) and described multiple sources of K (e.g. langbenite, potassium magnesium sulfate or seaweed) that are approved for organic production.

Liquid Organic Nutrient Sources Used in Container Production

Gaskell (2006) described the nature of many forms of liquid-based organic fertilizers and that with improvements to organic fertilizer processing technology; many farmers have reported acceptable yield and plant quality when using these nutrient sources. Williams et al. (2009) compared various liquid organic fertilizers and 20-20-20 to produce poinsettias and found similar plant growth at rates of 100 to 200 $mg^.L^{-1}$ N. Nelson et al. (2010) used a soybean-based liquid organic fertilizer versus conventional soluble inorganic regimens to produce comparable plant growth in petunia at a rate of 98 $mg^.L^{-1}$ N.

Organic and Inorganic Plant Growth Trials

Many studies have been conducted comparing organic to industry-standard inorganic fertilizer regimens. Some studies support grower experiences indicating that with proper management, organic fertilizers can be used to produce high quality crops during greenhouse production. However, there are also many studies reporting reduced plant quality with organic fertilizer use (e.g. Peet et al., 2004).

A reduction in plant quality when using organic nutrient sources may be due to multiple factors. Among these factors is the differing rate of nutrient mineralization from different organic sources. This difference contributes to the unpredictability of organic fertilizer performance.

Hartz et al. (2010) has explored nutrient release from three soluble organic fertilizers in an incubation study and greenhouse bioassay with turf in which he found that nitrification was rapid, with > 90% of mineral nitrogen in nitrate form after only 1-week of incubation at 25°C. Dry organic fertilizers such as fish waste, guano, and feather meal all have nitrogen content >10% dry weight and relatively rapid nitrogen mineralization in agricultural soils; studies show that 60 to 80% of the N is available within 4 to 8 weeks (e.g. Hartz and Johnstone, 2006). Gaskell (2006) described the same variability in nitrogen mineralization from liquid-based organic fertilizers and reported different mineralization rates at different substrate temperatures when using the same organic nutrient source.

Rippy et al. (2004) identified a common challenge reported when using liquid-based organic fertilizers in drip irrigation systems. A reduction in plant growth was attributed to clogged irrigation emitters when using organic fertilizers. Many drip irrigation systems were designed for use with thoroughly dissolved inorganic nutrients. Liquid organic nutrient sources will support growth of a variety of organisms, such as slime molds, that easily clog emitters. Inline irrigation filters would address this challenge, but they also represent an additional input cost of liquid-based organic production using micro-irrigation.

With the observed variability in organic nutrient mineralization rates, and conflicting evidence on the efficacy of organic fertilizers when compared to inorganic nutrient sources, the need for further investigation of organic fertilizers in horticultural production systems is evident.

Microbial Inoculants in Soilless Substrates

A common substrate component used in soilless production systems for greenhouse-produced crops is Canadian sphagnum peat moss. To thoroughly consider the impacts of an application of microbial inoculants to peat-based substrates, an understanding of the inherent microbial populations that can be attributed to commercially available sphagnum peat is required. In general, peat is considered to be less biologically active than field soils. Schmilewski and Carlile (2010) report that substantial microbial populations are not present in sphagnum peat due to the high lignin content and acidic tendencies inherent to the material. With the addition of fertilizer amendments such as dolomitic limestone and plant root-substrate interactions, sphagnum peat has the potential to sustain diverse microbial populations. A well-aerated, properly amended peat-based substrate contains adequate quantities of carbon for microbial

population establishment. With the addition of a carbon source (fertilizers/inoculant products), a C:N ratio that is adequate for microbial mineralization (C:N ≤ 20:1) of organic nitrogen is present in peat based production systems.

Categories of Microbial Inoculants

While a large diversity among microbial inoculant products is evident, some similarities between products can be identified. Inoculants are typically labeled as: general beneficial, bacterial or mycorrhizal, or as a bio-fungicide. The labels of bacterial and mycorrizhal inoculant products typically report specific species that are included in the product, while the 'general beneficial' labels may only list one or two microbial species under the pretense of proprietary constituents. The vagaries in content reporting by certain manufacturers of microbial inoculant products can prove challenging when trying to correlate observed improvements to plant growth with establishment of specific beneficial microbial populations.

Powder or liquid-based inoculants typically contain the spores or propagules of beneficial microbes and a sugar source (e.g. glucose, molasses) that could serve as a short-term food supply for microbial growth once the inoculant is applied to the substrate. A final common factor amongst inoculant products is a low nutrient concentration analysis of the inoculant products. While some inoculants report trace amounts of N-P-K, the typical microbial inoculant does not provide significant supplemental nutrition in the form of plant nutrients.

Beyond these common factors lie many differences in microbial inoculant products. Certain products also contain humic and fulvic acids that have been reported to positively affect plant growth (Arancon et al., 2006). Organic constituents such as kelp meal, compost extracts, and other parent materials can be present in various microbial inoculant products. Undoubtedly, properties inherent to specific inoculant products may have bio-stimulative effects on plant growth that extend beyond merely applying beneficial microbial species (Pillay and Nowak, 1997).

The benefits of introducing or encouraging microbial activity on plant health have been widely explored. Many studies show increases in plant development, nutrient uptake and disease suppression with the addition of beneficial microflora (e.g. on tomato, Larkin and Fravel, 2002). Vermicomposts have been used to inoculate lateritic soils, thereby improving nutrient content and enzymatic activity (Pramanik et al., 2010). Research by Kaya et al. (2007) has compared

plant growth results from organic nutrition, but the authors cited the need to investigate the role of microbial activity in the root medium as it pertains to nutrient mineralization.

These studies and others contribute to the horticultural production industry moving away from the 'sterile media' concept of plant nutrient management and towards the encouraging beneficial microbial populations in root media that may contribute to nutrient cycling and disease suppression.

Bacterial Inoculants

Supporting research has been completed in many similar production scenarios that can offer insight into potential interactions between a peat-based substrate and a microbial inoculant addition. Elad et al. (1987) reported increased growth response of tomato with the antagonism of soil-borne pathogens by rhizobacteria. Many species of bacteria, including *Bacillus* spp. and *Enterobacter* spp*.,* have been described as beneficial for plant growth in field soil and laboratory applications. If bacterial inoculant products contain viable propagules/spores of these known beneficial bacterial species, then the opportunity to incur the reported benefits for soilless crop production exists.

Fungal and Mycorrhizal Inoculants

A common fungal component of beneficial inoculants and bio-fungicide products are various strains of *Trichoderma harzianum*. *Trichoderma* spp. have been reported to aid plant growth via increased nutrient cycling and suppression of root pathogens via resource competition and direct predation (Windham et al., 1985). Bio-fungicides are becoming more common in commercial production with soilless substrates. Products such as RootShield Plus™ (Bioworks Inc., Victor, NY) are OMRI-listed for organic crop production. Applied as a preventative fungicide treatment, root disease pathogens are managed by applying *Trichoderma harzianum* strain T-22 and *Trichoderma virens* strain G-41. These organisms have been shown to suppress root pathogen activity via direct resource competition, pathogen antagonism/parasitism, and by stimulating plant growth via increased nutrient cycling. Bio-fungicides demonstrate effectiveness as a preventative measure with respect to root pathogens and are being marketed as offering 'general improvements' to plant growth. Some commercial processed substrate product lines include a pre-purchase incorporation of bio-fungicides (e.g. ProMix™).

Beyond the volume of general information about benefits to plant growth with the presence of beneficial microbial populations, Gravel et al. (2009) reported that rhizobacteria may interact with mycorrhizal fungi to increase root colonization and nutrient content of plant tissue. This research suggests potentially beneficial interactions when using a compliment of bacterial and mycorrhizal inoculant products.

Microbial Inoculants in Plant Production

A recently published study showed inconsistent improvements to some plant growth responses with certain inoculant products in soil-based greenhouse and field conditions (Russo and Fish, 2012). The authors asserted that growth improvements from inoculant use were inconsistent between trials and applications, and ultimately claimed "no particular benefit" from some of the inoculant products (Mpact^{TM}, Bio-S.I.^{TM}, PMSLA EO-12^{TM}, Compost tea, etc.) that were tested. This study outlines the challenges faced by horticultural producers: of the eight inoculants tested, some products showed no measurable impact on plant growth, and some inoculants contributed to subtle improvements in certain production scenarios.

The practice of applying microbial inoculants to a peat-based substrate, using either organic or inorganic nutrient sources while monitoring changes in multiple substrate conditions and plant growth response, is under-represented in current scientific publication. Further studies are required to address some of the complexities involved in a beneficial microbial inoculants effect in a peat-based production system. To investigate the conflicting results regarding fertilizer and inoculant performance, plant growth, nutrient release rates and general microbial activity of the peat-based substrate must be simultaneously observed and analyzed. Additionally, microbial population analysis of the inoculant products must accompany the research to verify microbial species content and viability. Considering the all of these results in tandem may aid in correlating observed changes in the measured response variables when viable beneficial microbial species are applied.

Hydroponic Crop Production

Hydroponics is a commonly used crop production practice around the world. As is the case with all plant production systems, the choice of nutrient sources for plant growth is complex. Regardless of the specific hydroponic system, the use of organic versus inorganic nutrient regimens dramatically influences system management. In addition, an increasing number

of supplemental additives that advertise increases in plant growth are available. As is the case in container production, plant growth supplements known as beneficial microbial inoculants have become an increasing share of the 'growth supplement' product market. Certain facets of nutrient management and microbial inoculants have been studied in hydroponic production systems.

Nutrient Regimens in Hydroponic Systems

Inorganic Nutrient Regimens

The majority of hydroponic crop production systems have been developed utilizing inorganic, salt-based nutrient regimens. Multiple studies have been completed that describe the optimum rate of inorganic nutrient applications for a multitude of ornamental and food crops using 'nutrient film technique' (NFT) hydroponic systems (Premuzic et al., 1998). The recommended nutrient rates are determined based on the premise of maximizing plant growth as opposed to simply resulting in healthy plant growth.

Organic Nutrient Regimens

With an increasing effort to incorporate sustainable practices into production systems, organic fertilizer regimens are being used more frequently by greenhouse vegetable producers. The incorporation of organic nutrients into hydroponic systems has not been without significant challenges. Research initially reported that organic fertilizers were not suitable for hydroponics as a result excess phytotoxins present in organic nutrient sources (Garland et al., 1993). Subsequent research outlined processing methods with organic fertilizers to increase nitrate concentrations, resulting in an organic fertilizer source that could be used in a hydroponics system. With a time frame of 50 days, researchers showed that predominately ammonium-based organic fertilizers could undergo microbial conversion, resulting in plant available nitrate from organic hydroponic solutions (Shinohara et al., 2011). These studies provide insight regarding the mineralization processes occurring in hydroponic solutions and demonstrate that adequate plant growth can be achieved using organic nutrient sources in a hydroponic system.

Private manufacturers have developed processing methods that allow for organic fertilizers to be mixed directly to hydroponic reservoirs. While these processed, commercially-available organic mixes offer negligible amounts of nitrate, marketable plant growth can be attained when using a full complement organic fertilizer regimen. Despite these improvements of

a more "user friendly" form of organic hydroponic nutrients, consistent production challenges remain.

These challenges include extreme pH fluctuation, inconsistent rates of nutrient mineralization and very high electrical conductivity of the nutrient solution from non-nutrient fertilizer constituents. Perhaps the greatest challenge to overcome when adopting organic fertilizers is maximizing plant growth such that it is comparable to growth resulting from using inorganic fertilizer regimens (Garland et al., 1997).

Despite the challenges reported with organic fertilizer use in hydroponic systems, guiding principles of hydroponic production should be environmental sustainability and maximizing benefit to human health as a result of proper nutrient management decisions. The ill effects of excess nitrate application to food crops, with regards to a sustainable nutrient management regimen and the potential human health hazard from excess nitrate levels in consumed plant tissue, further motivates the transition from inorganic, high nitrate nutrient regimes (Gent, 2003).

Although research has been completed on various aspects of organic and inorganic nutrient sources, further consideration of nutrient mineralization rates and efforts to increase plant growth response is required to address the challenges of organic fertilizer adoption.

Microbial Inoculants in Hydroponic Systems

Unique studies have been completed using NFT hydroponics with regard to microbial inoculants. Conflicting plant growth studies have been published, reporting the benefit or non-benefit of microbial inoculant addition. Measuring and analyzing differences in plant growth, reservoir nutrient concentrations, and general microbial activity as a result of microbial inoculant application to a constantly recirculating hydroponic system could potentially benefit crop production firms.

Bacterial Inoculants

Studies of bacterial inoculant use in hydroponic systems have shown increased plant disease resistance with product use (Rankin and Paulitz, 1994). These researchers reported improvements to plant growth as a result of both bio-stimulation and increases in nutrient processing. With a relatively brief establishment period, bacterial populations have the potential to establish and impact growth in both short and extended crop production cycles. Cirou et al. (2011) established protocols for bio-stimulation of beneficial bacteria in hydroponic potato roots,

citing methods to boost beneficial bacterial populations in a hydroponic rhizosphere. While many studies have been completed observing bacterial interactions with plant roots, few studies have evaluated the performance of commercially available microbial inoculant products in hydroponic lettuce production.

Mycorrhizal Inoculants

Some unique applications of mycorrhizal inoculants in NFT hydroponic systems for plant and inoculum production have been reported. NFT systems were used to produce roots as an inoculum source for red clover fields (Elmes et al., 1983). Plant roots were inoculated and grown in NFT troughs for 22 weeks. Roots harvested from the NFT troughs were shown to be viable mycorrhizal inoculum sources. Other studies have shown that modification to a standard NFT system provides a more stable environment for mycorrizhal establishment. Lee and George (2005b) reported that installing a glass bead matt provided a physical matrix for mycorrhizal establishment and proposed the necessity of a periodic root-dry down period to encourage myco populations.

Cordiki et al. (2005) evaluated the efficacy of commercially available mycorrhizal inoculants on sweet gum plant growth in nursery container production. This study cited improvements to plant growth responses when mycorrhizal inoculants were applied. However, different inoculant products improved different aspects of plant growth. The authors recommended further research to quantify specific inoculant product results in different production systems.

Similar to other inoculant product research, studies have been published citing no improvement to plant growth with mycorrhizal applications. Cwala et al. (2010) reported no improvements to plant growth in hydroponic tomato production, citing abundant nutrient availability as the likely cause of inoculant ineffectiveness.

A consistent theme of previously completed studies indicates a minimum time required for mycorhizzal population establishment. Studies cite a minimum of 8 to 10 weeks of plant growth after exposure to an inoculum source for viable mycorhizzal populations to be observed in the root system (e.g. Lee and George, 2005a). Crop production cycles such as hydroponic tomato production that exceed this time frame could potentially benefit from mycorrhizal inoculant addition. Conversely, these findings point to a potential shortcoming for mycorrhizal

inoculant use in some common vegetable production systems. Many crops like lettuce (Latuca spp.), basil (Ocimium spp.), and thyme (Thymus spp.) have production cycles that are typically 5 to 6 weeks in duration. Currently, there is little evidence that mycorhizzal populations can establish populations rapidly enough to offer potential benefit for short-term cropping cycles.

Ubiquity of Beneficial Microbial Organisms

Microbial inoculant products have been shown to contain spores or propagules of beneficial bacteria such as *Bacillus* spp. or *Enterobacter* spp. While applying these organisms provides the opportunity to establish populations of beneficial bacteria, many of these species are ubiquitous in nature. *Bacillus* spp. are common as a soil borne organism and in the digestive systems and excrement of mammals. A single *B. subtilis* endospore can remain viable for decades and is resistant to drought and solar radiation (Straiger and Losik, 1996).

Eighty percent of plant species form a symbiotic relationship with mycorrhiza (Wang, 2006). Fossilized root tissue dated at 400 million years old show the presence of mycorrhiza. Mycorrhizal inoculant products may contain both endo and/or ecto mycorrhizal propagules.

The majority of both bacterial and mycorrhizal organisms sold as inoculant products are ubiquitous in nature; they have adapted to colonize diverse environments. This reality may contribute to reduced efficacy of microbial inoculant products.

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Chapter 2 - **Evaluating Impatiens (*Impatiens walleriana*) Production in a Peat-based Substrate with Inorganic or Organic Fertilization and Microbial Inoculants**

Introduction

As more greenhouse producers turn to organic fertilizers as a component of sustainable production practices, information is needed to aid in managing nutrition when organic nutrient sources are used. Multiple studies have compared organic to industry-standard inorganic fertilizer regimens with varying results.

Increasingly, beneficial microbial inoculant products are being marketed as plant growth stimulators. Dozens of microbial inoculant products are available in the marketplace and are advertised to 'boost beneficial microbial populations.' Published research has described the results of microbial inoculant addition to various crop production systems; conflicting conclusions regarding inoculant product efficacy have been reported (Russo and Fish, 2012; Cwala et al., 2010).

Organic fertilizer effect and microbial inoculant product applications have been studied separately in many different production systems. Multiple studies have reported that organic fertilizers can be used to produce high quality crops during greenhouse production. For example, Nelson et al. (2010) compared a soybean-based liquid organic fertilizer versus conventional inorganic fertilizer to produce petunia (*Petunia* spp.) and cyclamen (*Cyclamen graecum)* and measured comparable plant growth at a medium rate of 98 mg$^.$L^{-1} N. Experimenting with pre-plant incorporation of turkey litter, Kraus and Warren (2000) described the effectiveness of a slow release, organic fertilizer in containerized nursery production. The litter was determined to be a viable phosphorous (P) and micronutrient source, but supplemental nitrogen (N) additions were required to produce comparable growth to the conventional nutrient control. Both of these studies indicate that with proper management, organic fertilizers can produce marketable plants. Both studies also described application rates of organic fertilizer application that proved to be detrimental to plant growth compared to an inorganic fertilizer control. Peet et al. (2004) reported decreased tomato (*Solanum lycopersicum)* growth when organic fertilizers were used in

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soilless substrates. This study also cited the difficulty in predicting substrate pH when using organic fertilizers with container-grown plants.

A reduction in plant quality when using organic nutrient sources may be due to multiple factors. One potential factor is the differing rate of nutrient mineralization from organic sources that contributes to the unpredictability of organic fertilizer performance. Dry organic fertilizers such as fish waste, guano, and feather meal all have relatively high nitrogen content (>10% dry weight) and relatively rapid N mineralization in agricultural soils; Hartz and Johnstone (2006) showed that 60 to 80% of the N is available within 4 to 8 weeks of application to the substrate. In contrast, a controlled release inorganic fertilizer such as Osmocote^{TM} typically demonstrates metered nutrient release of NH_4-N, NO_3-N, PO_4-P and K over the course of a production cycle.

As greenhouse producers choose to implement sustainable crop production practices, another management consideration is whether or not to include microbial inoculants or bio-fungicides as substrate amendments in their production regimes. Categories of microbial inoculants can include powder or liquid-based amendments that contain spores or propagules of beneficial bacteria such as *Bacillus subtilis* or mycorrhizal species such as *Glomus intradices* . A common fungal component of beneficial inoculants and bio-fungicide products are various strains of *Trichoderma harzianum*. *Trichoderma* spp. have been reported to aid plant growth via increased nutrient cycling and suppression of root pathogens by resource competition and direct predation (Windham et al., 1985). Some inoculant products may include processed organic constituents such as a compost tea or worm castings. In conjunction with applying microbial species, some inoculant products may also contain humic and fulvic acids, molasses, kelp meal and/or glucose. Constituents such as humic acids have been shown to positively effect plant growth responses (Arancon et al., 2006).

Russo and Fish (2012) outlined a significant challenge experienced by growers looking to incorporate an effective inoculant product. Of the eight microbial inoculant products tested on vegetable crop growth in soil-based greenhouse and field production systems, four products resulted in inconsistent increases in some plant growth responses, two resulted in no improvement over the control, and in some instances two other products were mildly detrimental to plant growth. This study described a potential limitation to inoculant application in typical field soil conditions in that a typical field soil has a microbial population and diversity that is much greater than that of any populations that could be applied via inoculant products.

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Additionally, Russo and Fish reported that bacterial populations have the opportunity to establish and affect substrate and plant quality within about four weeks of exposure to the substrate. Fungal species such as mycorrhiza or *Trichoderma* spp. require a longer period (4 to 8 weeks) to establish populations in the root zone (Corkidi et al., 2005).

Soilless horticultural production commonly uses an amended sphagnum peat or bark-based substrate and nutrient regimens that are applied to maximize plant growth. Soilless crop production systems that incorporate sphagnum peat moss are using a substrate that is described by Schmilweski and Carlile (2010) as less biologically active than soils. The high lignin content and acidity can limit the potential of sphagnum peat to support significant microbial populations without the addition of fertilizer, lime amendments and root/substrate interactions. The authors report that the low initial microbial activity of sphagnum peat, at the point of first use in the production environment, provides the opportunity for microbial population establishment, both beneficial and/or pathogenic.

Potential interactions between the two sustainable production practices of organic fertilizer use and inoculation with microbial inoculants merits further investigation in soilless substrate production systems. Reported reductions in plant growth responses and inconsistent substrate/fertilizer interactions when using various forms of organic fertilizers hinder the horticultural production industry's transition from a conventional inorganic fertilizer source to organic counterparts that are generally considered to be more sustainable. Research is needed to observe potential correlations between microbial population activity and the addition of organic fertilizers and/or microbial inoculants. Measuring changes to plant growth responses, substrate nutrient status, and substrate microbial activity with an application of beneficial microbial inoculant products in commonly used substrates like sphagnum peat may offer insights into conflicting results from previous research.

The specific objectives of these experiments were to: 1) measure general microbial activity and nutrient status of a peat-based substrate when two inorganic and two organic fertilizer treatments were used to produced impatiens; and 2) determine whether application of beneficial microbial inoculants influenced total microbial activity, substrate nutrient concentration, or impatiens growth.

Materials and Methods

Two experiments were conducted in the glass greenhouse range of the Throckmorton Plant Sciences Center, Manhattan, KS. Experiment 1 was conducted from 21 July (day 1) to 22 Sep 2011 (day 63) and experiment 2 was conducted from 22 Mar (day 1) and was completed on 24 May 2012 (day 63). Experimental designs for both experiments were randomized complete block (RCBD) with four blocks and four replications per treatment.

Experiment 1

Plants and Growing Environment

Seeds of *Impatiens walleriana* Hook. f. 'Super Elfin White XP' (Ball Seeds Chicago, IL) were sown to 288 plug trays filled with a peat-based substrate (Fafard 3B, Conrad Fafard, Inc., Agawam, MA) and lightly covered with vermiculite. Prior to sowing seeds to the plug tray, the peat and vermiculite were sterilized at 120°C for 20 minutes to eliminate microbial populations inherent to the substrate. Seeds were germinated under mist in a greenhouse with day temperature set-point of 30.5°C for 28 days.

The impatiens plugs were transplanted into 16.5 cm, 1.85 L round, azalea pots (Belden Plastics, St Paul, MN) with two seedlings per pots. The pots were transported to a 7.6 m x 7.6 m glass greenhouse room on day 1 and plugs were transplanted at to the treatment media on day 7 of the experiment. All equipment and surfaces had been previously sterilized with GreenShield (BASF, St Louis, MO) with the goal of reducing microbial contamination from surface contact.

Plants were grown under natural day lengths and under white-washed glass glazing to manage high temperatures. Temperature and relative humidity were monitored using HOBO Environmental Monitors (Onset Computer Company, Bourne, MA). Daytime (0500 to 1600 hrs) temperature of the production space averaged 29.5°C, with night temperatures (1601 to 0459 hrs) at 23.6°C and relative humidity ranged from 52% to 78%.

Minor outbreaks of thrips (*Frankliniella occidentalis)* required pesticide application. Pylon at a rate of 0.03ml per L water (BASF Co., Florham Park, NJ) was applied to the foliage once outbreaks were detected (day 16).

To ensure consistent irrigation across treatments, sentinel pots of each treatment were monitored by weight measurements to determine when the weight of the pot decreased by 25 to

35% of container capacity due to water loss. Leaching fraction was maintained between 15% and 25% by adding 650 to 800 ml irrigation solution when pots dropped within the target weight range.

Fertilizer Treatments

Experiment 1 consisted of 13 total treatments (Table 2-1). The fertilizer treatments included a commercial inorganic, controlled release fertilizer Osmocote[TM] (OSM) 14N-4.2P-11.6K (14-14-14; Everiss, Inc., Dublin, OH) and two organic fertilizers, Feather Meal (FM 12.8-0-0; 12.9N-0.6P-0.2K) and Blood Meal (BlM 13.6-0-0; 14.4N-0.6P-0.2K), both supplied by Boer Commodities, Inc. (Fresno, CA; Table 2-2).

The substrate consisted of 70 Canadian sphagnum peat (Premier Tech Horticulture Inc., Quakertown, PA) : 30 perlite (Thermo Rock East Inc., New Eagle, PA), by volume. All treatments received pre-plant amendments of Micromax[TM] (Scotts, Inc, Marysville, OH), dolomitic lime (Sun Gro Horticulture, Agawam, MA) and a surfactant (Suffusion Granular, OHP Inc., Mainland, PA). Organic fertilizer treatments also included bone meal (6.35N-14.5P-1.2K, Boer Commodities, Inc., Fresno, CA) and potassium magnesium sulfate (KMS; 0N-0P-18.3K, Diamond K Gypsum Inc., Richfield, VT) with application rates listed in Table 2-2. Organic nutrient application rates were designed to match the N-P-K application rates used in the OSM treatments. Nutrient analysis of the organic fertilizers was determined via replicate sample submission to the Agricultural Experiment Station Chemical Laboratories, Univ. Missouri-Columbia.

Inoculant Treatments

Autoclaved substrate was used in some treatments to evaluate the result of eliminating microbial populations inherent in commercially available Canadian sphagnum peat. After mixing the peat and perlite, the substrate was treated at 120°C for 15 min one day prior to mixing the treatments. Two commercial inoculant products were applied in combination with the fertilizer treatments. Sub Culture M (M) (General Hydroponics, Sebastopol, CA) was applied to the substrate as mycorrhizal fungi inoculant. Endo/ecto mycorrhizal species listed on the M product label included: *Glomus intradices, G. aggregatum*, *G.monosporum, Scleroderma citrinum,* and *S. cepa*, among others. Sub Culture M was applied at recommended label rate of 0.86 g·L^{-1} water. Sub Culture B (B; General Hydroponics, Sebastopol, CA) was applied as a beneficial bacterial

inoculant at $0.1g \cdot L^{-1}$. Species listed on the Sub Culture B label included: *Bacillus subtilis, B. lichenformis*, and *Streptomyces lydicus*. Both dry inoculant products also listed *Trichoderma harzianum* as a component. Inoculants were tested individually, as a combination of both, and as a combination of both at 5 times the recommended label rates during experiment 1.

Experiment 2

The methods used for experiment 2 were the same as in experiment 1 except as follows. In experiment 2, established seedlings of impatiens 'Tempo White' (PanAmerican Seeds Chicago, IL) were provided by a wholesale grower (Neosho Gardens, Council Grove, KS) as 288 plugs. Prior to planting, seedlings received an application of 50 $mg \cdot L^{-1}$ N fertilizer from Peter's 20N-4.4P-16.6K (Peter's 20-10-20 Peat-lite Special, JR Peters, Inc., Allentown, PA) and were maintained using mist applications of municipal water at 30 min intervals with 15 sec mist duration prior to transplanting. Plugs were planted to the treatments at day 1 of experiment 2 and were moved to the 7.6 m x 7.6 m production space under whitewashed glass glazing. Daytime (0500 to 1600 hrs) temperature of the production space averaged 26.2°C, with night temperatures (1601 to 0459 hrs) at 21.6°C and relative humidity ranged from 52% to 78%.

Experiment 2 included modifications to the fertilizer and inoculant treatments compared to Experiment 1 (Table 2-1). A treatment utilizing constant liquid fertilization (CLF) with 20N-4.4P-16.6K (JR Peters, Inc., Allentown, PA) was included. Plants receiving CLF nutrition were fertilized with 250 ml of 100 $mg \cdot L^{-1}$ N dissolved in de-ionized water at each irrigation (Table 2-2). All other treatments were irrigated with de-ionized water for the duration of the cropping cycle. The other fertilizer treatment modification involved a reduction in the rate of KMS that was incorporated into the organic nutrient regimens in experiment 2. The reduction in KMS application was based upon observation of greater than sufficient potassium concentration resulting from the rate used for experiment 1.

Experiment 2 included the inoculant product EM1™ (TeraGanix Alto, TX) as a liquid based microbial inoculum source applied at 3 $ml \cdot L^{-1}$ water. Specific organisms listed as contained in EM1 were limited to *Pseudomonas* spp., while the product label references 'dozens' of other beneficial organisms in a proprietary molasses-based liquid. Once experimental pots were placed into the production space, Sub Culture M and B were dissolved in deionized water,

EM1 was diluted in deionized water and both were applied as a drench of 250 mL inoculant solution to the top of the substrate.

Cost of inoculant products is based on pricing made available to the general public at the time of this publication. Sub Culture B and Sub Culture M were each $29.99 for 200 g quantities. The EM1 was purchased for $14.99 for 0.95 L. The input cost of the Sub Culture M/B combination was $0.04 per pot. The EM1 cost was $0.03 per pot. EM1 was the only inoculant to be OMRI^{TM} certified for organic production.

As a preemptive pest control measure, *Amblyseius cucumeris* ((Oudemans) Acarina: Phytoseiidae; (Thripex, Koppert B.V., The Netherlands) was applied as a biological control agent of thrips (*Frankliniella occidentalis)* populations on day 2 of the second experiment. Plants were not sprayed with insecticide in this study.

Growth Data

Plant growth measurements were taken at day 30 and day 63 of experiment 1 and day 63 of experiment 2. Plant height was measured from the rim of the pot to the top of the foliage canopy. Plant width was determined as the average of two measurements taken at right angles, perpendicular to each other. Once tissue was removed at the final harvest data collection, basal caliper measurements were taken from the exposed basal stem, 1.5 cm above the substrate surface, of both shoots per pot and reported as average basal caliper per pot. Whole above-ground shoot tissue was weighed and dried for 48 hours at 78°C after which dry weights were recorded. In addition to these measurements, experiment 2 also included measurements of leaf chlorophyll content (SPAD-502 meter, Minolta, Tokyo, Japan) of youngest, fully expanded leaves just prior to the tissue harvest.

Digital images from a fixed overhead perspective were taken and evaluated for floriferousness at final harvest. Ratings of floriferousness were assigned by two independent observers with 1 = none to very few flowers present and with 5 = maximum floral coverage. Rating scale used for evaluations is shown in Figure 2-1 Evaluations were averaged and evaluated for statistical differences.

Substrate Analysis

To measure changes in substrate nutrient levels as a result of the treatments, the Pour Thru^{TM} (Cavins et al., 2001) method was completed on days 7, 14, 21, 35, 49 and 63 of

experiment 1 and days 7, 14, 21, 28, 35, 42, 49, and 56 of experiment 2. The substrate was saturated to container capacity with distilled water or 100 mg·L^{-1} N nutrient solution in the CLF treatments of experiment 2. The substrate was allowed to equilibrate for 30 min, and then an additional 10 ml of distilled water was applied to the surface of the substrate to displace equilibrated root medium solution. In experiment 1, a Pour Thru^{TM} procedure was completed prior to planting the seedlings. In experiment 2, seedlings were planted 7 days prior to the first Pour Thru^{TM} analysis. The leachate was analyzed for pH and electrical conductivity (EC) using an Accumet XL20 (Thermo Fischer Scientific Inc. Pittsburg, PA). Leachates were submitted to the Soil Testing Laboratories at Kansas State University and analysis were completed for ammonium-N, nitrate-N, PO_4-P and K concentrations using an Alpkem RFA autoanalyzer with methods described by Hosomi and Sudu (1986).

To measure general microbial activity throughout the cropping cycle, substrate samples were collected and analyzed for CO_2-C evolved during an incubation period. Ten gram samples of substrate were taken from the root zone, 5 cm below the substrate surface when pots were at container capacity on days 7, 14, 21, 35, and 49 in experiment 1 and 7, 14, 21, 28, 35, 42, 49, and 56 in experiment 2. All visible root pieces present in each substrate sample were removed using sterilized tweezers to avoid incidental measurement of root respiration. Sealed substrate samples were incubated for 24 hours. The gas contained in the incubated substrate sample was analyzed for total carbon from CO_2 respiration (CO_2-C) by microbial activity using a gas chromatograph (GC-8A, Shimadzu Scientific Instruments, Columbia, MD). To measure respiration levels resulting from microbial respiration during the 24 hour incubation period, the chromatograph output was compared against a known standardized CO_2 gas mixture after subtracting the amount of ambient, atmospheric CO_2-C present in each sample.

Root proliferation late in the production cycle resulted in the omission of the last data collection for experiment 1. Root growth had become so prolific throughout the substrate that physical removal was not feasible. Root barriers constructed of laminated plastic bent to 90° angles were installed in pots at the beginning of experiment 2. The barriers reduced, but did not entirely exclude, root presence and allowed for thorough root removal at the last sample collection.

Inoculant Product Analysis

To determine the presence and viability of the species reported to be contained in the inoculum products, serial dilutions were prepared, plated and analyzed using PCR. A twenty percent dilution of Sub B and EM1 was prepared using nuclease-free water in a sterile 50 ml centrifuge tube. Forty microliters of the dilution was subsequently spread onto LB agar plate and incubated for 48 h at 30°C. Single colonies of the bacteria were visible on the LB plates after incubation for 48 h. Based on colony sizes and color single colonies were selected and streaked separately onto new LB agar plates for further purification. Single colonies from the subcultures were thereafter applied in a polymerase chain reaction (PCR) for amplification of their respective 16S genomic DNA regions. The PCR thermocycling protocols (MJ Research PTC-100 Peltier thermal cycler) were: 94°C for 2 min, followed by 31 cycles at 94°C for 40 s; 55°C for 1 min; 72°C for 10 s; then 72°C for 7 min, and finally held at 4°C. PCR reaction mixtures containing no DNA served as control. The PCR products were visualized with ethidium bromide (1 μl per 100 ml of Agarose) in 1% Agarose gel with ultraviolet light, cleaned and sequenced.

Approximately 2 grams of Sub Culture M was weighed-out and spread directly on a 9-cm-diameter Petri-plate containing one-fourth strength potato-dextrose agar (Difco Laboratories, MD) amended with tetracycline (10 mg/L) and streptomycin (10 mg/L) (designated as "1/4 PDA^{++}") (Biotech Research Grade, Fisher Scientific Inc., NJ). Two plates of the sample were prepared and maintained at 23°C in the dark to allow for fungal outgrowth. Fungal growths were visible after 24 h of incubation. Subcultures of five fungal colonies from the two plates were made onto fresh 1/4 PDA^{++} plates and later used for genomic DNA isolation.

Genomic DNA was isolated from fungal colonies isolated from Sub Culture M using a modified method of Jiangfeng et al. (2005). Isolates were grown from 5 mm-diameter potato dextrose agar-mycelial discs at 25°C for 5 days in the dark in complete media (modified from Correll et al. (1987) and containing per liter of distilled H_2O: sucrose, 30 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $NaNO_3$, 2 g; N-Z amine A (casein), 2.5 g; yeast extract (Difco), 1.0 g; 10 ml vitamin solution (contained per liter of 50% ethanol: thiamine HCl, 100 mg; riboflavin, 30 mg; pyridoxine HCl, 75 mg; D-pantothenate Ca, 200 mg; *p*-aminobenzoic acid, 5 mg; nicotinamide, 75 mg; choline Cl, 200 mg; folic acid, 5 mg; D-biotin, 5 mg; and *myo*-inositol, 4 g); 0.2 ml trace element solution. The trace element solution contained (per 95 ml of distilled H_2O) – citric acid, 5 g; $ZnSO_4 \bullet 7H_2O$, 5 g; $FeNH_4)(SO_4)_2 \bullet 6H_2O$, 1 g; $CuSO_4 \bullet 5H_2O$, 0.25 g;

MnSO$_4$•H$_2$O, 50 mg; H$_3$BO$_4$, 50 mg; and NaMoO$_4$•2H$_2$O, 50 mg. After incubation, mycelia were collected by filtration in a 16.5 cm diameter filter paper (KenAG Non Gauze milk filter) and ground into fine powder in pre-chilled mortars and pestles with liquid nitrogen.

The powder was transferred into a 1.5 ml micro-centrifuge tube and filled to the 0.5 ml mark. Next, 700 µl of 65°C 2% cetyltrimethylammonium (CTAB) solution and 7 µl of 2-mercaptoethanol were added to each tube. The tubes were vortexed briefly (2 to 3 sec) to disperse any clumps of mycelia, placed in a 65°C incubator for 10 min, briefly (2 to 3 sec) vortexed again to further homogenize the mixtures in the tubes, and returned back into the 65°C incubator for an additional 20 min. At the end of the incubation periods, 350 µl of chloroform:iso-amyl alcohol (24:1 v/v) was added to the tubes and vortexed briefly to thoroughly mix the aqueous and organic phases that formed in the tubes. The tubes were mixed gently by hand for an additional 5 minutes then centrifuged at 13,500 × g for 6 min to separate the organic and aqueous phases. 600 µl of the aqueous (upper) phase was transferred into a fresh, sterile 1.5 ml micro-centrifuge tube.

The genomic sequences collected from the PCR anaylsis of the prepared inoculant samples were compared to known sequences using the BLAST^{TM} Database. Query coverage for base pair matches was recorded. The resulting coverage from comparing colony growth from Sub Culture B when compared to known genomic sequences showed a ≤ 90% match to *Bacillus* sp. Of the five *Bacillus* species listed on the product label: *B. subtilis, B. pumilis, B. cerus, and B. licheformis* were confirmed as present and viable using PCR analysis. The EM1 inoculant product showed a 95% matching coverage for *Lactobacillus* sp. and *Enterobacter* sp.

All inoculant products used were submitted to Kansas State University Soil Testing Laboratory (Manhattan, KS) for complete nutrient profile analysis. LECO TruSpec analysis for C:N ratio and nitric perchloric digest using an ICP Spectrometer (720-ES, Varian, Ltd., Mulgrave AUS) was performed to assess total P, K, Ca, Mg, Zn, Fe, Mn and Cu concentrations. Nutrient additions from microbial inoculant products were not always negligible. Sub Culture M had significant levels of N, K and Ca (Table 2-3). Sub Culture B and EM1 contained much less supplemental nutrition than was measured in the Sub Culture M.

Statistical Procedures

Data were analyzed by sample or harvest date unless otherwise indicated using the PROC MIXED procedure of SAS ver. 9.1.3 (SAS lnstitute, Inc., Cary, NC). Pairwise comparisons of the treatment effects were made across fertilizer treatments using a Tukey-Kramer adjustment and statistical significance level $p \leq 0.05$. Orthogonal contrasts were performed on subsets of the data to measure differences between inoculated and non-inoculated treatments, and between autoclaved and regular treatments.

Results & Discussion

Plant Growth, Experiment 1

Fertilizer Treatment Effect

Differences in plant growth occurred between the main fertilizer treatments (OSM, FM, BlM). Plants were commercially salable across treatments. Effect of the fertilizer treatments will be discussed as the primary factor affecting plant growth. Changes in plant growth as a result of inoculant addition will be discussed with comparisons to non-inoculated treatments within the same fertilizer regimen.

At the mid-crop data collection of experiment 1, there were no differences observed in diameter or height between the three fertilizer treatments (Table 2-4). At the end-crop harvest, the OSM-treated plants were smaller than those fertilized with organic nutrients in experiment 1 based on dry weight and, to a lesser extent, basal caliper and plant diameter. The greatest dry weight was observed in the BlM (12.7 g) treatments followed by FM (11.9 g), with OSM resulting in the least dry weight (8.3 g). Additionally, increases in basal caliper measurements were observed when comparing BlM to OSM (Table 2-4). All other measured plant growth parameters among fertilizer treatments were not significantly different.

Significant differences in floriferousness of the impatiens plants were observed across treatments in experiment 1. The OSM treatments resulted in a greater flowering response when compared to the organic regimens (Fig. 2-2). With the greatest rating for floral coverage, the OSM treatments always rated higher in floral coverage than the FM and in all but one BlM treatments (Fig. A-1).

Inoculant Treatment Effect

Inoculant addition resulted in increased plant growth when used in conjunction with organic fertilizers in some instances (Table 2-4 and 2-5). At the mid-harvest, a significant increase in plant height resulted in the BlM + M/B 5X treatments when compared to the standard BlM treatment. Only one instance of significant changes to plant growth was observed with the addition of microbial inoculants to the organic fertilizer treatments in experiment 1 at end-harvest. The greatest dry weight was recorded in FM + M/B 5X treatment and was significantly greater than all other FM treatments. Differences in plant growth were not significant as a result of microbial inoculant addition in the Osmocote^{TM} treatments at the end- harvest (Table 2-4). A reduction in plant diameter was observed as a result of autoclaving in the AC OSM treatment when compared to the standard OSM or OSM + M/B treatments (Table 2-4). The autoclaved treatments resulted in decreased plant growth when compared to non-autoclaved treatments (orthogonal contrast p=0.03).

Substrate Nutrient Analyses, Experiment 1

Substrate pH and Electrical Conductivity (EC)

Substrate pH was influenced by fertilizer treatment in experiment 1 (e.g. App. A-4). The target pH range of 5.5 to 6.5 was the goal of the dolomitic lime application rate. BlM resulted in the highest pH, with FM initially measured at 6.2 and OSM at 5.5 in experiment 1 (Fig. 2-3). Increased substrate pH occurred as a result of the autoclaving, as shown in FM (Fig. 2-4). The substrate pH was increased with the autoclaving process in all three fertilizer treatments in experiment 1 (App. A-4).

The EC of the substrate was different as a result of fertilizer treatments (Fig. 2-5). The organic treatments (FM, BlM) experienced greater electrical conductivity than was measured in the inorganic treatments in experiment 1 (Fig. 2-5). The OSM (controlled release prill) effectively regulated release of nutrients over the course of the cropping cycle in experiment 1. The organic nutrient sources had a higher percentage of readily available nutrients, most notably from the KMS as a mineral nutrient source, and this resulted in high substrate EC measurements and nutrient analysis at day 7 of experiment 1. The only difference in substrate EC with the addition of microbial inoculants in experiment 1 was in the BlM treatments. The BlM + M/B and

AC BlM treatments had a lower EC at day 7 when compared to their respective standard BlM treatment (Fig. 2-6).

While the organic treatments tended to result in a higher pH and EC compared to the inorganic treatments after transplanting the impatiens plugs to the substrate, the irrigation practices with a leaching fraction of 15% to 25% and the weekly Pour Thru^{TM} extraction aided in flushing excess soluble salts from the organic (FM, BlM) treatments.

Substrate Ammonium Analysis

A considerable difference in ammonium concentrations resulted from the fertilizer treatments (App. A-8). The organic nutrient sources of FM and BlM had higher concentrations (200 to 275 mg$^.$L^{-1} on day 7) of NH$_4$-N present in the substrate through day 49 of experiment 1 than did the OSM (50 mg$^.$L^{-1} on day 7; Fig 2-7). The organic materials were high in proteins that are mineralized to NH$_4$-N. Significant increases in NH$_4$-N concentrations in the substrate were observed at day 7 of the experiment with the autoclaving process compared to their respective standard or inoculated fertilizer treatments via orthogonal contrasts across all treatments in experiment 1 (e.g. FM treatments shown in Fig 2-8, p=.02). When organic matter is subject to heat treatment, the resulting breakdown of structural components causes a release of ammonium. Substrate ammonium would then either be taken up by the plant, converted to ammonia in very small quantities as the process is limited by a substrate pH of 5.5 to 6.0, or become subject to nitrification and available for plant uptake or loss via leaching (Bothe et al., 2006).

Substrate Nitrate Analysis

Differences in NO$_3$-N concentrations as a result of the fertilizer treatment occurred in experiment 1 (App. A-10). On day 7 of experiment 1, OSM treatments resulted in about 35 mg$^.$L^{-1} more NO$_3$-N at day 7 of the experiment than was recorded in the organic treatments (Fig. 2-9). At day 35, the organic nitrogen sources FM and BlM had been mineralized to NO$_3$-N and resulted in increased NO$_3$-N levels compared to OSM treatments.

Some differences in the concentrations of NO$_3$-N in the substrate were recorded as result of the AC + FM treatments in experiment 1. Increased NO$_3$-N concentrations were observed at day 35 and day 42 in the AC FM treatment (Fig. 2-10). This treatment also resulted in the greatest initial concentration of NH$_4$-N, which would then be subject to potential conversion to

NO_3-N. No other significant differences in NO_3-N concentrations were observed as a result of the inoculant treatments in experiment 1.

Substrate Phosphorous Analysis

Osmocote[TM] supplied more PO_4-P than did the organic treatments at days 7 and 14 of experiment 1 (Fig. 2-11). However, the 12 mg.L^{-1} PO_4-P resulting from the organic treatments was sufficient for optimum plant growth. No changes in substrate concentrations of phosphate were observed as a result of the inoculant treatments in experiment 1 (App. A-12).

Substrate Potassium Analysis

Substantial differences were measured in the concentrations of potassium in the experiment 1 as a result of the fertilizer treatment (App A-14). A large difference was observed in levels of potassium in the substrate between the Osmocote[TM] and the organic fertilizer treatments. Osmocote[TM] released 50 mg.L^{-1} K at day 7 of the experiment (Fig. 2-12). The KMS used as the primary potassium source for the organic treatments resulted in 300 to 350 mg.L^{-1} K concentration in the substrate at day 7 of the experiment 1. As a soluble mineral, the KMS was much more readily available once applied to the substrate. Although the rates of applied potassium were calculated to be equal across all treatments, the organic fertilizer treatments had a much greater amount of available potassium at the initiation of the experiment than was available from the OSM treatments. The nature of the KMS amendment was certainly a contributing factor to increase in substrate EC in experiment 1.

Plant Growth, Experiment 2

Fertilizer Treatment Effect

The plants experienced some salt stress early in the growth phase in the organic fertilizer treatments in experiment 2. The stressed plugs recovered to produce growth that was similar to control treatments after three weeks.

In experiment 2, plant growth resulting from fertilization with OSM or CLF fertilizer treatments was not different (Table 2-5). BlM amended treatments resulted in smaller plants when compared to those fertilized with OSM or CL with respect to basal caliper, fresh and dry weights. These growth measurements were significantly lower in the organic treatments when

compared to the inorganic fertilizer regimens. This is may be attributed to the salt stress early in the growth phase in the organic fertilizer treatments as no initial plant stress was observed in the inorganic fertilizer regimens. The BlM treatments generally resulted in higher SPAD readings when compared to inorganic nutrient regimens, but were not greater than the standard OSM treatment (Table 2-5).

Inoculant Treatment Effect

Plant growth was greater in only the following instances with the addition of microbial inoculants and in one instance decreased plant growth in experiment 2. Repeating the trend of experiment 1, there were no observed changes to plant growth in the OSM treatments with the addition of microbial inoculants (Table 2-5). Increases in basal caliper were recorded with the presence of microbial inoculants in the CLF + M/B (11.8 mm) and AC BlM + M/B (10.4 mm) treatment, compared to their respective standard fertilizer treatment (9.0mm, 7.9mm).

A decrease in plant growth with respect to dry weight was observed in the CLF + M/B when compared to the standard CLF treatment (Table 2-5). This was the only instance of a reduction in plant growth with the addition of microbial inoculant products in either experiment.

A higher SPAD measurement was recorded in the CLF + M/B treatment when compared to the standard CLF treatment. A reduction in SPAD meter readings was observed in the OSM + M/B treatment when compared to the standard OSM treatment (Table 2-5).

Substrate Nutrient Analyses, Experiment 2

Experiment 2 included a CLF regimen and a reduced pre-plant potassium amendment compared to experiment 1. Stark contrasts in substrate nutrient levels were observed when evaluating inorganic and organic fertilizer treatments between experiments. The fertilizer treatment (OSM, CLF, BlM) was the most influential factor with respect to significant changes in the properties of the substrate leachate. Microbial inoculant treatments frequently affected no significant differences in substrate conditions in experiment 2.

The highest pH occurred in the organic fertilizer regimens in both experiments and the lowest overall pH was observed in the CLF treatments in experiment 2 (Fig 2-13 and 2-14). The OSM treatments resulted in a similar substrate pH in both experiments of 5.0-5.5 (Figs. 2-3, 2-13). The substrate pH was increased to 5.5 with the autoclaving process in the CLF treatments in experiment 2 (Fig. 2-14).

Substrate EC was 2.8 in OSM and 3.8 in BlM treatments (Fig. 2-15). The constant liquid feed resulted in the lowest initial EC (0.5) and increased substrate salt concentrations with continuous application of nutrients to an EC of 1 by day 42 (Fig 2-15). A unique response in substrate EC was observed in one treatment as a result of microbial inoculant addition in experiment 2. The OSM + M/B had a higher initial EC than the other OSM treatments (Fig 2-16). This was the only instance in either experiment where significant change in substrate EC resulted from inoculant product addition.

Substrate Ammonium Analysis

In experiment 2, the OSM prill released a much greater amount of NH_4-N (250 mg$^.$L^{-1} on day 7) versus observations from experiment 1. The CLF treatments had a significantly lower ammonium concentration (20 mg$^.$L^{-1}) than the OSM or BlM treatments at day 7 (Fig. 2-17). By day 28 there were no differences in ammonium concentration between the OSM and CLF treatments. The organic treatments had higher concentrations of NH_4-N present in the substrate until day 42, by day 49 the CLF had the highest level of NH_4-N in the substrate (Fig. 2-17).

There were no differences in substrate ammonium concentrations with the addition of microbial inoculant products in either experiment (App. A-9).

Substrate Nitrate Analysis

The limited NO_3-N concentrations (<.05 mg$^.$L^{-1}) of BlM treatments was similar in both experiments (Figs. 2- 9 and 2-18). The OSM treatment resulted in the greatest amount of NO_3-N (225 mg$^.$L^{-1}) at day 7 of experiment 2. The CLF NO_3-N concentration was less than the OSM, but greater than the BlM. There was no difference in NO_3-N concentration between the OSM and CLF treatments as the OSM dropped in NO_3-N concentration at day 14. By day 21 the NO_3-N concentration had increased in the organic treatments, similar to the NO_3-N concentration in the OSM. CLF treatments had the greatest NO_3-N concentration at day 21 (Fig 2-18). The repeated trend of increasing NO_3-N concentrations from organic nutrient sources after 21 days of the cropping cycle continued through day 42. At day 49, the CLF and the organic treatments had higher levels of NO_3-N than the OSM, with no differences in substrate NO_3-N concentration as a result of inoculant addition in either experiment (App. A-11).

Substrate Phosphorus Analysis

OSM treatments in experiment 2 showed similar trends of rapid nutrient release in the substrate P analysis. 100 $mg^.L^{-1}$ P was measured at day 7 to 140 $mg^.L^{-1}$ P at day 14 (Fig. 2-19). By day 49 of experiment 2, the CLF treatments had accumulated the highest concentration of phosphorus, and no differences were observed across treatments at day 56. No changes in substrate concentrations of phosphate were observed as a result of the inoculant treatments in either experiment (App. A-13).

Substrate Potassium Analysis

In experiment 2, reducing KMS application rates from 8.7 $g^.L^{-1}$ to 3.2 $g^.L^{-1}$ more closely matched the available substrate K concentrations between the OSM and BlM fertilizer treatments. With the reduced rate of KMS used in experiment 2 in conjunction with the increased nutrient release rate from OSM, there was no difference in substrate concentrations of potassium between the BlM and OSM treatments (Fig 2-20). The CLF had significantly less potassium in the substrate until day 56 of the experiment (Fig. 2-20). There were no changes in substrate analysis of potassium with the addition of microbial inoculants (App. A-15).

CO$_2$ Respiration, Experiments 1 and 2

Substantially higher CO_2 respiration levels were observed with the incorporation of organic fertilizers at day 7 in both experiments (shown in Fig. 2-21 and 2-22). Serving as both a stable food source and a potential inoculum source, organic fertilizer sources resulted in an increase microbial activity regardless of inoculant addition.

At day 7 of experiment 2 the BlM treatments resulted in a significantly higher concentration of CO_2-C from incubated substrate samples (Fig 2-22). There was increased respiration observed in the BlM treatments through day 14. At day 7 OSM and CLF treatments were not different. At day 28 the OSM had a significantly lower respiration rate when compared to the other fertilizer treatments, for the remainder of the experiment, no significant differences in CO_2-C respiration were observed. No significant differences were observed in CO_2-C as a result of the inoculant treatments in the experiment 2 (App. A-17).

An increase in CO_2 respiration was measured as a result of autoclaving in the OSM treatments in experiment 1 (Fig 2-23). With the degradation of organic matter in the presence of extreme heat, an increased food supply for microbial utilization was likely created.

Discussion

In both experiments, inorganic and organic nutrient regimens resulted in substantially different substrate pH, EC and N-P-K nutrient concentrations. In experiment 1, organic and inorganic fertilizers resulted in comparable plant growth; in experiment 2, organic fertilizers resulted in some mild reductions in plant growth. The salt stress that was observed in the organic treatments in experiment 2 was partially remediated by the leaching of soluble salts from the substrate, allowing the young plants to recover and produce adequate growth.

The nutrient release pattern of each fertilizer source offers insight into the challenges inherent in transitioning from one nutrient regime to another. Crop producers who have grown accustomed to the consistent release of plant available nutrients from an Osmocote[TM] prill, or to the steady increase of nutrient levels in the substrate with repeated CLF applications, will observe much different results with organic fertilizer use. In both experiments, protein-based organic fertilizers resulted in relatively high concentration of ammonium early in the production cycle. After 28 to 42 days, microbial conversion of ammonium to nitrate was observed in both organic nutrient regimens and in both experiments. In contrast, the OSM and CLF treatments began the cropping cycle with nitrate available for plant uptake.

The most notable effect of nitrate differences between treatments was the resulting floriferousness of the mature plants in experiment 1. The balance of N forms resulting from the Osmocote[TM] may have contributed to the increase in flower production when compared to organic treatments (Fig. 2-2) in experiment 1. Supplemental nitrogen additions from the Sub Culture M inoculant product resulted in no difference between floriferousness ratings between BlM + 5x rate of inoculants when compared to Osmocote[TM] treatments in experiment 1

The autoclaving procedure altered the physical and chemical properties of the peat-based substrate and consistently reduced plant growth when compared to respective non-autoclaved or inoculated treatments in experiment 1. The increased ammonium concentration of the autoclaved peat may have caused a reduction in plant growth measurements, but did not prove to be

excessively detrimental to the plants. A reduction in plant growth with the AC treatment was not observed to the same degree in experiment 2.

In considering the increases to certain plant growth measurements with microbial inoculant additions, alternative possibilities for changes in plant growth with inoculant addition deserve exploration. The Sub Culture and EM1 inoculant products used in the two experiments contained other compounds in addition to listed beneficial microbial populations. Subculture M contains kelp meal, humic and fulvic acids. These particular substrate constituents have demonstrated the potential to improve plant growth in previous studies (e.g. Arancon et al., 2006). It is feasible to consider that beneficial microbial populations inherent to these two inoculant products had the opportunity to establish and provide benefits to plant growth. Additionally, other constituents of the inoculant products may have had a direct impact on plant growth as well.

Although no nutrient specifications were reported on the Sub Culture M product label, considerable nutrient concentrations are inherent to the product (Table 2-3), perhaps most notably the additional N. This addition of supplemental nutrients from inoculant product incorporation is a likely cause of minor and inconsistent increases to plant growth in certain treatments. The changes in plant growth were predominately observed with the addition of Sub M at a 5x label rate, and this product was shown contain macro and micronutrient concentrations that would likely affect plant growth.

While the inoculant products contained beneficial organisms that have been shown to process plant nutrients, no consistent differences in substrate nutrient levels were attributed to inoculant addition. In a non-limiting nutrient environment, as is common in horticultural production, a reduction in the observed benefit could be anticipated from enhanced nutrient cycling, when compared to production scenarios where nutrients are limiting.

Comparing the CO_2-C respiration of the various fertilizer and inoculant treatments in a soilless substrate to reported respiration rates from other production systems can provide context for the data. A healthy, native prairie land soil, rich in organic matter and microbial populations was recorded to have a microbial respiration of 24 to 96 µg CO_2-C (g^{-1}substrate $^.$ $24hr^{-1}$) (Williams et al., 2010). The same soil produced a maximum of 240 µg CO_2-C (g^{-1}substrate $^.$ 24 hr^{-1}) with glucose supplementation. The organic nutrient sources in experiment 1 resulted in respiration rates that were within this reported range. The BlM treatments recorded a much

greater respiration at day 7 of experiment 2. The reason for the drastic increase in respiration in BlM treatments in experiment 2 versus the measured respiration from BlM in experiment 1 is unknown. This context indicates that the observed increases to microbial respiration with organic fertilizer addition were typically, but not always, within the reported limit of a healthy, microbially-active substrate. The additional glucose applied as part of the Sub Culture B inoculant would be consumed rapidly and would likely be exhausted in the 7 days prior to the first substrate sample collection.

Conclusion

The challenges described in previous research were observed in these studies with organic fertilizer incorporation. Although comparable plant growth was measured in some instances when using organic fertilizers, changes to plant architecture as a result of organic fertilizer were evident in the case of floriferousness. Fresh and dry shoot weights and basal caliper measurements were similar between inorganic and organic fertilizers in experiment 1. The reduction in plant growth when using organic fertilizers in experiment 2 was likely related to transplantation of plugs prior to a leaching event that ultimately contributed to reduced plant growth. Our experience suggests that growers could leach salts from pre-plant application of organic fertilizers to an appropriate level prior to transplanting plugs and achieve impatiens growth that matches controlled-released inorganically fertilized plants. While leaching of excess salts may remedy excess nutrient concentrations in the substrate, environmental implications of nutrient loss from production systems should not be neglected. Excess soluble salt levels present when using a pre-plant incorporation of dry organic fertilizers may justify the growing trend of applying a lower concentration of pre-plant organic nutrient sources while supplementing plant nutritional needs throughout the cropping cycle with complimentary liquid organic fertilizers. Using a combination dry pre-plant/liquid supplement organic nutrient regimen may reduce initial soluble salt exposure to transplanted plugs, and subsequently supplement organic nutrients in a manner that better matches plant nutrient uptake.

Floral crop producers may deem the potential reduction in floriferousness with the use of organic fertilizers a reduction in salable plant quality. The potential for increased flower production with inoculant product addition to organic fertilizer, as shown in the 5x inoculant rate, may be a viable economic investment for some crop producers. Growers that are

considering incorporation of organic fertilizers and beneficial microbial inoculants are best served to consider previous research pertaining to their specific production scenario. Growers must also be aware of organic guidelines with respect to inoculant products, should organic crop production compliance be a goal of the firm.

While microbial inoculant addition contributed inconsistent increases in certain plant growth responses, the predominating factor affecting growth was likely the supplemental N addition or humic/fulvic acids as part of microbial inoculant products. The inoculant products tested in these experiments were proven to contain beneficial microbial species, a significant change to microbial respiration was not observed as the result of inoculant product application. Inoculant product addition may be considered an insurance policy against pathogen invasion via applications of known pathogen antagonists. Growers aiming to maintain organic certification must ensure that inoculant products meet organic production standards, and that the additional cost of inoculant product incorporation is appropriate for their specific production system.

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Tables and Figures

Table 2-1. Summary of treatments from experiments 1 and 2. n=4

Experiment 1
Inorganic Control treatments with Osmocote™
Autoclaved Peat + Osmocote (AC OSM)
Peat + Osmocote (OSM)
Peat + Osmocote + SubCulture M + B (OSM + M/B)
Organic treatments with Feather Meal (FM), Bone Meal (BnM), and KMS
Autoclaved Peat + FM, BnM, KMS (AC FM)
Peat + FM, BnM, KMS (FM)
Peat + FM, BnM, KMS + SubCulture B (FM + B)
Peat + FM, BnM, KMS + SubCulture M (FM + M)
Peat + FM, BnM, KMS + SubCulture M + B (FM + M/B)
Peat + FM, BnM, KMS + 5X label rate SubCulture M + B (FM + M/B 5X)
Organic treatments with Blood Meal (BlM), Bone Meal (BnM), and KMS
Autoclaved Peat + BlM, BnM, KMS (AC BlM)
Peat + BlM, BnM, KMS (BlM)
Peat + BlM, BnM, KMS + SubCulture M + B (BlM + M/B)
Peat + BlM, BnM, KMS + 5X label rate SubCulture M + B (BlM + M/B 5X)
Experiment 2
Inorganic treatments with Osmocote™
Autoclaved Peat and Osmocote (AC OSM)
Autoclaved Peat + Osmocote + SubCulture M + B (AC OSM + M/B)
Peat and Osmocote (OSM)
Peat + Osmocote + SubCulture M + B (OSM + M/B)
Inorganic CLF treatments
Autoclaved Peat CLF (AC CLF)
Autoclaved Peat + CLF + SubCulture M/B (AC CLF + M/B)
Peat + CLF (CLF)
Peat + CLF + SubCulture M/B (CLF + M/B)
Organic treatments with Blood Meal (BlM), Bone Meal (BnM), and KMS
Autoclaved Peat + BlM, BnM, KMS (AC BlM)
Autoclaved Peat + BlM, BnM, KMS SubCulture M/B (AC BlM + M/B)
Peat + BlM, BnM, KMS (BlM)
Peat + BlM, BnM, KMS + EM1 (BlM + EM1)
Peat + BlM, BnM, KMS + SubCulture M/B (BlM + M/B)

Table 2-2. Nutrient amendment application rates for experiments 1 and 2.

Nutrient Source	Nitrogen Source	Rate (g·L^{-1})	Phosphorous Source	Rate (g·L^{-1})	Potassium Source	Rate (g·L^{-1})
Osmocote^{TM} (OSM)	OSM	7.7	OSM		OSM	
14-14-14						
Feather Meal (FM)	FM	9.3	Bone Meal	14.1	KMS	8.7
12.9N-0.6P-0.2K			6.35N-14.5P-1.2K		0N-0P-18.3K	
Blood Meal (BlM)	BlM	8.3	Bone Meal	14.1	KMS	8.7/3.2 *
14.4N-0.6P-0.2K			6.35N-14.5P-1.2K		0N-0P-18.3K	
Peters^{TM} CLF*	CLF	0.5	CLF	-	CLF	-
20-10-20 Peat Lite						
All Treatments						
Dolomitic Lime	-	5.5	-	-	-	-
Micro Max^{tm}	-	0.6	-	-	-	-
Suffusion	-	0.6	-	-	-	-

*CLF Treatment and altered KMS application rate used in experiment 2.

Table 2-3. Nutrient addition with label rate application of inoculant products in experiment 1 and 2.

Nutrient	EM1™	Sub Culture B™	Sub Culture M™
Total N (%)	ND	0.31	0.79
Total C (%)	ND	34.2	26.5
P (mg·pot^{-1})	0.03	0.01	0.05
K (mg·pot^{-1})	0.67	0.07	5.4
Ca (mg·pot^{-1})	0.25	0.01	1.02
Mg (mg·pot^{-1})	0.08	0.004	0.41
S (mg·pot^{-1})	0.29	0.02	0.61
Cu (mg·pot^{-1})	0.0004	0.0001	0.002
Fe (mg·pot^{-1})	0.006	0.006	0.96
Mn (mg·pot^{-1})	0.003	0.001	0.01
Zn (mg·pot^{-1})	0.001	0.0004	0.004

Table 2-4. Fertilizer [Osmocote^{TM} (OSM), Feather Meal (FM) or Blood Meal (BlM)] and inoculant treatment [Autoclave (AC), Sub Culture M and B (M, B, M/B or M/B 5x)] effect on bedding impatiens growth in experiment 1 at mid-crop (day 30) and end-crop (day 60); n=4.

Treatment	Diameter	Height	Basal Caliper	Fresh Wt	Dry Wt
	(cm)	(cm)	(mm)	(g)	(g)
Mid Crop					
AC OSM	22.2a[y]	5.75b	.	.	.
OSM	24.8a	7.5ab	.	.	.
OSM + M/B	26.5a	7.0ab	.	.	.
AC FM	23.9a	7.2ab	.	.	.
FM	23.7a	6.8ab	.	.	.
FM + B	24.8a	7.4ab	.	.	.
FM + M	25.7a	7.5a	.	.	.
FM + M/B	27.4a	8.8ab	.	.	.
FM + M/B 5x	27.4a	7.7ab	.	.	.
AC BlM	23.4a	6.3b	.	.	.
BlM	23.6a	6.3b	.	.	.
BlM + M/B	25.3a	5.8b	.	.	.
BlM + M/B 5x	27.5a	7.3a	.	.	.
LSD*	1.1	.5			
End Crop					
AC OSM	31.8b[y]	8.8a	8c	131.5a	6.5c
OSM	31.1b	9.5a	8.8bc	152.3a	8.3bc
OSM + M/B	31.0b	10.3a	10.3bc	153.0a	8.4c
AC FM	32.3ab	9.5a	10.3bc	168.5a	10.1b,d
FM	34.3ab	11.3a	11.3ab	191.3a	11.9ab,d
FM + B	34.4ab	11.3a	12.3a	201.5a	12.4ab,d
FM + M	34.2ab	10.8a	12.3a	189.8a	13.1ab,de
FM + M/B	33.0ab	12.0a	13.5a	208.5a	13.7ab,de
FM + M/B 5x	34.3ab	10.5a	13a	212.3a	14.8a,e
AC BlM	32.8ab	10.8a	10.3b	160..3a	9.8bc
BlM	34.1ab	10.0a	12.5a	180.5a	12.7ab
BlM + M/B	33.0ab	10.8a	11.8a	196.0a	14.6a
BlM + M/B 5x	35.1a	11.0a	11.3a	202.3a	14.1ab
LSD*	0.6	0.6	0.6	10.8	3.9
LSD FM**					.8

*LSD reported as standard error of the Least Squared Means of the entire data set. ** LSD reported as standard error of the Least Squared Means of feather meal treatments. [Y] Any two means within a column not followed by the same letter are significantly different. Significance reported reflects Tukey-Kramer adjusted pairwise comparisons (p≤0.05).

[Y] Any two means within a column not followed by the same letter are significantly different.

Table 2-5. Fertilizer [Osmocote^{TM} (OSM), Constant Liquid Feed (CLF) or Blood Meal (BlM)] and inoculant treatment [Autoclave (AC), Sub Culture M and B (M, B, M/B or M/B 5x)] effect on bedding impatiens growth in experiment 2 at end-crop (day 63). n=4

Treatment	Diameter (cm)	Height (cm)	Basal Caliper (mm)	Fresh Wt (g)	Dry Wt (g)	SPAD
			End Crop			
AC OSM	29.7a^y	17.6a	10.3bc	145.4a	9.3a	44.4abc
AC OSM + M/B	28.1ab	17.4a	11.4ab	130.3a	8.1a	35.5c
OSM	29.2ab	17.0a	9.8bc	133.9a	7.8a	46.0a
OSM + M/B	29.1a	17.5a	10.1ab	122.5a	7.2a	42.1bc
AC CLF	28.4a	16.8a	9.0bc	150.8a	7.6a	38.2bc
AC CLF + M/B	29.9a	18.4a	11.8ab	136.1a	8.2a	38.2bc
CLF	28.0a	18.0a	8.8cd	147.1a	7.6a	38.9bc
CLF + M/B	26.0a	17.5a	10.8b	138.4a	5.78b	51.9a
AC BlM	25.5a	17.9a	7.9d	87.9b	6.81a	52.1a
AC BlM + M/B	25.8a	17.8a	10.4abc	113.3ab	6.48a	51.6a
BlM	22.8b	16.6a	7.4cd	88.9b	3.62b	48.4ab
BlM + EM1	23.1b	16.9a	9.9cd	74.9b	4.38b	53.5a
BlM + M/B	26.3ab	18.3a	8.9cd	115.3ab	6.6ab	47.1ab
LSD*	1.2	0.6	0.5	11.1	0.4	2.1

^Y Any two means within a column not followed by the same letter are significantly different.

*LSD reported as standard error of the Least Squared Means of the entire data set. Significance reported reflects Tukey-Kramer adjusted pairwise comparisons; (p≤.05).

Figure 2-1. Rating scale (1-5) for floriferousness evaluations at end-crop (day 63) of experiment 1.
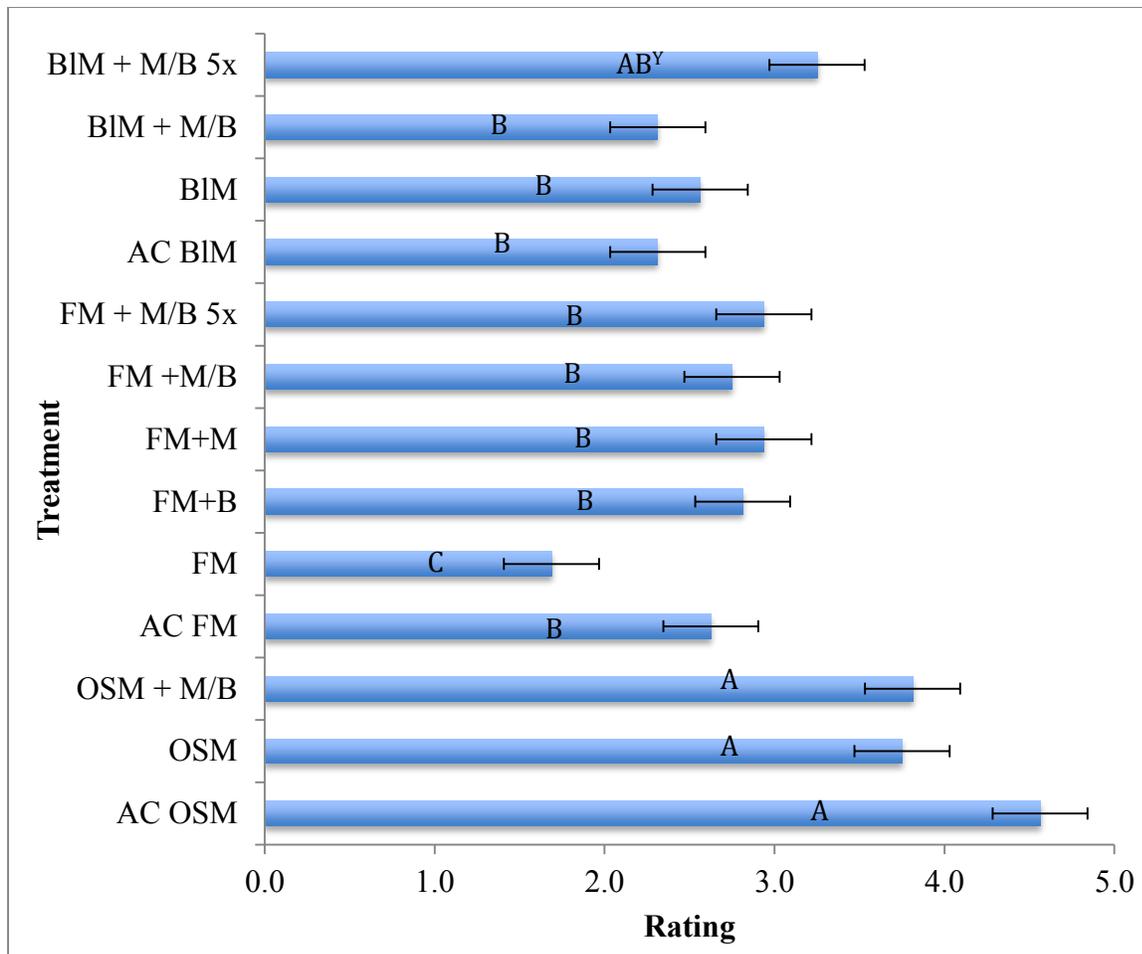
Figure 2-2. Fertilizer [Osmocote™ (OSM), Feather Meal (FM) or Blood Meal (BlM)] and inoculant treatment [Autoclave (AC), Sub Culture M and B (M, B, M/B or M/B 5x)] effect on Floriferousness in experiment 1.

[Y] Any two means within a row not followed by the same letter are significantly different. LSD reported as standard error of the Least Squared Means of the entire data set. Significance reported reflects Tukey-Kramer adjusted pairwise comparisons. (p≤.05); n=4.
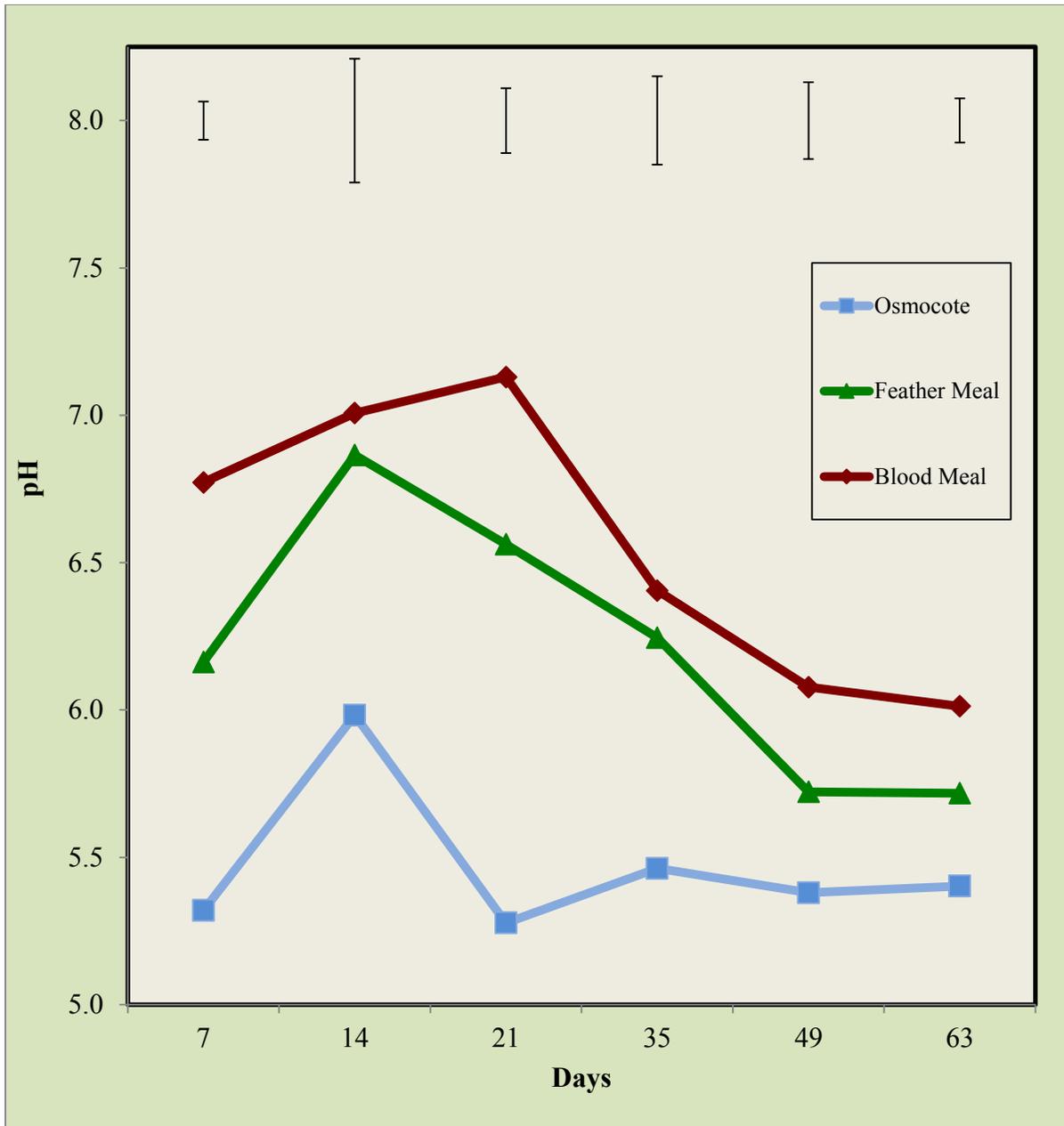
Figure 2-3. Fertilizer treatment effect on substrate pH in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.

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Figure 2-4. Comparison of autoclaved (AC) feather meal treatment to feather meal treatment substrate pH in experiment 1.

Standard error of the least squares means estimate = 0.63; n=4.
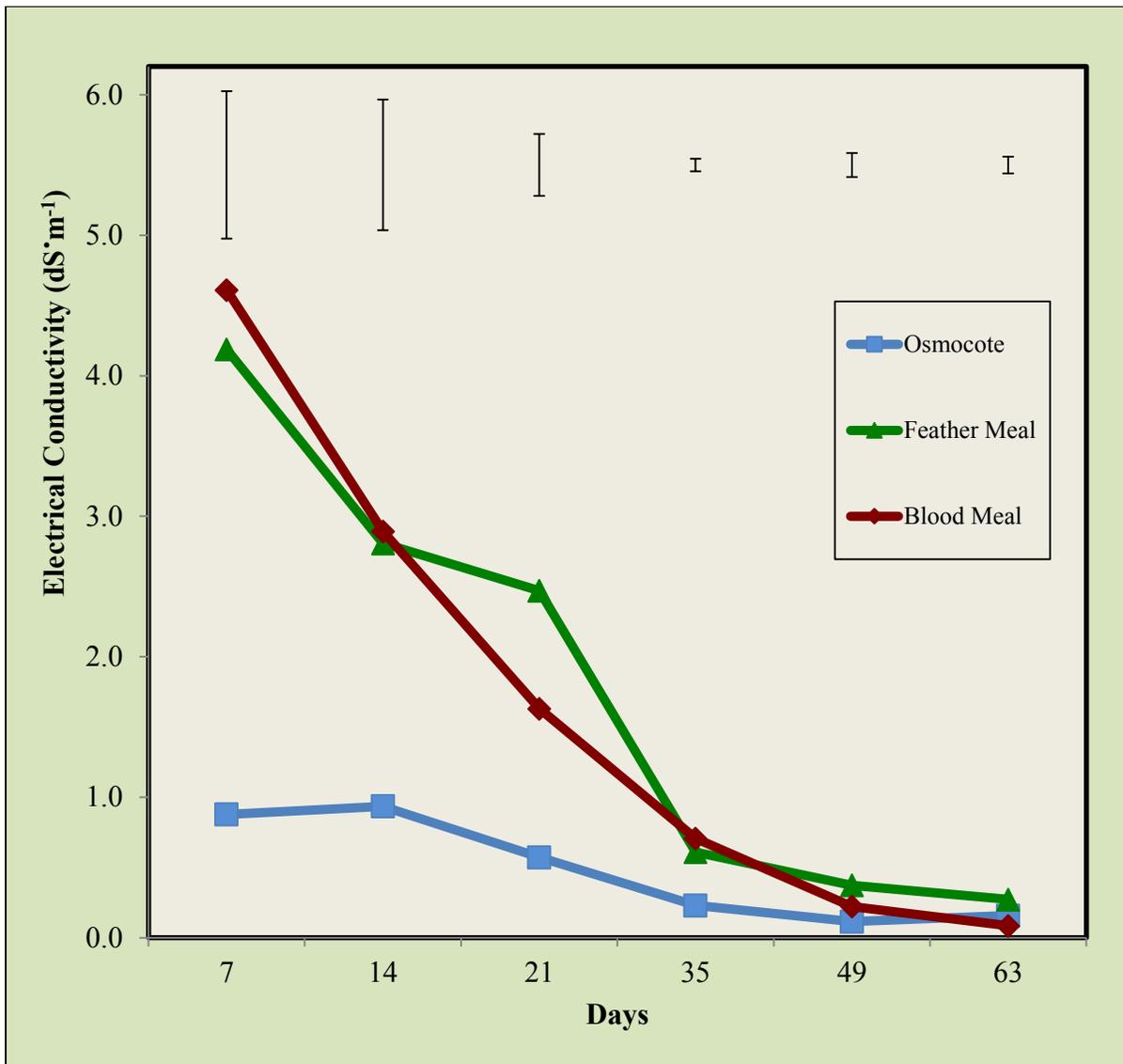
Figure 2-5. Fertilizer treatment effect on substrate EC in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
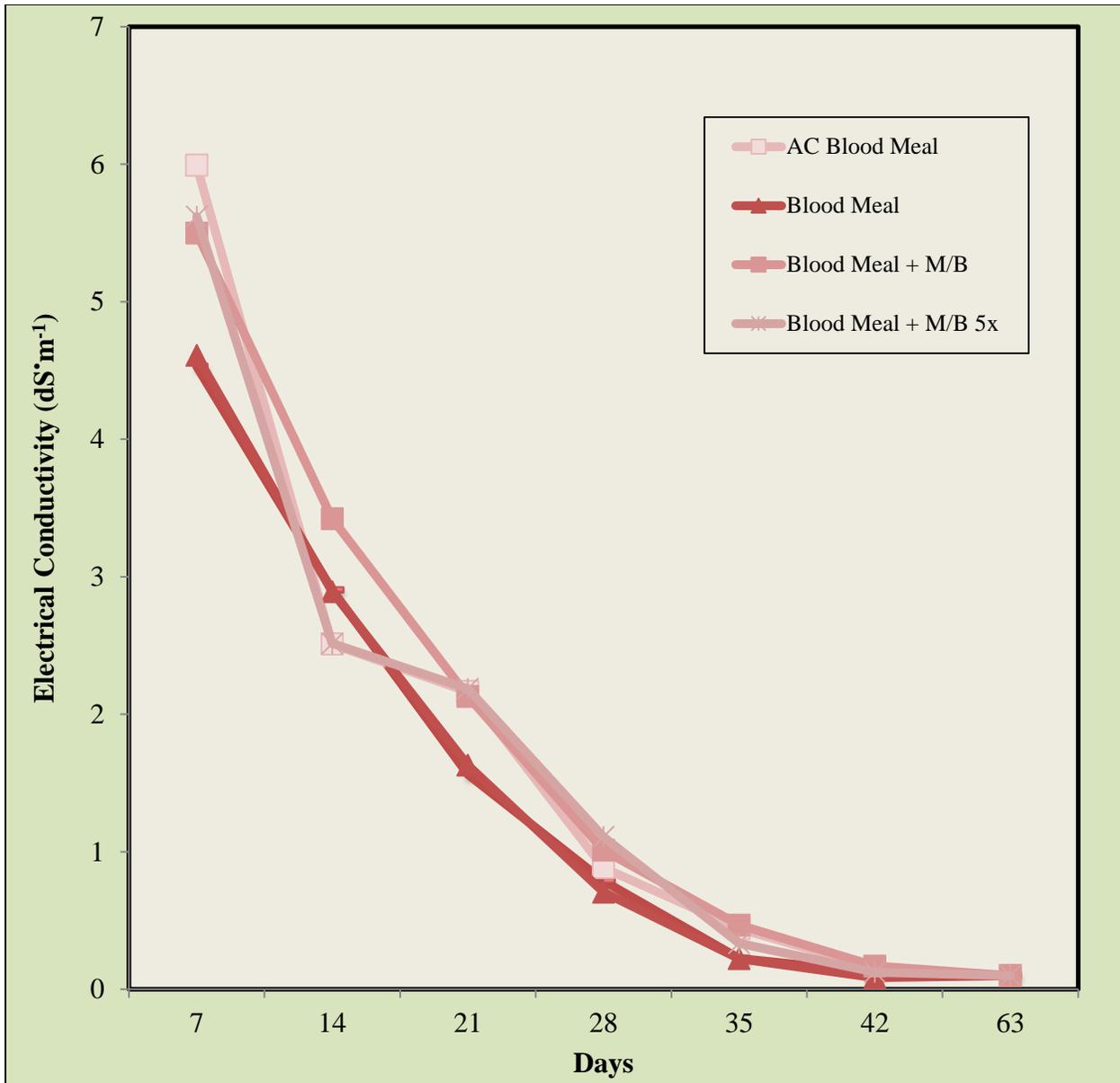
Figure 2-6. Effect of blood meal and inoculant treatments [autoclaved (AC) or Sub Culture M/B (M/B)] on substrate EC in experiment 1.

Standard error of the least squares means estimate =0.79; n=4.
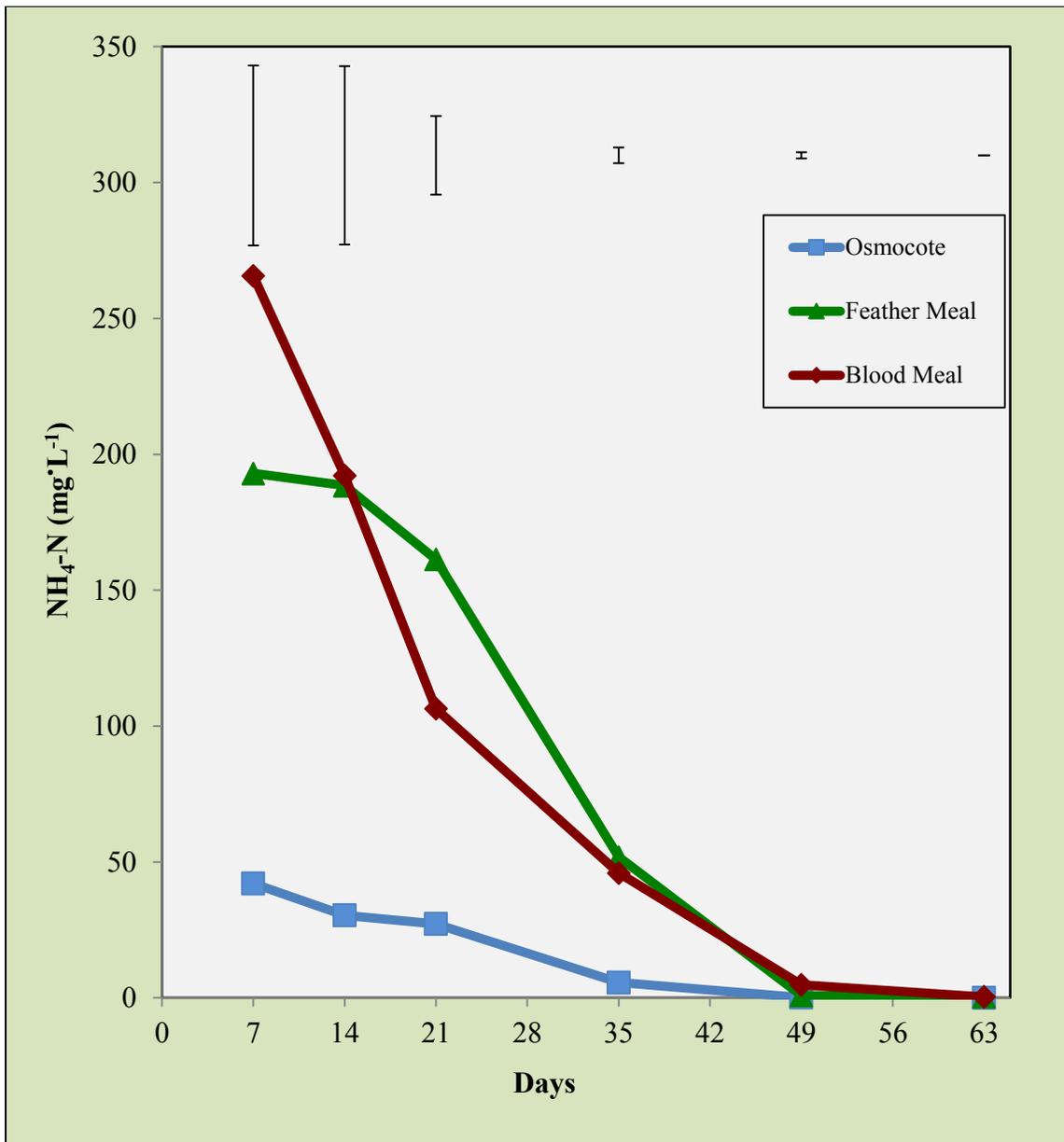
Figure 2-7. Effect of fertilizer treatment on substrate ammonium concentrations in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
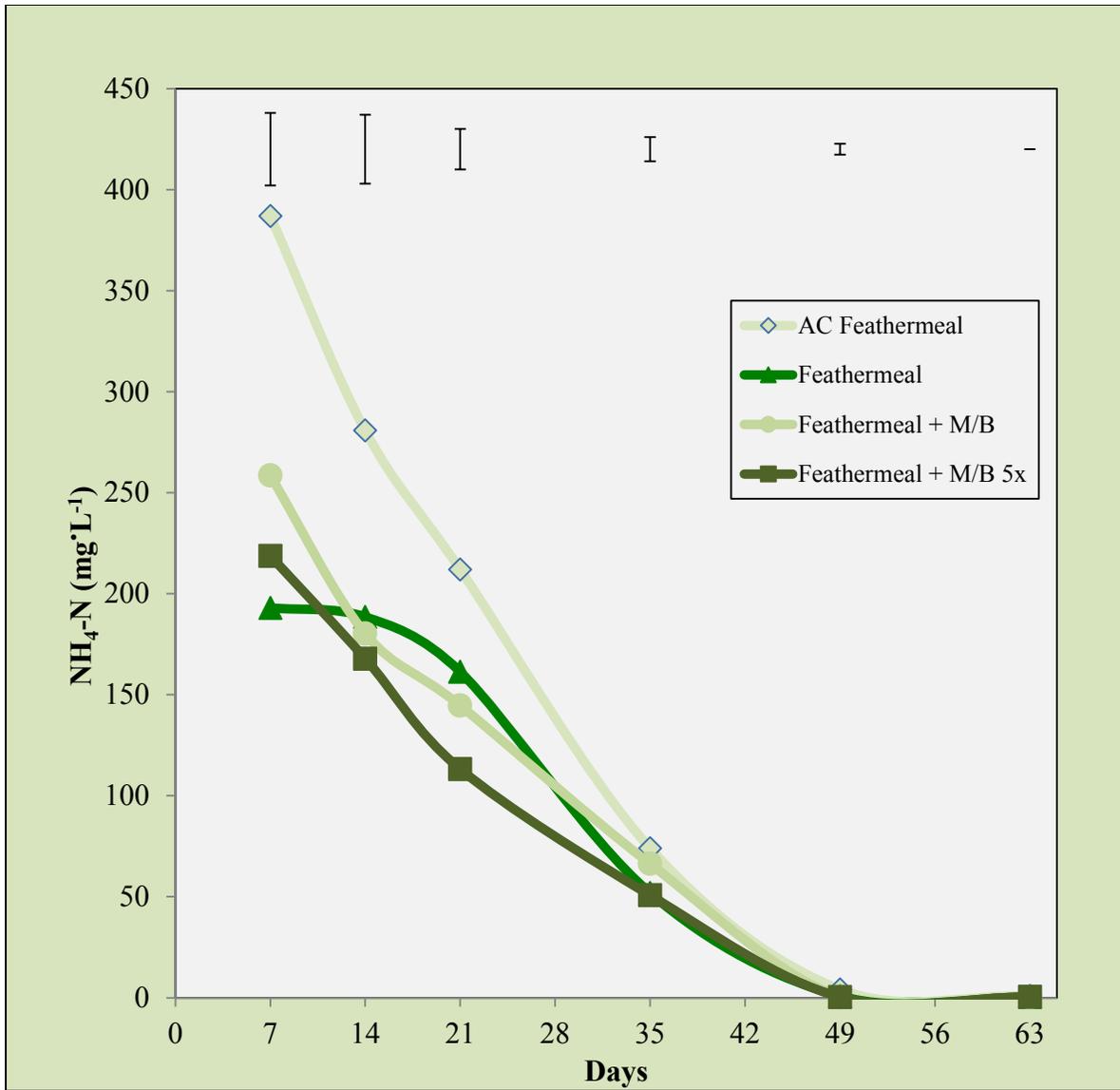
Figure 2-8. Effect feather meal and inoculant treatment [autoclaved (AC) or Sub Culture M/B (M/B)] on substrate NH₄-N concentration in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
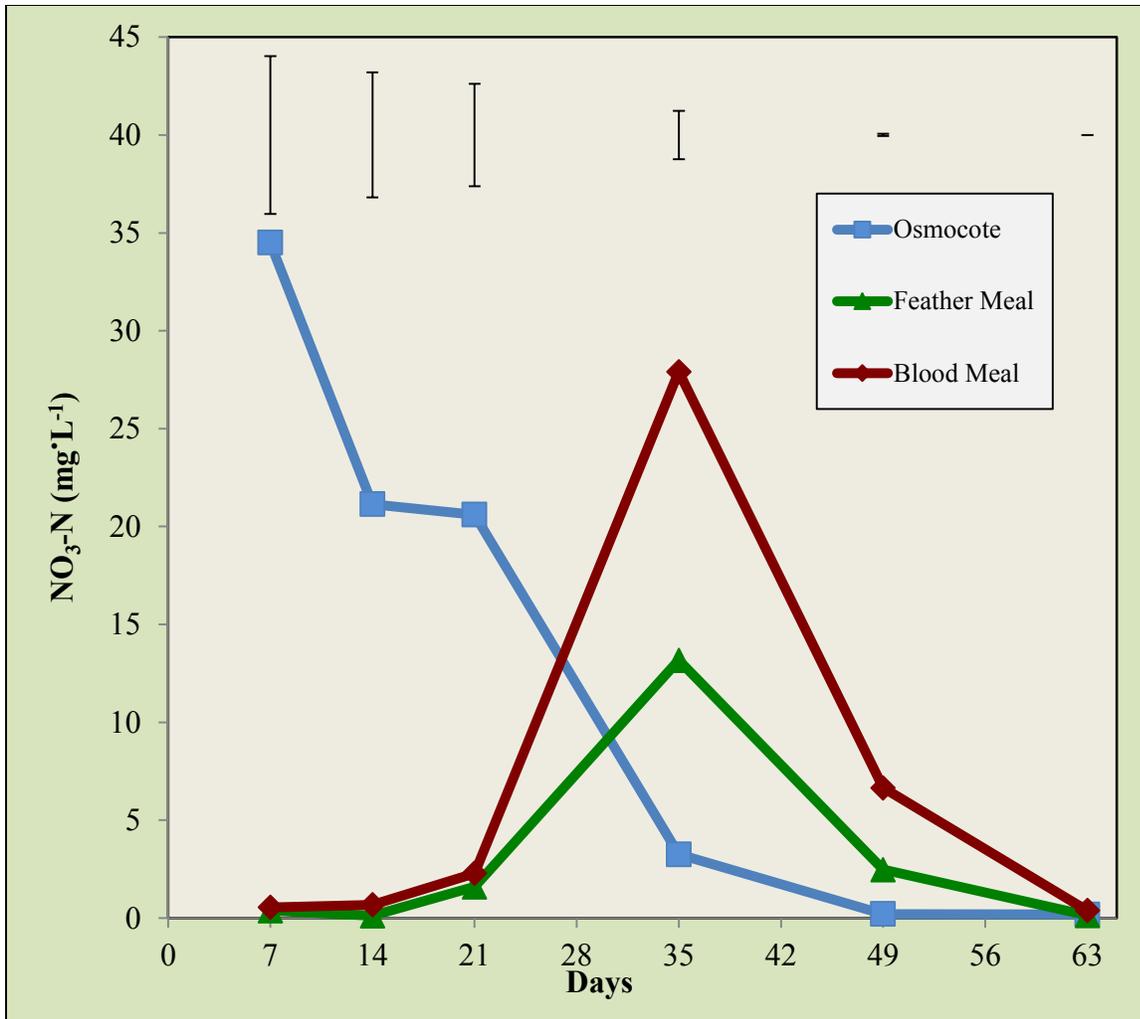
Figure 2-9. Effect of fertilizer treatment on substrate NO₃-N concentration in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
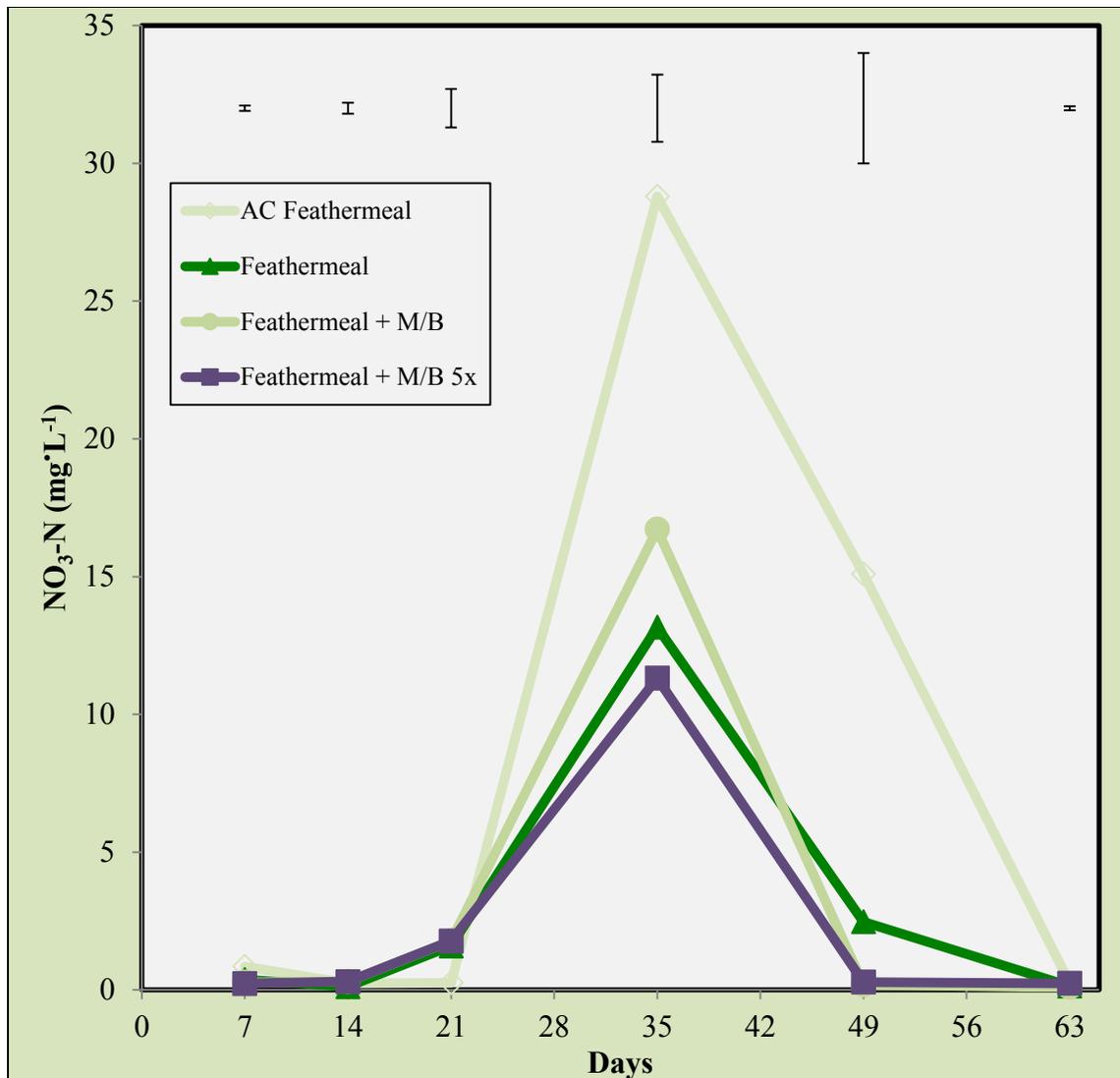
Figure 2-10. Feather meal and inoculant treatment [autoclaved (AC) or Sub Culture M/B (M/B)] effect on substrate NO₃-N concentration in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
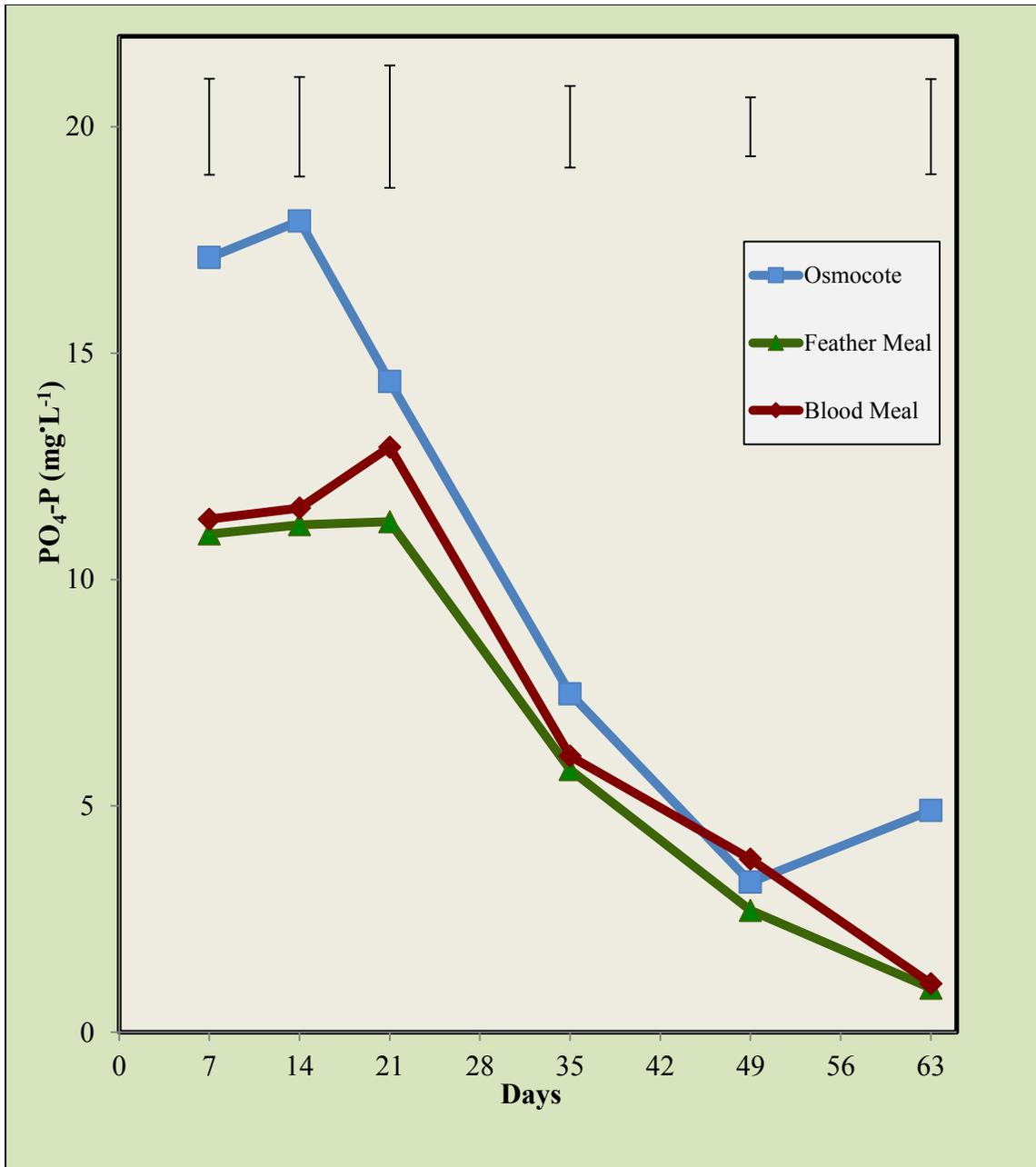
Figure 2-11. Fertilizer treatment effect on substrate PO$_4$-P concentration in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
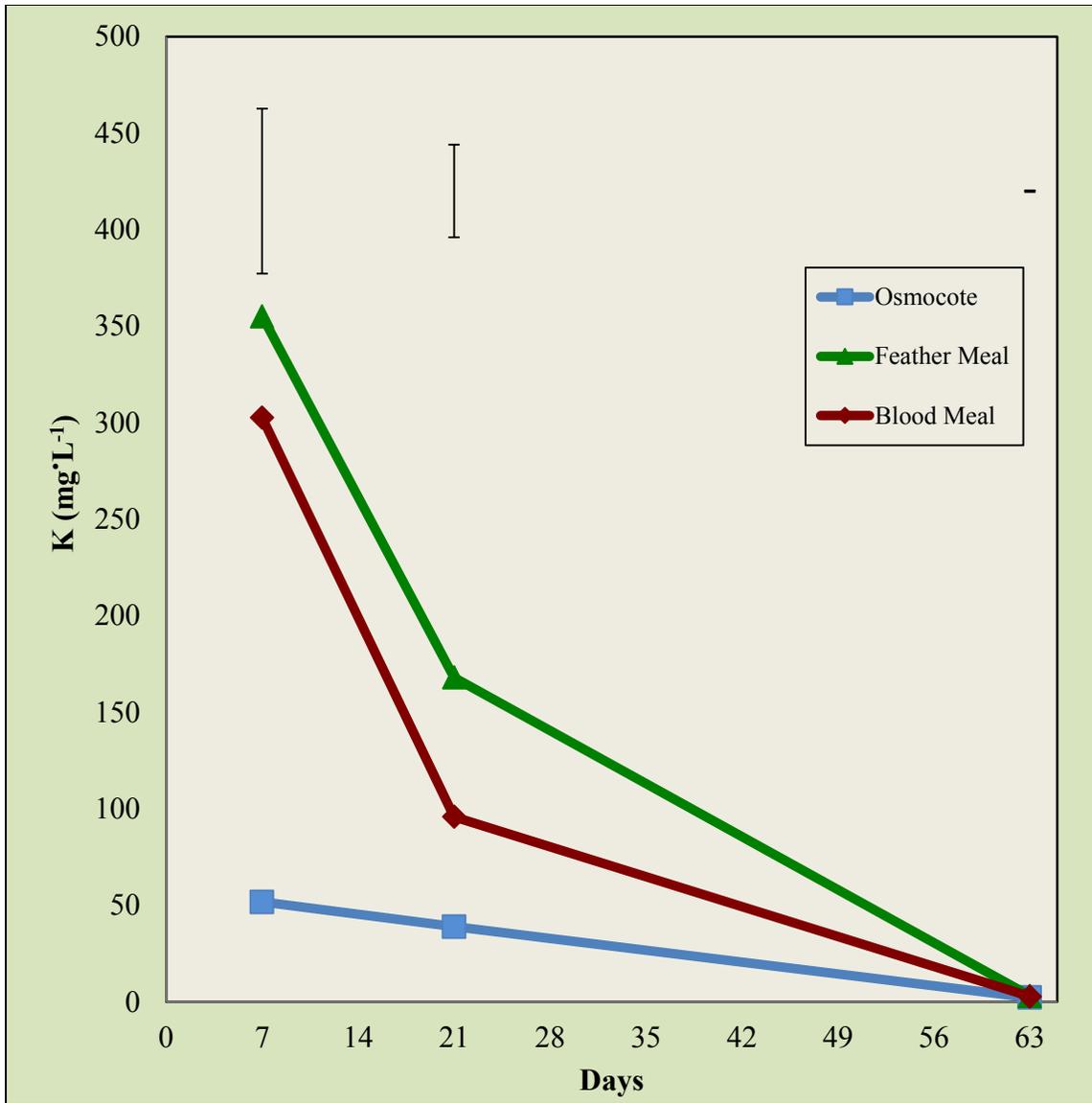
Figure 2-12. Fertilizer treatment effect on substrate K concentrations in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
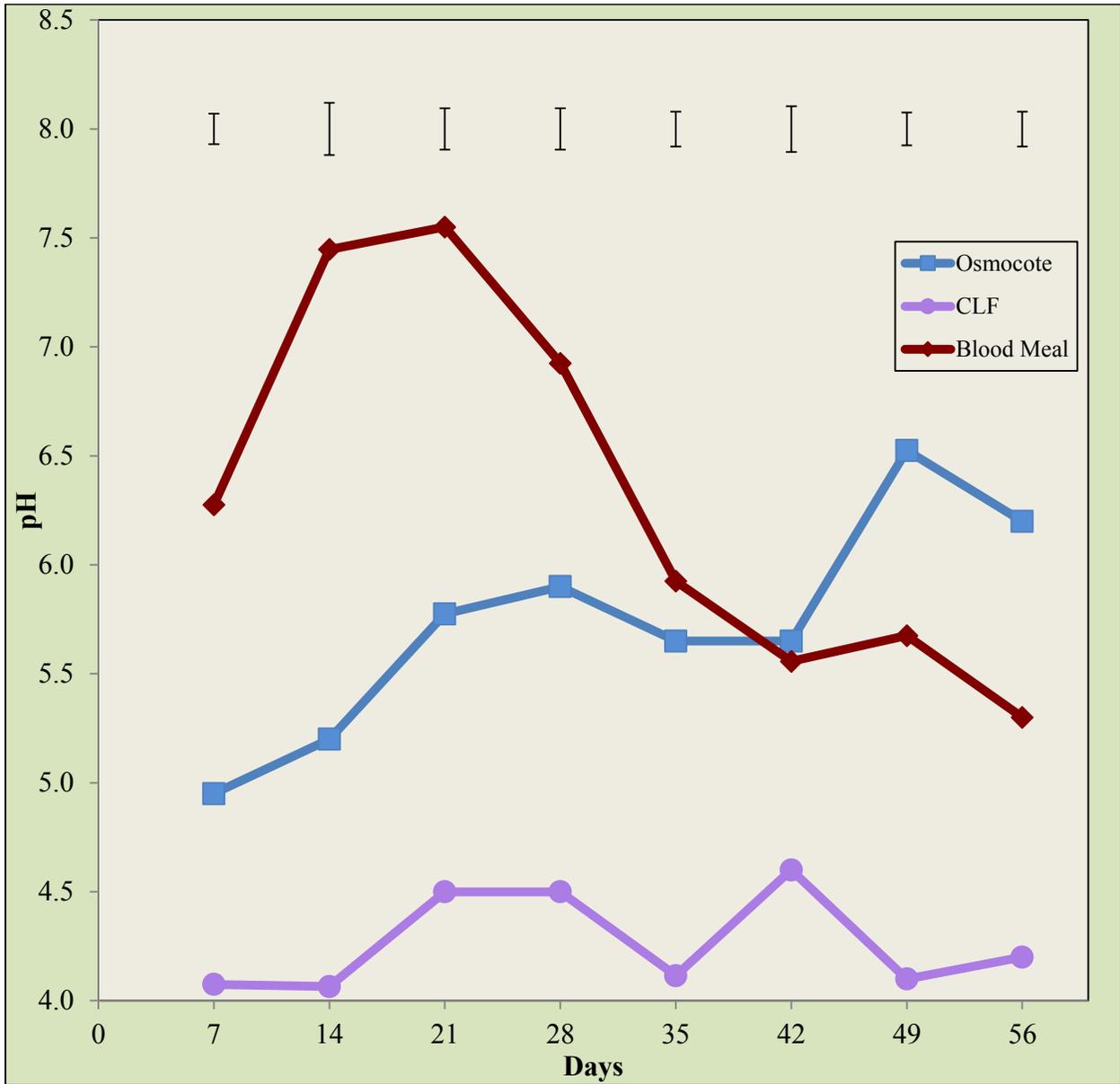
Figure 2-13. Fertilizer treatment effect on substrate pH in experiment 2.

Bars report a 'by date' standard error analysis of the least squared means; n=4.
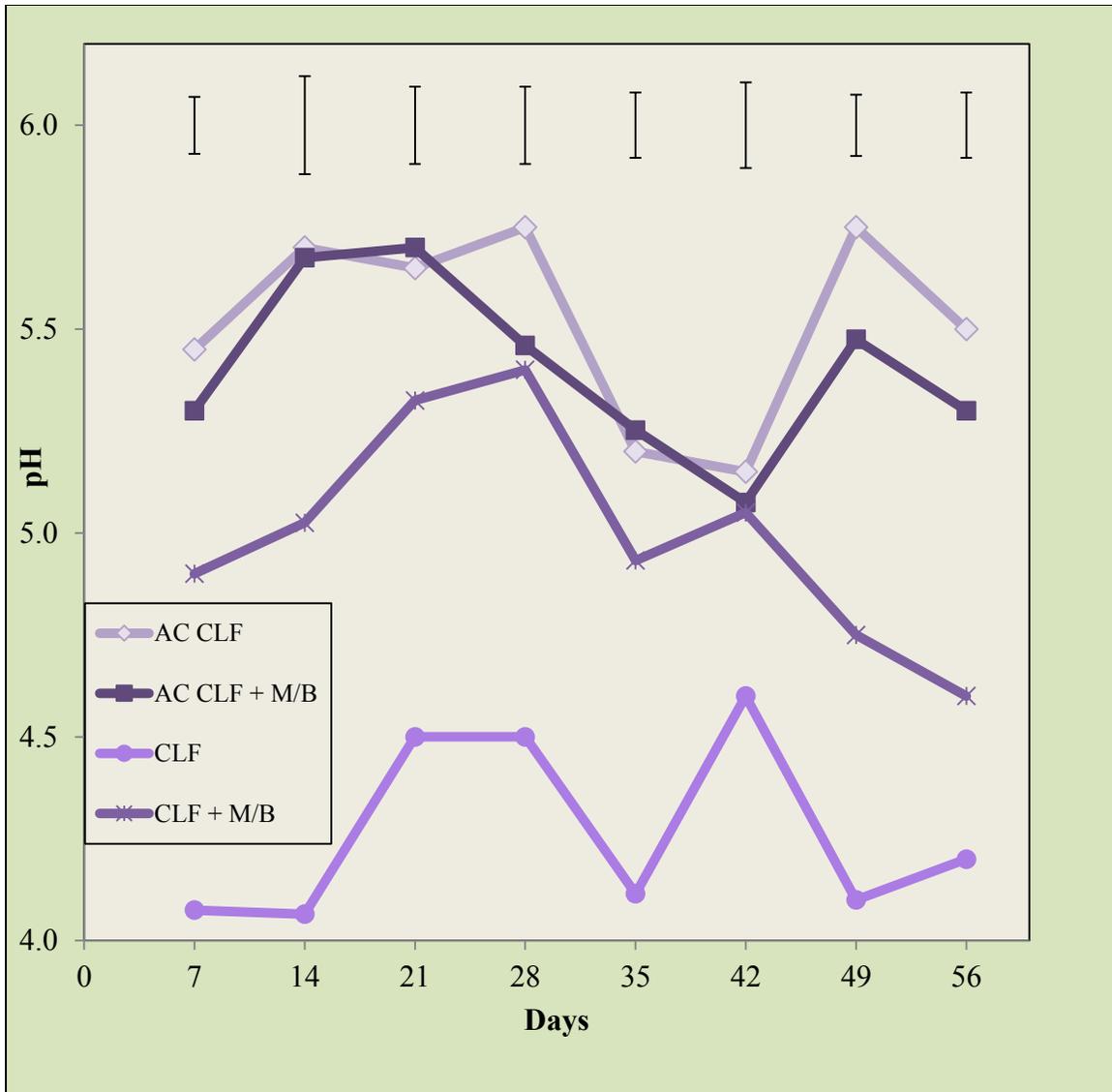
Figure 2-14. Effect of constant liquid feed (CLF) and inoculant treatments [autoclaved (AC) or Sub Culture M/B (M/B)] on substrate pH in experiment 2.

Bars report a 'by date' standard error analysis of the least squared means; n=4.
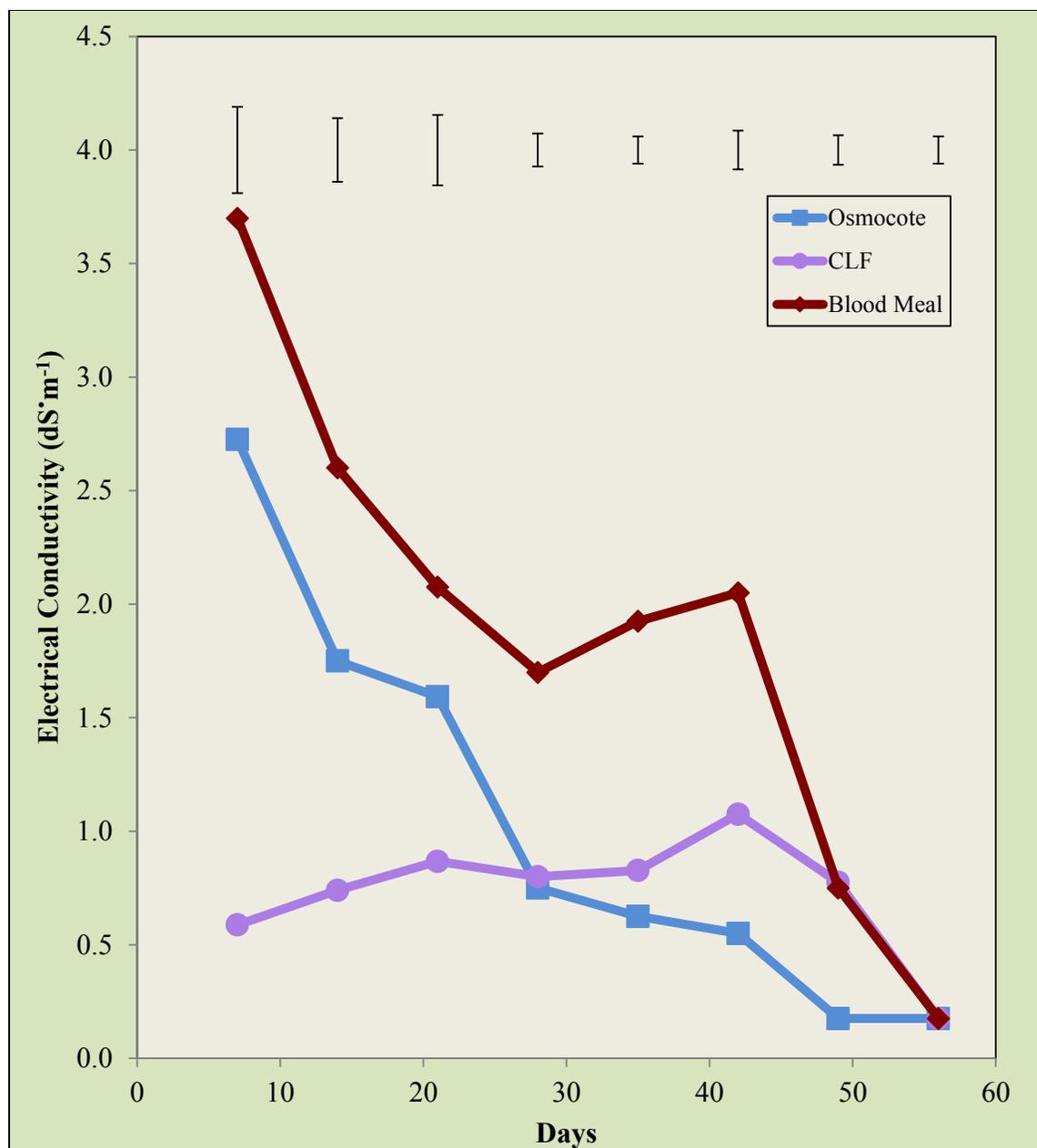
Figure 2-15. Effect of fertilizer treatment on substrate EC in experiment 2.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
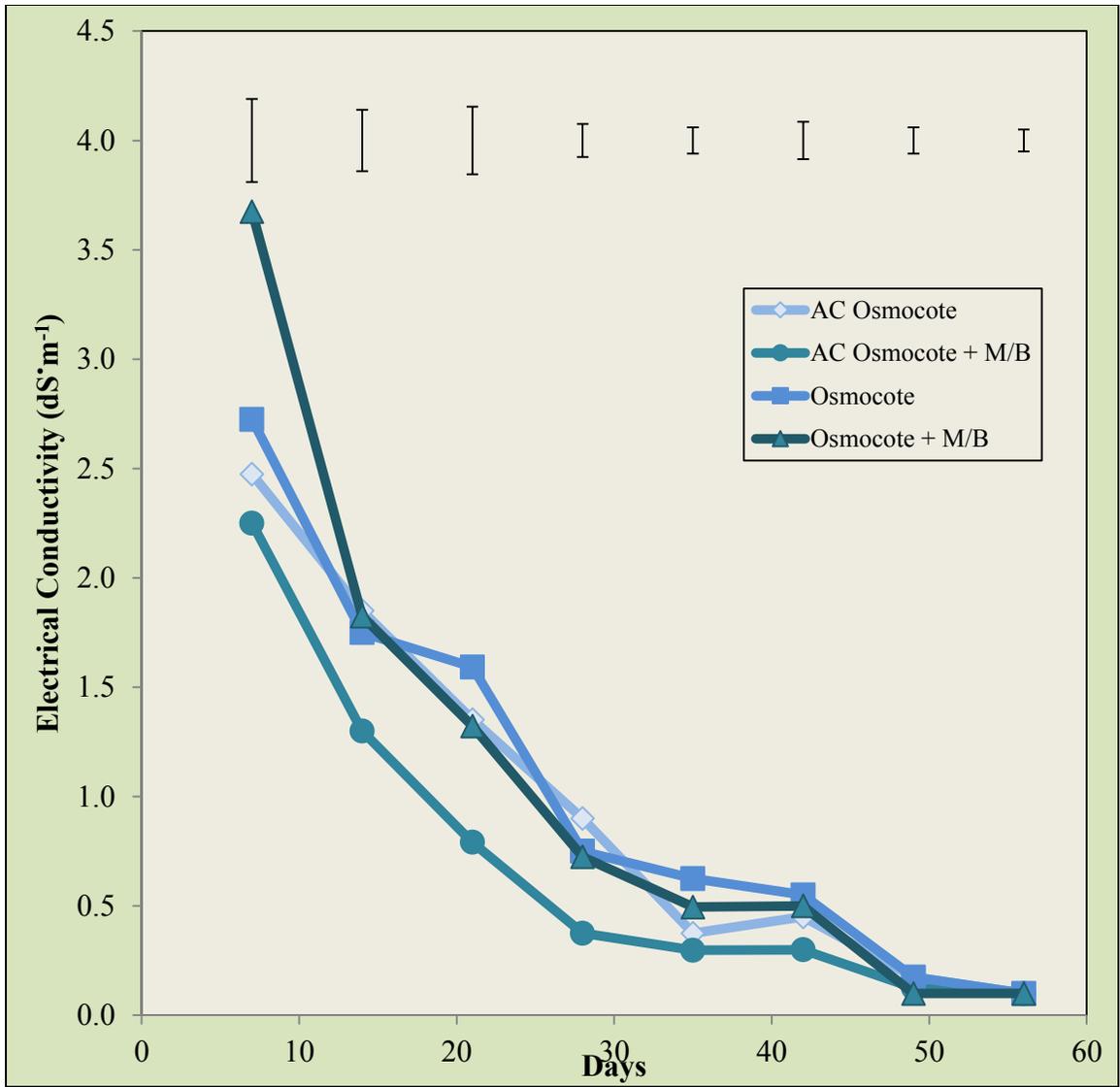
Figure 2-16. Effect of inoculant treatments [autoclaved (AC) or Sub Culture M/B (M/B)] on substrate EC in the Osmocote^{TM} treatment in experiment 2.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
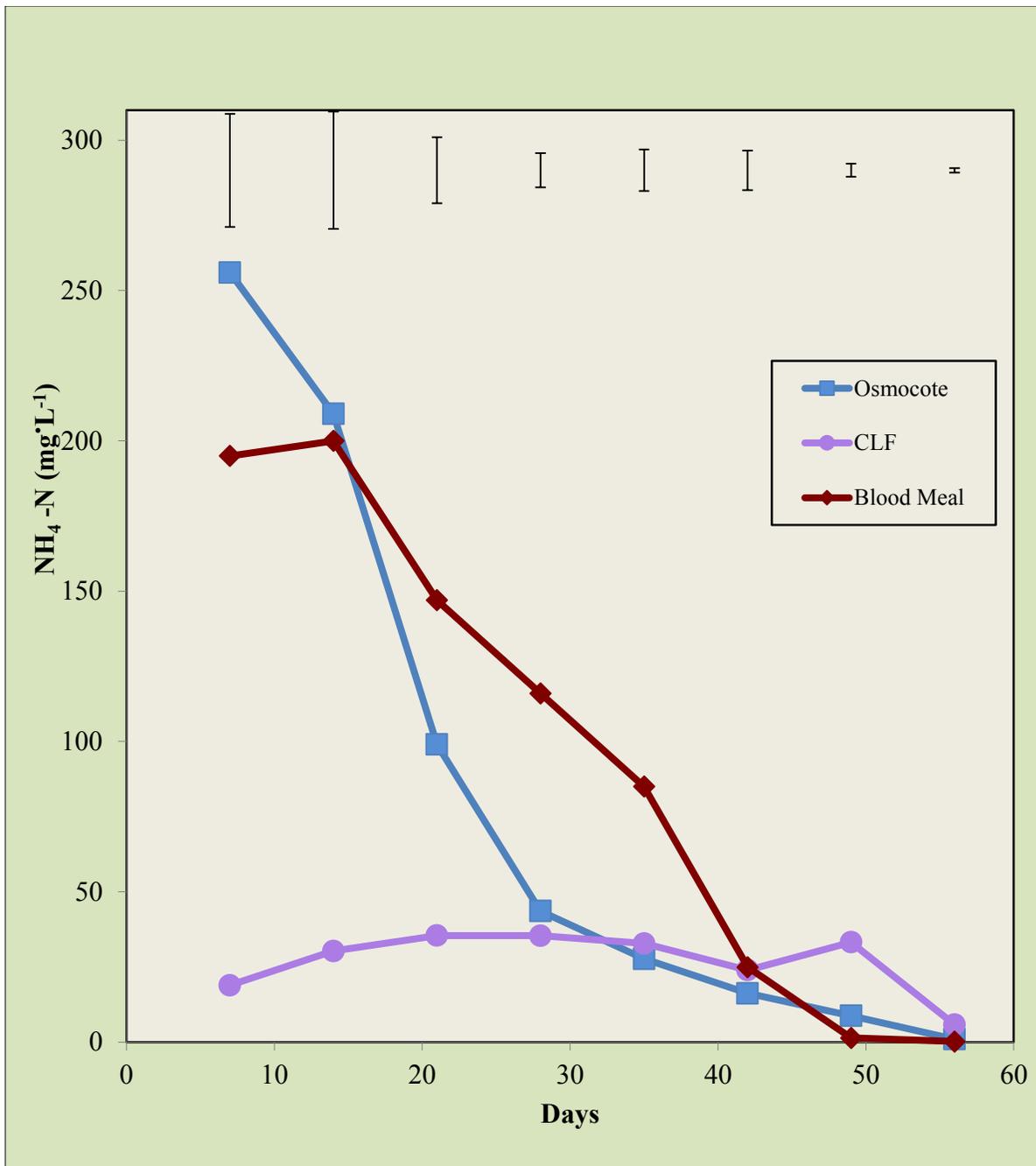
Figure 2-17. Fertilizer treatment effect on substrate NH₄-N concentration in experiment 2.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
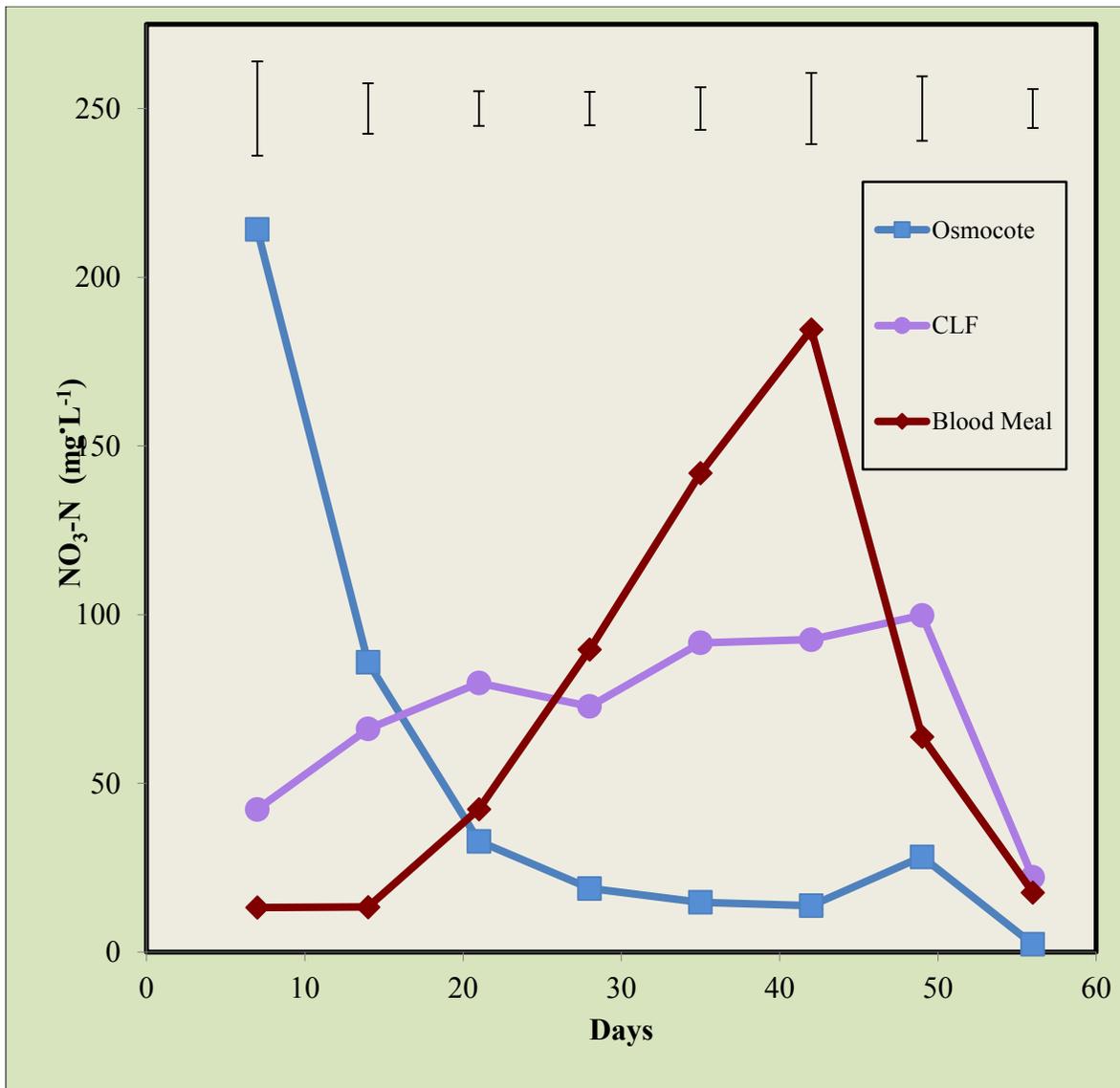
Figure 2-18. Fertilizer treatment effect on substrate NO₃-N concentration in experiment 2.
Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
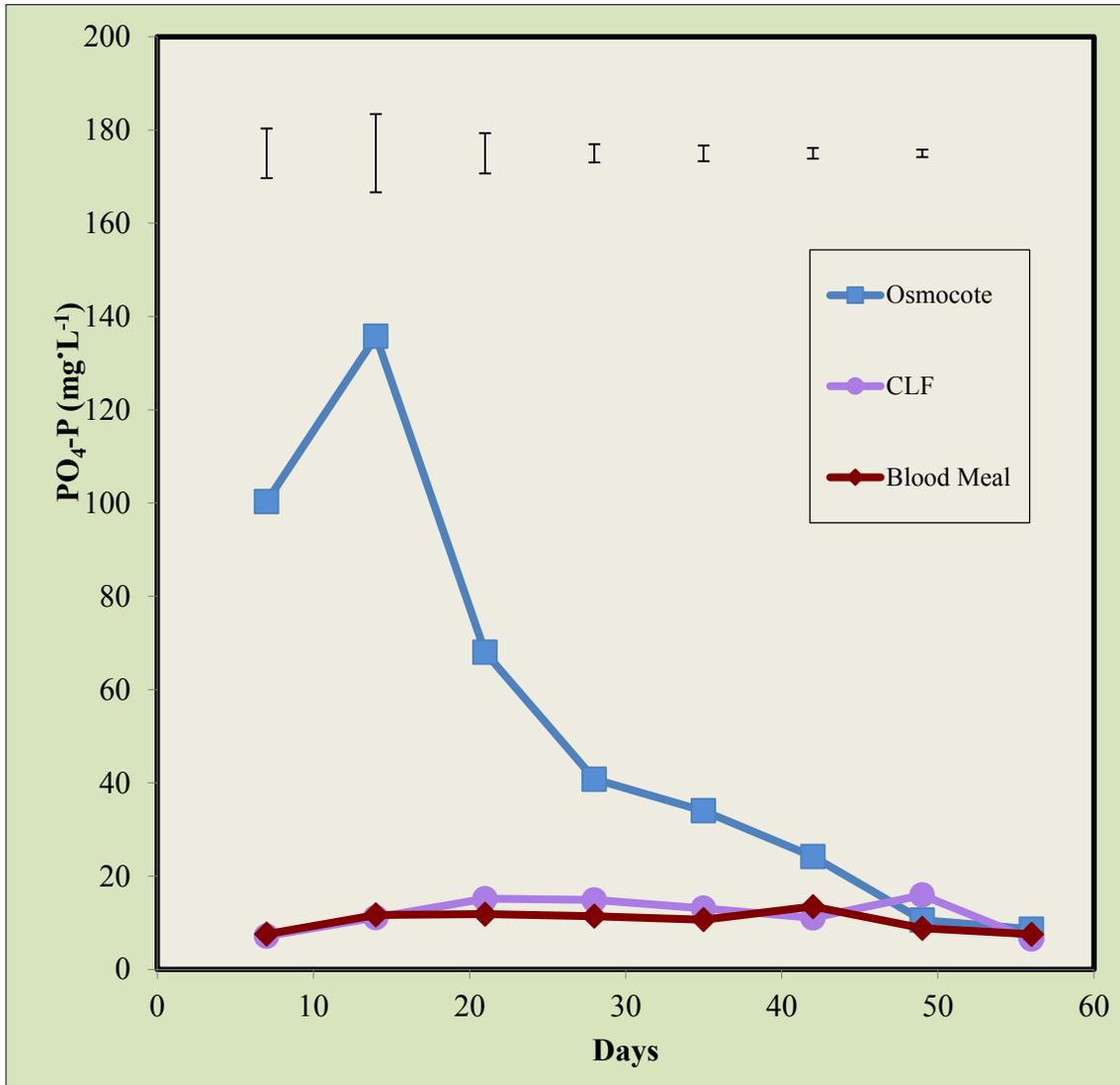
Figure 2-19. Fertilizer treatment effect on substrate PO4-P concentration in experiment 2.
Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
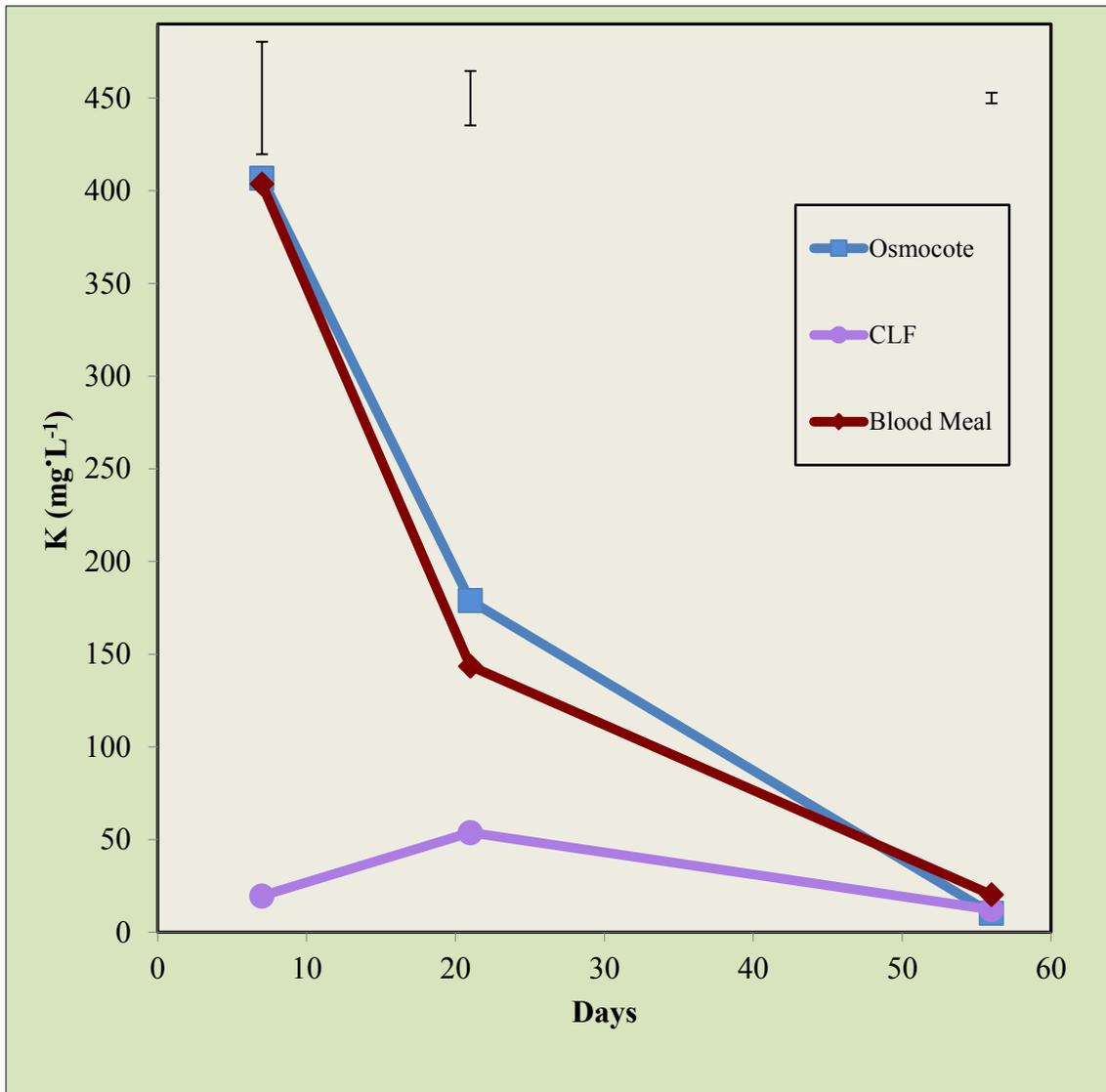
Figure 2-20. Fertilizer treatment effect on substrate K concentrations in experiment 2.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
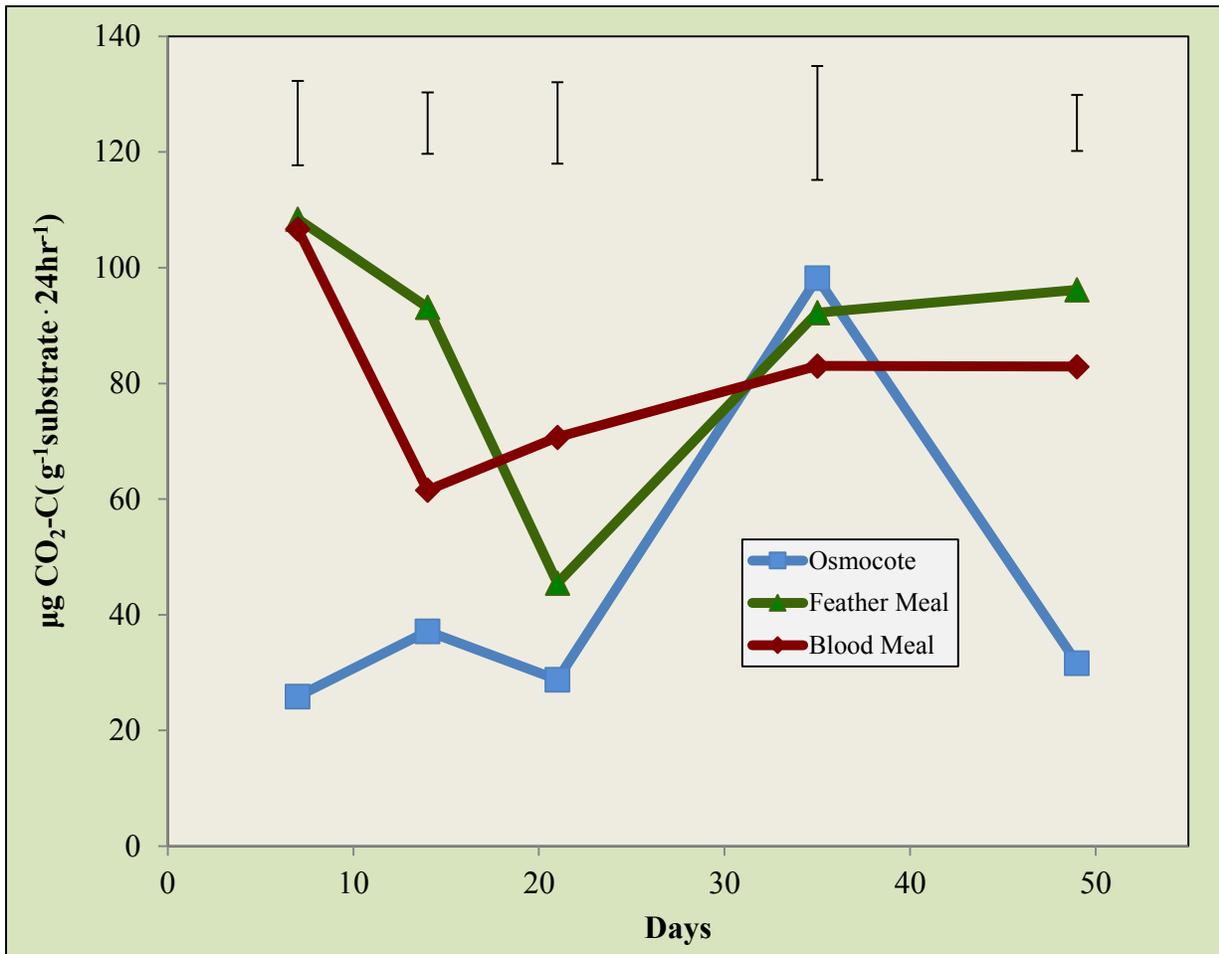
Figure 2-21. Fertilizer treatment effect on CO_2-C respiration from microbial activity in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
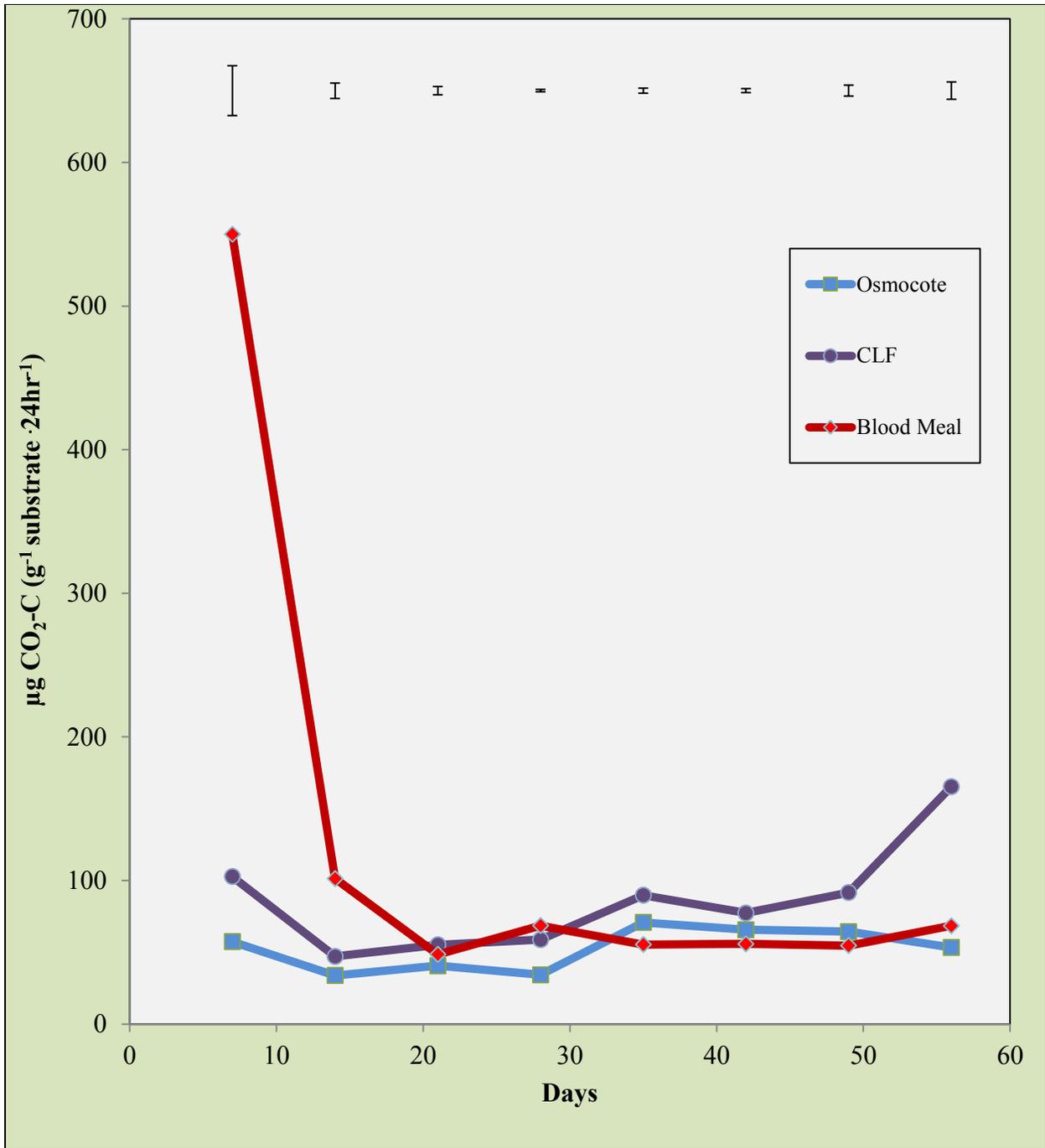
Figure 2-22. Fertilizer treatment effect on CO_2-C respiration from microbial activity in experiment 2.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.
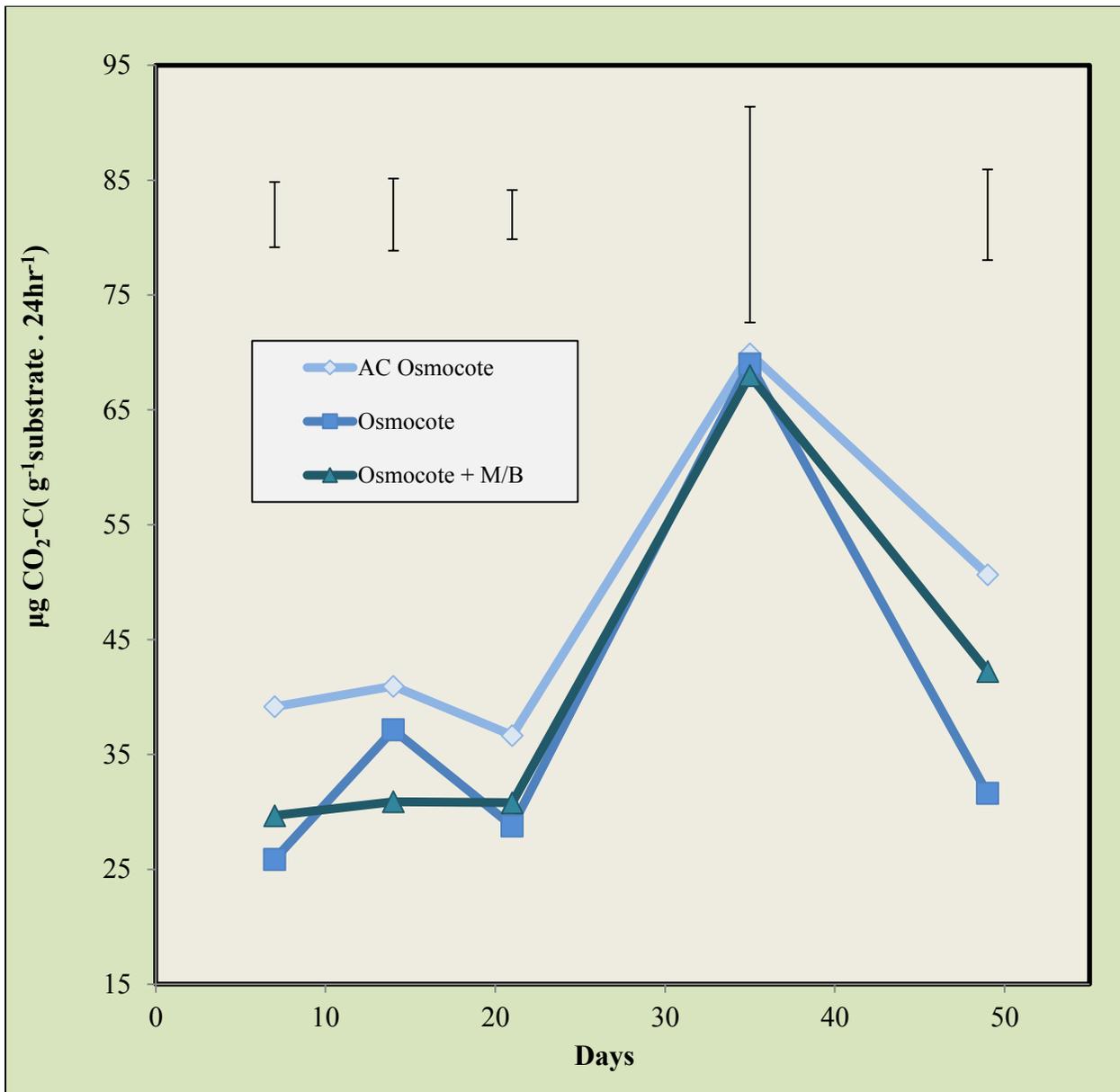
Figure 2-23. Osmocote^{TM} and inoculant treatment [autoclaved (AC) or Sub Culture M/B (M/B)] effect on CO$_2$-C respiration from microbial activity in experiment 1.

Bars indicate a 'by date' standard error analysis of the least squared means; n=4.

Chapter 3 - Organic or Inorganic Fertilization of Butterhead Lettuce (*Latuca sativa*) with or without Microbial Inoculants in an NFT Hydroponic System

Introduction

Persistent challenges have been reported when organic fertilizer sources are used in hydroponic crop production systems. These challenges include extreme pH fluctuation when managing organic nutrient mixes, inconsistent nutrient mineralization rates and varying nutrient concentrations with regards to electrical conductivity (EC) measurements of the nutrient solution. Compared to the performance of conventional, inorganic nutrient sources for nutrient film technique (NFT) production, organic nutrient regimens are much more variable.

Some research has focused attention on organic fertilizer incorporation in NFT hydroponics. Garland et al. (1997) showed that waste residue contained excess organic compounds and phytotoxins that proved to be deleterious to plant growth in closed hydroponic systems. Garland et al. (1997) identified the primary challenge to overcome when adopting organic fertilizers as maximizing plant growth using organic nutrient sources in a manner that is comparable to the resulting plant growth from using inorganic fertilizers. Similarly, comparing lettuce (*Latuca sativa*) growth from organic waste and processed fish emulsion regimens to conventional nutrients, Atkin and Nichols (2004) found reduced growth rates in organic hydroponic treatments.

With the goal of improving performance and consistency of organic fertilizers, efforts have been made to develop processing techniques that provide suitable organic nutrient sources for hydroponic crop production. The majority of organic nutrient sources are derived from proteins that provide NH_4-N and very little NO_3-N. Shinohara et al. (2011) reported on the development of processing methods for liquid organic fertilizers that increased levels of NO_3-N via a 50 day microbial conversion treatment. Using advanced processing techniques, private industry has begun to market organic nutrient sources with full complements of macro and micronutrients for use in hydroponic systems. When applied at recommended application rates, marketable plant growth can be attained by using organic nutrient components in a NFT production system.

Despite improvements in organic fertilizer performance in hydroponic systems, many crop producers are considering supplemental products that may potentially increase plant growth. Microbial inoculant products are becoming more numerous and diverse. Inoculant products are listed as containing spores or propagules of beneficial microbial species. Beneficial bacterial and fungal species have been identified as beneficial for plant growth. Increased resistance to root rot in hydroponic crop production has been reported with the addition of rhizobacterial populations (Rankin and Paulitz, 1994). Other studies have shown the ability to establish beneficial mycorrhizal colonies in NFT hydroponic systems, provided a physical matrix is present and 12 hour root dry-down periods are established (Lee and George, 2005).

With a goal of exploiting beneficial microbial populations, commercially available inoculant products are advertised to 'boost' beneficial microbial populations, benefit plant growth and suppress disease and insect pest damage. Inoculant products are marketed for use in soil and in soilless/hydroponic crop production. Products may include beneficial bacteria and mycorrhizal fungi in a liquid or powder carrier. Studies have evaluated microbial inoculant products in soil-based and nursery container production. A recent study reported no benefit from mycorrhizal inoculant addition in non-nutrient limiting production systems (Cwala et al., 2010). Russo and Fish (2012) claimed that no consistent benefit from inoculant addition was observed. Corkidi et al. (2005) reported varied improvements to sweetgum (*Liquidambar styraciflua*) plant growth with a 14 week exposure to commercial mycorrhizal inoculant products. A large inoculant product base and conflicting research results on the efficacy of certain microbial inoculants suggest that further experimentation is required.

Evaluating organic fertilizers and microbial inoculant products as two components of sustainable crop production may offer insight towards addressing the challenges of organic fertilizer use in a closed NFT vegetable production system. The objectives of this research were to 1) characterize nutrient solution pH, EC, NH_4-N, NO_3-N, and general microbial activity when inorganic and organic nutrient regimens were used to produce butterhead lettuce (*Latuca sativa*) in a NFT system; 2) determine if addition of microbial inoculants affects lettuce growth in a NFT system; and 3) evaluate petiole nitrate concentrations of lettuce produced with the different nutrient regimens. Results may provide producers an opportunity to make more informed management decisions regarding use of organic fertilizers and/or some microbial inoculants.

Materials and Methods

Four hydroponic experiments were conducted in the glass greenhouse range of Kansas State University's Throckmorton Plant Sciences Center. Experiment "day 1" occurred when lettuce transplants were set in hydroponic troughs. The dates of the experiments were as follows: Experiment 1 from 9 Jan (day 1) to 24 Feb (day 40); Experiment 2 from 27 Mar (day 1) to 4 May (day 40); Experiment 3 from 29 May (day 1) to 3 July (day 35), and Experiment 4 from 12 Sept (day 1) to Nov. 15 (day 63). Experimental designs for all experiments were randomized complete block (RCBD) with three blocks and three replications. An experimental unit consisted of one NFT trough and nutrient solution stock tank with six heads of lettuce. Experiment 1 consisted of four inorganic nutrient treatments, with the final three experiments consisting of a combination of inorganic and organic fertilizer treatments (Table 3-1).

Plants and Growing Environment

Butterhead lettuce (*Latuca sativa* L. 'Fidel' (Paramount Seeds, Stuart, FL) was used in Experiment 1 and 'Rex' (Paramount Seeds, Stuart, FL) was used in all of the other experiments. Seeds were germinated and grown in 5 cm diameter, 75 cm^3 black net pots (OS Plastic, Stone Mountain, GA). For seed germination, pots were filled solely or partially with loose rockwool (Grodan, Hedehusene, Denmark), as follows. In experiments 1 and 4, net pots were filled completely with rockwool. For experiments 2 and 3, net pots were filled with LECA clay pebbles (Hydroton, Oketau, Germany) except for the upper 3 cm, which was filled with rockwool. Seeds were germinated under mist and fertilized with 100 mg$\cdot$L^{-1} N from 20 N-4.4 P-16.6 K (JR Peters, Inc., Allentown, PA) as a starter nutrient source. Fourteen days after germination, the seedlings were transplanted to hydroponic troughs and remained in the system for the duration of the experiments. Plants were grown with natural day lengths and under whitewashed glass glazing to manage high temperatures. Temperature and relative humidity were monitored using HOBO Environmental Monitors (Onset Computer Company, Bourne, MA). In experiment 1, daytime (0500 to 1600hrs) temperature of the production space averaged 24.2°C, with night temperatures (1601 to 0459 hrs) at 20.6°C and relative humidity ranged from 48% to 63%. In experiment 2, daytime (0500 to 1600 hrs) temperature of the production space averaged 27.4°C, with night temperatures (1601 to 0459 hrs) at 23.9°C and relative humidity ranged from 52% to 68%. In experiment 3, Daytime (0500 to 1600 hrs) temperature of the

production space averaged 35.3°C, with night temperatures (1601 to 0459 hrs) at 31.2°C and relative humidity ranged from 73% to 86%. In experiment 4, daytime (0500 to 1600 hrs) temperature of the production space averaged 22.5°C, with night temperatures (1601 to 0459 hrs) at 20.1°C and relative humidity ranged from 59% to 65%.

Minor outbreaks of thrips (*Frankliniella occidentalis)* were managed with applications of a tank mix of azadirachtin (Azatin' OHP Inc., Mainland, PA) and *Beauveria bassiana* strain GHA (Botanigard[TM], BioWorks, Inc,.Victor, NY) once per study on day 14 of Experiment 1, day 12 of experiment 2, day 8 of the experiment 3 and at day 19 of experiment 4; a single application was sufficient to control thrips populations.

Hydroponic Culture

A nutrient film technique (NFT) system was used for all four experiments. Constantly recirculating nutrient solution was delivered to PVC troughs (7.6 cm x 6.4 cm x 170.2 cm) via an ECO 264 submersible pump (Sunlight Supply, Vancouver, WA) using 1.27 cm black poly-vinyl tubing (Hydrofarm, Grand Prairie, TX). Eighteen liter reservoirs (RoPack, Fullerton, CA) were used for all experiments except Experiment 4, during which 100 L Lexton reservoirs (Lewis Bins, Oconomowoc, WI) were used. The nutrient solution in the reservoirs was constantly aerated using one ECO Plus 15.2 cm^3 air stone (Sunlight Supply, Vancouver, WA) and one 70 $L^.hour^{-1}$ aquarium air pump.

The water source for all experiments was a municipal source from the City of Manhattan, Kansas' water treatment facility. Alkalinity was reported as 45 $mg^.L^{-1}$ $CaCO_3$ equivalent which did not buffer the water pH of 8.9. The hardness was reported at 110 $mg^.L^{-1}$, EC = 0.4 $ds^.m^{-1}$, Na = 30 $mg^.L^{-1}$ and Cl = 35 $mg^.L^{-1}$.

Fertilizer Treatments

All four experiments included an inorganic nutrient regimen (Inorg) adapted from optimal nutrient rates published by Cresswell (1991) for NFT lettuce production (Table 3-2). In experiment 1, inorganic nutrients were mixed to an electrical conductivity (EC) of 2.2 (high phase) from the initiation of the experimental treatments. To better suit the nutritional needs of the lettuce plants at different stages of development, in experiments 2, 3 and 4 a reduced inorganic nutrient concentration was applied to young plants, and after two weeks of growth, an increased nutrient concentration was used for the more mature plants. The initial regimen (low

phase) was used for 14 days at the beginning of the production cycle with an EC of about 1.1. After two weeks of established plant growth the 'high phase' nutrient regimen was implemented for the remainder of the production cycle.

Experiments 2, 3 and 4 included organic fertilizer regimens (Org) to compare to the inorganic treatments (Table 3-2). In experiment 2 Espartan [(2.7N-3.3P-2.6K; Kimitec (Almeria, Spain) distributed by HortAmericas (Euless, TX)] was used as the sole organic component (Table 3-3). With each experiment, the organic nutrient regimens were modified based on previous results to better optimize growth response. In experiments 3 and 4, additional organic amendments were added to increase organic fertilizer performance with regards to plant growth. In experiment 3, Espartan^{TM}, Caos^{TM}(10.5% Ca), Tunda Mix^{TM}(0.1% Cu, 2.5% Fe, 1.4% Mn, 0.1% Mo, 0.2% Zn) and potassium magnesium sulfate (KMS; 0N-0P-18.3K, Diamond K Gypsum Inc., Richfield, VT) were mixed as a full part organic regimen at both an initial 1.8 EC and then increased to 2.2 EC after 14 days. In experiment 4, Bombadier (8N-0P-0K Kimitec (Almeria, Spain) distributed by HortAmericas (Euless, TX)) was added as a supplemental organic nitrogen source (Table3-3).

Nutrient solution pH was adjusted using 0.8 N HCl to decrease pH and 0.8 N NaOH to increase pH; both were added using a 'Finnpipette' 1 to 5 ml pipet (Fischer Scientific Inc., Pittsburg, PA). Best efforts were made to maintain a reservoir pH within the range of 5.5 to 6.0. Reservoir volumes were maintained consistently amongst all treatments using equal volume tap water additions for reservoir dilution. In the first three experiments, 18 L reservoirs were filled with 12 L of solution and in experiment 4, the 100 L reservoir volume was filled with 80 liters of nutrient solution.

Inoculant Treatments

An UV filtration treatment (UV) was incorporated in experiments 1, 2 and 3, as a control treatment to observe a treatment effect that would limit microbial population growth within the hydroponic nutrient solution. An 'Advantage Ultraviolet Sterilizer 2000' (Aqua Ultraviolet, Temecula, CA) was connected to the nutrient supply line, subjecting the reservoir nutrient solution continuously to UVB spectrum light.

Inoculant products tested in the hydroponic growth trials included: Sub Culture M^{TM} with Sub Culture B^{TM} (0.1 N-0.04 P-0.02 K) combination (M/B), both from General Hydroponics (Sebastool, CA); and EM1 (EM1; TeraGanix, Alto, TX). The Sub Culture M and B product combination represented an endo/ecto mycorrhizal fungi inoculant and bacterial inoculant mix with several fungal (e.g. *Glomus intradices, Glomus aggregatum and Trichoderma harzianum*) and bacterial (e.g. *Bacillus subtilis, B. pumilis)* species. The Sub Culture M was applied to the reservoirs at 0.37 g·L^{-1} water in combination with Sub Culture B at 0.1 g·L^{-1} water at every nutrient solution change.

EM1 was OMRI certified as a proprietary blend of beneficial microorganisms including such species as *Lactobacillus* spp. EM1 was applied at 7.7 ml·L^{-1} water. Cost of inoculant products is based on pricing made available to the general public at the time of this publication. Sub Culture B and Sub Culture M were $29.99 for 200 g quantities. The EM1 was purchased for $14.99 for 0.95 L.

Inoculant Product Analysis

Inoculant products were submitted to Kansas State University Soil Testing Laboratory (Manhattan, KS) for total nutrient analysis. LECO TruSpec analysis for C:N ratio and nitric perchloric digest done by ICP Spectrometer (720-ES, Varian Ltd, Mulgrave AUS) was performed to assess P, K, Ca, Mg, Zn, Fe, Mn and Cu concentrations on TSS samples as described by Csuros (1997). Nutrient additions from microbial inoculant products were not always negligible. Sub Culture M inoculant had significant concentrations of N, K and Ca (Table 3-4 and 3-5). The Sub Culture B and EM1 products contained much less supplemental nutrition than was measured in the Sub Culture M. Only the EM1 inoculant product was listed as OMRI^{TM} certified for organic crop production.

To determine the presence and viability of the species reported to be contained in the inoculum products, serial dilutions were prepared, plated and analyzed using polymerase chain reaction (PCR). A 20% dilution of Sub B and EM1 was prepared using nuclease-free water in a sterile 50 ml centrifuge tube. Forty microliters of the dilution was subsequently spread onto LB agar plates and incubated for 48 h at 30°C. Single colonies of the bacteria were visible on the LB plates after incubation for 48 h. Based on colony sizes and color, single colonies were selected and streaked separately onto new LB agar plates for further purification. Single colonies from the

subcultures were thereafter applied in a PCR for amplification of their respective 16S genomic DNA regions. The PCR thermocycling protocols (MJ Research PTC-100 Peltier thermal cycler) were: 94°C for 2 min, followed by 31 cycles at 94°C for 40 s; 55°C for 1 min; 72°C for 10 s; then 72°C for 7 min, and finally held at 4°C. PCR reaction mixtures containing no DNA served as control. The PCR products were visualized with ethidium bromide (1 µl per 100 ml of Agarose) in 1% Agarose gel with ultraviolet light, cleaned and sequenced.

Approximately 2 g of Sub Culture M was weighed-out and spread directly on a 9-cm-diameter Petri-plate containing one-fourth strength potato-dextrose agar (Difco Laboratories, MD) amended with tetracycline (10 mg$\cdot$L^{-1}) and streptomycin (10 mg$\cdot$L^{-1}) (designated as "1/4 PDA^{++}") (Biotech Research Grade, Fisher Scientific Inc., NJ). Two plates of the sample were prepared and maintained at 23°C in the dark to allow for fungal outgrowth. Fungal growths were visible after 24 h of incubation. Subcultures of five fungal colonies from the two plates were made onto fresh 1/4 PDA^{++} plates and later used for genomic DNA isolation.

Genomic DNA was isolated from fungal colonies isolated from Sub Culture M using a modified method of Jiangfeng et al. (2005). Isolates were grown from 5 mm-diameter potato dextrose agar-mycelial discs at 25°C for 5 days in the dark in complete media (modified from Correll et al. (1987) and containing per liter of distilled H$_2$O: sucrose, 30 g; KH$_2$PO$_4$, 1 g; MgSO$_4$.7H$_2$O, 0.5 g; KCl, 0.5 g; NaNO$_3$, 2 g; N-Z amine A (casein), 2.5 g; yeast extract (Difco), 1.0 g; 10 ml vitamin solution (contained per liter of 50% ethanol: thiamine HCl, 100 mg; riboflavin, 30 mg; pyridoxine HCl, 75 mg; D-pantothenate Ca, 200 mg; *p*-aminobenzoic acid, 5 mg; nicotinamide, 75 mg; choline Cl, 200 mg; folic acid, 5 mg; D-biotin, 5 mg; and *myo*-inositol, 4 g); 0.2 ml trace element solution. The trace element solution contained (per 95 ml of distilled H$_2$O) – citric acid, 5 g; ZnSO$_4$•7H$_2$O, 5 g; FeNH$_4$)(SO$_4$)$_2$•6H$_2$O, 1 g; CuSO$_4$•5H$_2$O, 0.25 g; MnSO$_4$•H$_2$O, 50 mg; H$_3$BO$_4$, 50 mg; and NaMoO$_4$•2H$_2$O, 50 mg. After incubation, mycelia were collected by filtration in a 16.5 cm diameter filter paper (KenAG Non Gauze milk filter) and ground into fine powder in pre-chilled mortars and pestles with liquid nitrogen.

The powder was transferred into a 1.5 ml micro-centrifuge tube and filled to the 0.5 ml mark. Next, 700 µl of 65°C 2% cetyltrimethylammonium (CTAB) solution and 7 µl of 2-mercaptoethanol were added to each tube. The tubes were vortexed briefly (2 to 3 sec) to disperse any clumps of mycelia, placed in a 65°C incubator for 10 min, briefly (2-3 sec) vortexed again to further homogenize the mixtures in the tubes, and returned back into the 65°C incubator

for an additional 20 min. At the end of the incubation periods, 350 μl of chloroform:iso-amyl alcohol (24:1 v/v) was added to the tubes and vortexed briefly to thoroughly mix the aqueous and organic phases that formed in the tubes. The tubes were mixed gently by hand for an additional 5 minutes then centrifuged at $13,500 \times g$ for 6 min to separate the organic and aqueous phases. 600 μl of the aqueous (upper) phase was transferred into a fresh, sterile 1.5 ml micro-centrifuge tube.

The genomic sequences collected from the PCR anaylsis of the prepared inoculant samples were compared to known sequences using the BLAST[TM] Database. Query coverage for base pair matches was recorded. The resulting coverage from comparing colony growth from Sub Culture B when compared to known genomic sequences showed a $\leq 90\%$ match to *Bacillus* spp. Of the five *Bacillus* spp. listed on the product label: *B. subtilis, B. pumilis, B. cerus, and B. licheformis* were confirmed as present and viable using PCR analysis. The EM1 inoculant product showed a 95% matching coverage for *Lactobacillus* spp. and *Enterobacter* spp.

Data Collected

Plant Growth

To determine the fertilizer and microbial inoculant treatment effect on plant growth, the following data were collected: fresh and dry weights of both leaf and root tissue, and root length at end-crop harvest. Plants were harvested and weighed individually on day 40 of experiment 1, day 35 of experiments 2 and 3, and at day 63 of experiment 4. In experiments 2 and 3, roots were separated from the LECA clay pebbles and rockwool to assess accurate root measurements, as well as shoot mass. Shoot and root tissues were dried for 48 hours at 78°C to determine dry weights.

Nutrient Solution Analyses

Depending on the study, pH and EC of the solutions in the reservoirs were measured twice per day to almost daily using a hand-held pH/EC meter (Hanna Instruments, Ann Arbor, MI). Bi-weekly calibration of the meter was completed to ensure consistent equipment performance. Using a dual-ion probe with a 'CleanGrow CG001' Meter (Little Island Cork, Ireland) NH_4 mg·L^{-1} and NO_3 mg·L^{-1} were measured daily in all but experiment 4. The electrodes were cleaned and calibrated daily. Selected duplicate nutrient solution samples were submitted to Kansas State University Soil Testing Laboratories (Manhattan, KS). Results from the portable

ion probe and the lab analysis are highly correlated and show similar nutrient concentrations from the same sample (Table 3-6). In general, the CG001 meter provided results consistent with traditional nutrient analysis, though probe aging resulted in omission of the 7 final days of reservoir nitrogen analysis data collected in experiment 3.

In experiment 3, nutrient solution samples were analyzed for P and K concentrations from inorganic and organic nutrient sources at days 10, 15, 25, and 35. Nutrient analyses of the reservoirs were sampled beginning with non-inoculated inorganic treatments, followed by non-inoculated organic, and concluding with inoculated treatments. Both meters were rinsed with de-ionized water and dried between treatments to avoid contamination of non-inoculated treatments. All nutrient solution sampling was completed within a 15 minute time period.

Microbial Analyses

To measure CO_2 respiration from microbial activity within the nutrient solution, 10 ml nutrient solution samples were sealed and incubated for 48 hours. The gas from the incubated sample was analyzed for CO_2-C concentration using a gas chromatograph (GC-8A, Shimadzu Scientific Instruments, Columbia, MD). Chromatograph readings were compared against a known concentration of 10% CO_2 gas, subtracting the CO_2-C content of the ambient air, to calculate the quantities of CO_2-C that were respired from microbial activity within the sample solution.

To estimate CO_2-C respiration from an established hydroponic system, containing mature root systems, in the absence of microbial inoculants, inoculants were withheld from the experiment 1 treatments for 12 days prior to tissue harvest (Day 28). No significant change in CO_2-C respiration across inorganic treatments was observed without the presence of inoculants. No analytical benefit of this treatment alteration was identified; this practice was discontinued for the final three experiments.

To gain an understanding of pathogen levels that were present in the NFT hydroponic system and attempt to explain differences in growth results, reservoir samples were submitted the Guelph Laboratories (Ontario, Canada) for a DNA MultiScan of known plant pathogens (e.g. *Fusarium oxysporum, F. solani, P. drechsleri, and Pythium* spp.*)* from experiment 3. Results were reported on a scale from 1 to10 indicating incidence and severity of pathogens. Reported values of 1 to 3 indicate low incidence of scanned pathogens, 4 to7 moderate pathogen

incidence, and 8 to 10 indicating high incidence. The results reported relatively low levels of pathogen incidence in all treatments and there was no significant difference in reported pathogen levels across experimental treatments (Table 3-7).

To determine root colonization from mycorrhizal inoculant product application, root samples from experiment 4 were collected and stained for microscopic observation. Fresh root tissue was collected from established plants. Root piece sections were harvested and placed in 5 cm x 5 cm sampling screens. The samples were rinsed in deionized water and soaked in 5% KOH for 20 minutes under heat. Sample screens were rinsed with 1% HCl and heated with Typhan blue dye for 15 minutes. Stained root pieces were analyzed under a 'SG3500' (Nikon Inc., Tokyo, Japan) microscope and a Nikon 'C-FMC' dissecting microscope (Nikon Inc., Tokyo, Japan). Multiple sections from each root sample were observed.

Leaf Petiole Nitrate Analysis

To analyze differences in petiole concentrations of NO_3-N, leaf petioles were harvested and analyzed on days 35, 49, 56, and 63 of experiment 4. A 5 cm length of leaf petiole tissue was harvested from the youngest fully expanded leaf from three lettuce heads per trough. The three petioles were combined and mashed using a garlic press. The petiole sap was placed on sampling tissue atop a CARDY meter (Horiba Ltd., Japan) for NO_3-N analysis (Hartz et al., 1994).

Statistical procedures

All data were analyzed by date using SAS ver. 9.1.3 (SAS Institute Inc., Cary, NC) using PROC MIXED procedure. Adjusted pairwise comparisons of the treatment effects were made across fertilizer treatments within each respective experiment. For experiments 2 and 3, Tukey-Kramer adjusted pairwise comparisons were made within inorganic and organic treatments.

Results and Discussion

Plant Growth

The Inorg nutrient treatments resulted in greater shoot growth in all experiments when compared to the Org treatments with respect to dry weight (Table 3-8). In experiment 1, no differences in growth response were measured as a result of the inoculant treatment effect. All four Inorg treatments supplied ample nutrition for plant development, and with little disease or

insect pressure, the opportunity for improvement with inoculant addition was likely reduced in experiment 1.

In experiment 2, the inoculant treatments resulted in no significant growth differences in the Inorg, Inorg + UV or Inorg + M/B. Inoculant addition contributed to increased fresh and dry shoot weights and dry root weight in the Org + M/B treatment when compared to the standard Org treatment (Table 3-8). The nutrient levels of the Org treatments in experiment 2 were low, so supplemental N from Sub Culture M may have resulted in a measureable increase in plant growth.

Despite the incorporation of a complete organic nutrient regimen in experiments 3 and 4, Inorg treatments provided the greatest dry weight when compared to Org treatments. In experiment 3, inoculant addition resulted in increased in plant growth when compared to the standard treatment. The Inorg + M/B nutrient regimen resulted in an increase in fresh and dry shoot weight and root length when compared to the standard Inorg treatment (Table 3-8). In the organic nutrient treatments, tissue and root fresh weight, tissue dry weight and root length were increased as a result of the Org + UV and Org + M/B treatments over the Org treatments.

In experiment 4, fresh weights were comparable (Table 3-8) across Inorg and Org treatments with the addition of the supplemental N source (Bombardier[TM], Kimitec). Both Org treatments produced a marketable counterpart to the Inorg treatments in the same production system. As was the case in all experiments, dry weight was the greatest in the Inorg treatments. No significant differences in plant growth measurements were observed with inoculant addition in experiment 4.

Nutrient Status of the NFT Reservoirs

Electrical Conductivity

The EC of the nutrient solutions across the four experiments was monitored and maintained daily. Inoculant addition resulted in no significant difference in reservoir EC between the four Inorg treatments in experiment 1 (Fig 3-1). In experiment 2, utilizing only the single organic nutrient component, EC was higher in the Inorg treatments at all times during the cropping cycle compared to Org treatments (Fig. 3-2). In experiment 3 the full complement organic regimen resulted in a higher EC during the first two weeks when compared to the inorganic, 'low phase' regimen (Fig. 3-3). When the inorganic nutrient concentrations were

increased to the 'high phase' EC of the cropping cycle, the Org and Inorg EC levels were generally comparable in experiment 3 (Fig 3-4) and experiment 4 (data not shown).

EC fluctuations were influence by two main factors. Early in the cropping cycle, EC would typically increase as a result of reservoir evaporation. With a relatively low nutrient uptake at this stage of plant growth, an increase in EC over time was observed. As plant size increased, EC would typically decrease following a change of reservoir solution to fresh nutrient regimens, with increasing plant uptake of nutrients. Increased plant size increased the frequency of applied fresh water dilution, which also affected a decrease in nutrient solution EC. The EC was restored to desired levels when fresh nutrient regimens were applied to replenish reservoir nutrient concentrations and when tap water dilutions to reduce excessive EC measurements.

pH

In general the pH of all Inorg treatments was consistently managed across all four experiments between 5.0 and 7.0. The reservoir pH was subject to fluctuation via plant nutrient uptake and acidification processes. Addition of tap water to maintain desired EC levels minimally affected nutrient solution alkalinity. Inorganic nutrient sources responded consistently to HCl/NaOH additions and remained relatively stable throughout the production cycle. There were no differences recorded in pH measurements among Inorg or Org treatments with the addition of UV or inoculant products in all four experiments (Fig. 3-5 to 3-9).

The Org treatments were consistently subject to extreme pH fluctuations following a change of reservoir solutions to a fresh nutrient regimen. Upon mixing a fresh set of organic nutrients, HCl was added to obtain the desired initial pH of 5.5. Within 24 hours the reservoir pH increased to 6.8 to 7.5 and remained at this level until further acid injections were made (e.g. Fig 3-7, 3-8, 3-9). The trend of increasing pH was consistently observed, which indicates a management challenge involved with organic fertilizers.

A reaction in the nutrient solution that drives increases in pH of fresh organic nutrient regimens is N mineralization. Through microbial processing of proteins that are prevalent in organic fertilizer sources, plant available ammonium is liberated from proteins to ionic form in the nutrient solution, resulting in an increase in solution pH (Bothe et al., 2006). Although plant uptake of cationic nutrients and nitrification processes can decrease solution pH, the release of

OH⁻ anions through ammonification results in a net pH increase upon application of fresh nutrient regimens.

NH₄-N

The Inorg treatments resulted in higher NH_4-N concentrations in the nutrient solution than was present in the organic nutrient treatments in experiments 1,2 and 3 (Fig. 3-10 to 3-14). The organic regimen used in experiment 3 provided the most similar NH_4-N concentration (40 $mg^.L^{-1}$) to that of the inorganic fertilizer regimen (65 $mg^.L^{-1}$; Fig 3-14).

In experiment 1, inoculant addition resulted in significantly lower NH_4-N concentrations measured in the reservoir at days 9, 10 and 26 ($p \leq .05$) throughout the production cycle (Fig 3-10). Experiments 2 and 3 resulted in a similar trend at which NH_4-N was significantly decreased with the addition of microbial inoculants to Inorg treatments [days 8, 10, 25 experiment 2 and days 15, 31 and 32 of experiment 3 ($p \leq .05$)].

In the organic regimens, inoculant addition had a similar effect on NH_4-N concentrations in the reservoir. In experiment 2 at days 7, 25, and 27 the Org + M/B treatment measured less NH_4-N when compared to the Org treatment ($p \leq .05$). In experiment 3, the Org treatment consistently had the greatest NH_4-N concentration when compared with the Org + UV or Org + M/B treatments (Fig.3-10). Typically, lower NH_4-N concentrations were observed in treatments that had the greatest plant growth responses.

NO₃-N

There was a substantial difference in the levels of NO_3-N present as a result of the fertilizer treatments. The inorganic nutrient regimens had 100 times the NO_3-N concentration upon mixing a fresh nutrient set (Fig. 3-15 to 3-21). Initially, organic nutrient sources offered very little NO_3-N and were subject to minimal increases in nitrate concentration 2 to 4 days following nutrient application to the reservoirs.

In experiment 1, no consistent changes to NO_3-N concentrations were observed with addition of microbial inoculants to inorganic treatments (Fig. 3-15). In experiment 2, NO_3-N concentrations in the reservoir were increased as a result of M/B addition at multiple instances in both inorganic and organic nutrient regimens (Fig. 3-16 and 3-17). In experiment 3, NO_3-N concentrations were increased with inoculant addition at multiple times during the low phase and high phase nutrient regimens (Fig. 3-18 to 3-21).

Org treatments contained NO_3-N concentrations that were very low ($\leq 5mg^.L^{-1}$ NO_3-N). The addition of the Sub Culture M product that contains supplemental N sources would likely increase plant available NO_3-N or NH_4-N in the nutrient solution. Supplemental N additions from inoculant product application were evident in the nutrient solution analyses.

Leaf Tissue NO_3-N Analysis

Petiole NO_3-N concentrations were significantly different between organic and inorganic nutrient regimens in experiment 4 (Fig. 3-22). The organic treatments had lower concentrations of petiole NO_3-N at all points during the experiment ($p \leq 0.05$). Inoculant product addition had no significant effect on petiole NO_3-N content when compared with a respective non-inoculated treatment.

The reduction in petiole NO_3-N concentrations in Org treatments when compared to conventional, inorganic nutrient sources indicates potential benefits to organic fertilizer use in hydroponic production.

Phosphorus and Potassium

In experiment 3, the P concentration of the Inorg treatments was higher (46 $mg^.L^{-1}$) than that of Org treatments (10 $mg^.L^{-1}$; Fig. 3-23). The organic fertilizer regimens supplied sufficient P for healthy plant growth with the Espartan component. No changes in P concentration were observed as a result of inoculant treatments, despite supplemental P (0.22 $mg^.L^{-1}$) additions in treatments that received the Sub Culture M/B inoculant product combination.

Potassium concentrations were comparable in experiment 3 with the incorporation of the KMS to the organic regimen. The KMS served as a viable organic K source that was readily available upon mixing to reservoir nutrient solutions. No significant differences in K concentrations were observed as a result of inoculant treatments despite supplemental K concentrations found in the Sub Culture M product (Fig. 3-24).

Analysis of Microbial Activity

CO₂-C Respiration

Differences in CO_2-C respiration from microbial activity within nutrient solution samples were measured as a result of the fertilizer and inoculant treatment effects. CO_2-C respiration measurements were not consistently affected by microbial inoculant addition during experiment 1 (Fig. 3-25). In experiment 2, the Inorg + M/B treatment resulted in increased CO_2-C respiration of incubated solution samples when compared to the Inorg treatment at days 7 and 21.

In experiment 3, the Org treatments and Inorg + M/B treatments had comparable CO_2-C respiration at day 1. At these times the Org and Inorg + M/B measured greater CO_2-C respiration when compared to the Inorg and Inorg + UV treatments, coinciding with application of fresh nutrients and inoculant product (Fig 3-26 and 3-27). It was common in both experiments 2 and 3 that the greatest CO_2-C respiration was measured upon application of fresh nutrient/inoculum sources. As the nutrient solution was exposed to constant recirculation, plant root/nutrient solution interaction, and with consumption of the microbial food supply, the CO_2-C respiration response decreased with nutrient solution age.

Root Colonization

No mycorrhizal colonization was evident under microscopic analysis of root samples from any experiment 4 treatments. Mycorrhizal colonies have been observed to require 70 to 80 days to reach 80% colonization in NFT production systems. This time frame for population establishment would not be met by short cropping cycle production, but could be advantageous in crops that require a longer production cycle such as woody ornamentals (e.g. Corkidi, 2005) Root support matrices and dry down periods, which were not present in these experiments, have been shown to encourage mycorrhizal establishment. However, differences in root structure were observed across fertilizer regimens. Fibrous root structures and increased root hair incidence were observed more frequently in organic nutrient regimens when compared to inorganic nutrient regimens (Fig 3-28, 3-29).

Discussion

With respect to the comparison of nutrient regimens, inorganic nutrients typically resulted in greater plant growth when compared to organic nutrients. With comparable fresh weights measured when a full complement of organic nutrients + supplemental organic nitrogen was used in experiment 4; processed organic fertilizers demonstrated viable and sustainable production potential. Achieving comparable plant growth with drastically reduced petiole NO_3-N concentration from organic fertilizer use may increase marketability with respect to consumer preference. Consumer concerns regarding tissue nitrate concentrations of food crops are driving interest towards improving plant growth responses when using organic nutrient sources. Increased public attention, specifically in European markets, has pressured food crop producers towards reducing tissue concentrations of nitrate. Konstantopoulou et al. (2012) described methods to reduce N rates while maintaining marketable yields in hydroponic lettuce production. Zhao et al. (2003) cited reductions in tissue nitrate concentrations with organic nitrogen sources when compared to inorganic sources.

Although some increases in plant growth were measured in inoculated treatments, the true causes of the growth responses are subject to speculation. The significant changes in plant growth were observed with the addition of the Sub Culture M product, but this material was analyzed to contain 0.8% Total N, and increased concentrations of P, K, Ca, and Fe when compared to the other inoculant products used. This analysis could account for the increased frequency at which no changes in growth were observed in inorganic treatments, where plant available nutrients were not limited. In the few instances where significant increases to growth were observed in inorganic treatments receiving inoculants, other properties of the products maybe influencing plant growth. Humic and fulvic acids, which are listed as being present on the Sub Culture M product label, have been reported to positively affect plant growth responses in previously published research (Arancon et al., 2006).

The greatest impact on CO_2-C respiration was the presence of organic fertilizers. Providing an ample and stable food source, the organic nutrient constituents where the most influential factor on microbial activity in these experiments. The increase in respiration with organic fertilizer applications is consistent with our results from experiments conducted in a peat-based substrate (Chapter 2). The application of inoculant products did increase CO_2-C respiration when compared to the standard Inorg treatments upon application of the fresh

nutrients and inoculum products in experiments 2 and 3. The increase in respiration in inorganic treatments with the application of microbial inoculant products is due to the rapid consumption of applied carbon (glucose) contained in the inoculant product. Immediately upon application of the inoculant products to inorganic nutrient regimens, the CO_2-C respiration was greatest followed by a sharp decline in respiration. Once a fresh inoculant treatment was applied a similar trend was observed.

Conclusion

The establishment of beneficial organisms within a rhizoshpere could offer potential benefits to plant growth in an NFT system. However, in the absence of disease pressure or low nutrient level scenarios, the direct effect of root colony establishment on plant growth results may be indiscernible. This could account for the observed inconsistency of inoculant efficacy. The specific results of inoculant product application to different production systems are too complex to identify reliable benefits. If a production system is subject to disease pressure, or limited nutrients, the potential for benefit from applied inoculants may be increased.

Regardless the reasons for the observed changes in plant growth, microbial inoculant addition did significantly increase plant growth in most of the organic nutrient regimens. Crop producers looking to increase yield when using organic fertilizers may find benefit in incorporating beneficial microbial inoculant products. While the diversity in microbial inoculant products makes specific recommendations impossible, making an effort to understand all potential implications from a specific microbial inoculant product applied to a specific production system can provide crop producers a more informed choice when considering inoculant product incorporation.

With increasing public awareness regarding lowering nitrate levels in food crops for human consumption, organic nutrient use and benefits inherent may be increased. Considering that marketable plant growth can be achieved with such a drastic reduction in both nutrient solution and petiole NO_3-N concentrations, conventional hydroponic production systems utilizing described rates of inorganic nutrients are inconsistent with both sustainable nutrient management practices and with public sentiment regarding preferred nutritional quality of the food product. The large divergence in NO_3-N levels used in conventional and solely organic lettuce production offers just cause to pursue an integrated nutrient management approach.

Although not certified under current organic guidelines, an integrated nutrient management approach may provide increases to plant growth beyond solely organic production systems while offering a reduction in overall mineral nitrate usage and petiole NO_3-N concentrations.

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Tables and Figures

Table 3-1. Treatment summary for hydroponic NFT trials.

Hydroponic Experimental Treatments
Experiment 1
Treatment 1: Inorganic Control (Inorg)
Treatment 2: Inorganic + UV solution sterilant (Inorg + UV)
Treatment 3: Inorganic + Sub Culture M/B (Inorg + M/B)
Treatment 4: Inorganic + EM1 (Inorg + EM1)
Experiment 2
Treatment 1: Inorganic Control (Inorg)
Treatment 2: Inorganic + UV solution sterilant (Inorg + UV)
Treatment 3: Inorganic + Sub Culture M/B (Inorg + M/B)
Treatment 4: Organic (Org)
Treatment 5: Organic + Sub Culture M/B (Inorg + M/B)
Experiment 3
Treatment 1: Inorganic Control (Inorg)
Treatment 2: Inorganic + Sub Culture M/B (Inorg + M/B)
Treatment 3: Organic Control (Org)
Treatment 4: Organic + UV solution sterilant (Org + UV)
Treatment 5: Organic + Sub Culture M/B (Org + M/B)
Experiment 4
Treatment 1: Inorganic Control (Inorg)
Treatment 2: Inorganic + Sub Culture M/B (Inorg + M/B)
Treatment 3: Organic (Org)
Treatment 4: Organic + Sub Culture M/B (Org + M/B)

Table 3-2. Nutrient concentrations for inorganic regimens in all experiments and full complement organic regimens used in experiment 3 (Exp 3) and experiment 4 (Exp 4), in mg·L^{-1} H$_2$O.

	Low phase EC Inorganic (mg·L^{-1})	High phase EC Inorganic (mg·L^{-1})	1.8 EC Organic Exp 3 (mg·L^{-1})	2.2 EC Organic Exp 3 (mg·L^{-1})	1.9 EC Organic Exp 4 (mg·L^{-1})	2.3 EC Organic Exp 4 (mg·L^{-1})
NO$_3$-N	75	150	≤ 5	≤ 5	≤ 5	≤ 5
NH$_4$-N	20	40	43	53	44	67
PO$_4$-P	20	40	21	26	10	13
K	105	210	85	93	60	77
S	42.5	85	60	60	60	60
Ca	60	120	69	69	69	69
Mg	25	50	30	30	30	30
Cu	0.05	0.1	0.26	0.26	0.2	0.2
Zn	0.05	0.1	0.26	0.52	0.52	0.52
Fe	1.5	3.0	6.5	6.5	6.5	6.5
B	0.25	0.5	-	-	-	-
Mo	0.025	0.05	0.26	0.26	0.26	0.26
Mn	0.25	0.5	3.6	3.6	3.6	3.6

Table 3-3. Rates of organic fertilizer (ml·L^{-1} H$_2$O) in experiments 2, 3 and 4.

Organic Fertilizer Regimens	EC = .8 Exp 2	EC = 1.8 Exp 3	EC = 2.2 Exp 3	EC = 1.9 Exp 4	EC = 2.3 Exp 4
Espartan™	1.0 ml·L^{-1}	1.6 ml·L^{-1}	2.4 ml·L^{-1}	0.7 ml·L^{-1}	1.0 ml·L^{-1}
Tunda™	-	0.3 ml·L^{-1}	0.3 ml·L^{-1}	0.3 ml·L^{-1}	0.3 ml·L^{-1}
Caos™	-	0.7 ml·L^{-1}	0.7 ml·L^{-1}	0.7 ml·L^{-1}	0.7 ml·L^{-1}
Bombadier™	-	-	-	0.7 ml·L^{-1}	1.0 ml·L^{-1}
KMS	-	0.2 g·L^{-1}	0.2 g·L^{-1}	0.02 g·L^{-1}	0.02 g·L^{-1}

Table 3-4. Macronutrient analysis of inoculant products.

Inoculant Product	Total N %	Total C %	P mg·L^{-1}	K mg·L^{-1}
EM1™	ND	ND	40	899
Sub B™	0.31	34.2	0.03	0.32
Sub M™	0.79	26.5	0.22	21.7

Table 3-5. Micronutrient analysis of inoculant products.

Inoculant Product	Ca mg·L^{-1}	Mg mg·L^{-1}	S mg·L^{-1}	Cu mg·L^{-1}	Fe mg·L^{-1}	Mn mg·L^{-1}	Zn mg·L^{-1}
EM1™	339	101	383	.5	9	4	1.4
Sub B™	0.05	0.02	0.001	0.0004	0.02	0.002	0.002
Sub M™	4.1	1.6	2.4	0.001	3.8	0.04	0.002

Table 3-6. Nitrogen analysis of solution sample (1-1, 1-2, etc.) comparison between Kansas State University (KSU) Soil Testing Lab and the portable 'CG0001' CleanGrow meter.

	KSU Soil Testing Laboratory		'CG0001' CleanGrow Meter	
Solution Sample	NH_4-N mg·L^{-1}	NO_3-N mg·L^{-1}	NH_4-N mg·L^{-1}	NO_3-N mg·L^{-1}
1-1	31.9	128.6	35.0	179.9
1-2	32.9	135.1	51.0	149.0
1-3	29.8	114.2	38.0	133.9
2-1	38.7	144.9	46.0	181.0
2-2	37.2	131.0	47.0	122.1
2-3	35.1	130.4	46.0	121.0
3-1	21.2	96.6	23.0	134.3
3-2	33.7	136.7	46.0	203.2
3-3	31.3	131.8	38.0	203.4
4-1	33.8	126.7	42.0	167.5
4-2	33.9	132.5	40.0	150.6
4-3	34.1	127.8	40.0	112.4
5-1	11.1	0.5	12.0	0.1
5-2	16.7	0.3	18.0	0.0
5-3	12.3	0.5	14.0	0.1

Statistical correlation (r) between laboratory and portable meter analysis = .96

Table 3-7. Average pathogen incidence values (1 to 3 = low incidence, 4 to 7 = moderate incidence, 8 to 10 = high incidence) in organic (Org) and inorganic (Inorg) treatments, with and without microbial inoculants [Ultraviolet (UV) or Sub Culture M/B (M/B)] as reported by University of Guelph Laboratories in experiment 3.

Treatment	*Fusarium oxysporum*	*Fusarium Solani*	P. *drechsleri*	*Pythium* spp
Org	2a[y]	3.0a	-	.5
Org + UV	3.7a	4.0a	1	-
Org + M/B	1.7a	1.0a	-	-
Inorg	0.7a	0.7a	-	-
Inorg + M/B	1a	1.7a	-	-
LSM*	0.01	0.03	0.01	0.02

*LSM reported as standard error of the Least Squared Means. Significance reported reflects Tukey-Kramer adjusted pairwise comparisons; n=3, (p≤.05).

[Y] Any two means within a column not followed by the same letter are significantly different.

Table 3-8. Treatment effect on plant growth in hydroponic NFT butterhead lettuce (*Latuca sativa*) experiments at end-crop harvests of day 40 in experiment 1, day 35 in experiment 2, day 35 in experiment 3, and day 63 in experiment 4. n=3

Treatment	Tissue Fresh Weight(g)	Root Fresh Weight (g)	Tissue Dry Weight (g)	Root Dry Weight (g)	Root Length (cm)
Experiment 1					
Inorg	84.0a^y	6.7a	4.3a	0.6a	65.2a
Inorg + UV	114.0a	12.0a	5.0a	0.8a	45.7a
Inorg + M/B	102.3a	9.3a	4.3a	0.7a	53.6a
Inorg + EM1	60.0a	8.7a	3.1a	0.6a	63.4a
LSM*	15.8	1.6	0.6	0.2	1.5

Experiment 2					
Inorg	151.2a^y	51.7a	7.9a	3.8a	22.1a
Inorg + UV	189.7a	52.9a	12.3a	3.8a	23.1a
Inorg + M/B	159a	58.9a	7.6a	3.0a	25.8a
Org	19.7d	19.4d	1.7d	1.6d	15.4d
Org + M/B	34.4c	33.3c	2.1c	3.3c	22.8c
LSM Inorg**	8.8	2.2	1.4	0.5	0.04
LSM Org***	3.7	3.4	0.4	0.5	2.5

Experiment 3					
Inorg	245.1c	17.8c	8.6c	1.61b	41.7c
Inorg + M/B	281d	24.1c	9.7d	1.86b	54.7d
Org	154.9b^y	19.2b	5.8b	1.5b	26.3b
Org + UV	187a	40.7a	7.5a	2.3b	38.7b
Org + M/B	183.5a	41.5a	6.8ab	2.6a	52.3a
LSM Inorg**	9.1	2.9	1.2	0.87	5.1
LSM Org***	6.9	3.8	2.5	1.9	2.9

Experiment 4					
Inorg	266.7a^y	-	11.2a	-	-
Inorg + M/B	274.9a	-	10.0a	-	-
Org	176.9a	-	6.0b	-	-
Org + M/B	222.2a	-	6.9b	-	-
LSM*	21.8	-	0.6	-	-

*LSM reported as standard error of the Least Squared Means. Significance reported reflects Tukey-Kramer adjusted pairwise comparisons. (p≤.05)

**LSM reported as standard error of the Least Squared Means for Inorganic treatments. Significance reported reflects Tukey-Kramer adjusted pairwise comparisons. (p≤.05)

***LSM reported as standard error of the Least Squared Means for Organic treatments. Significance reported reflects Tukey-Kramer adjusted pairwise comparisons. (p≤.05)

^{Y} Experimental means within a column not followed by the same letter are significantly different
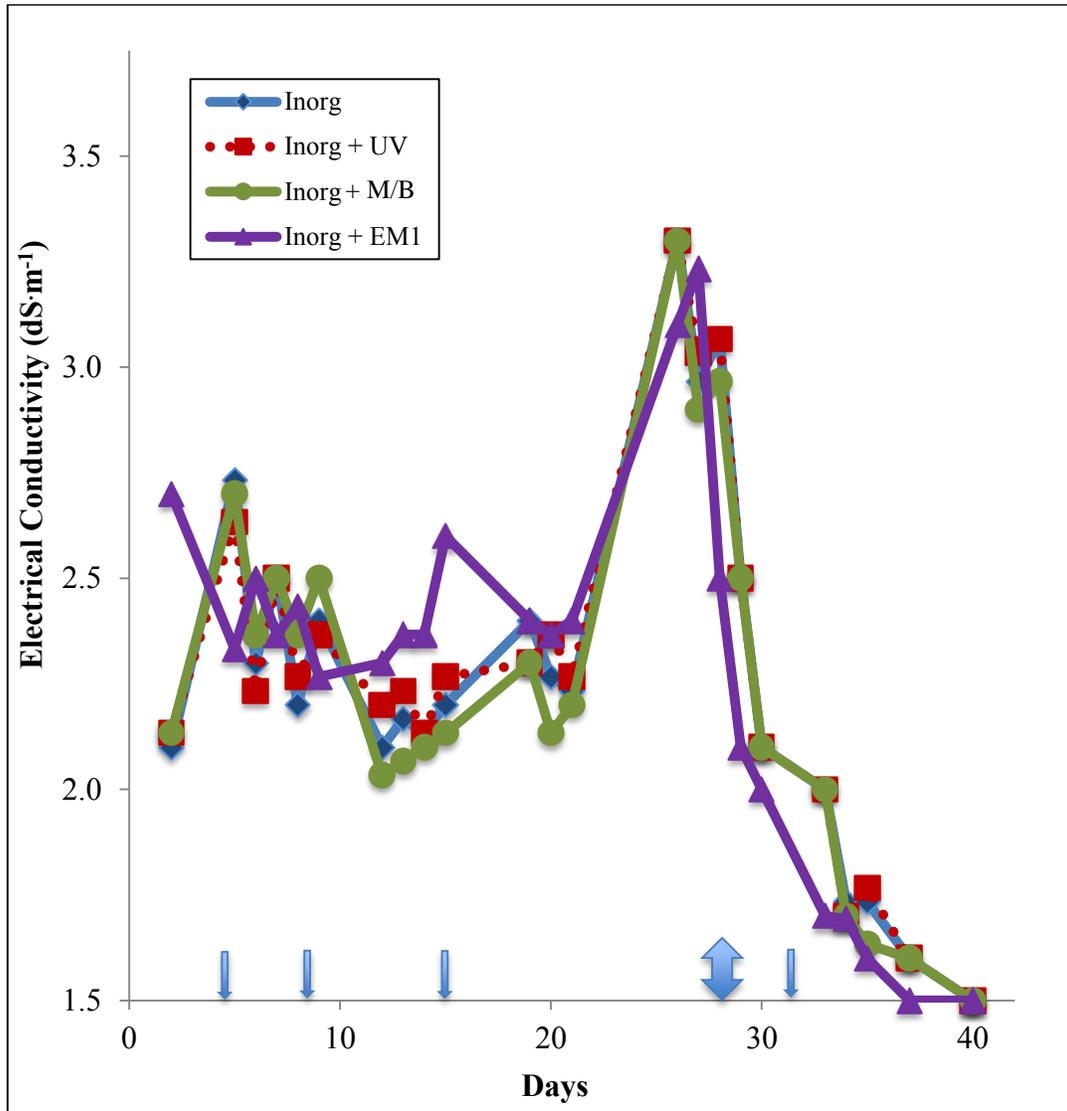
Figure 3-1. Inoculant treatment [Ultraviolet (UV), Sub Culture M/B (M/B) or EM1 (EM1)] effect on inorganic (Inorg) nutrient solution EC in experiment 1.

Standard error of the least squares means estimate = 0.06; n=3.

⬇ = application of tap water 'top up'

⬍ = application of fresh nutrient regimen.
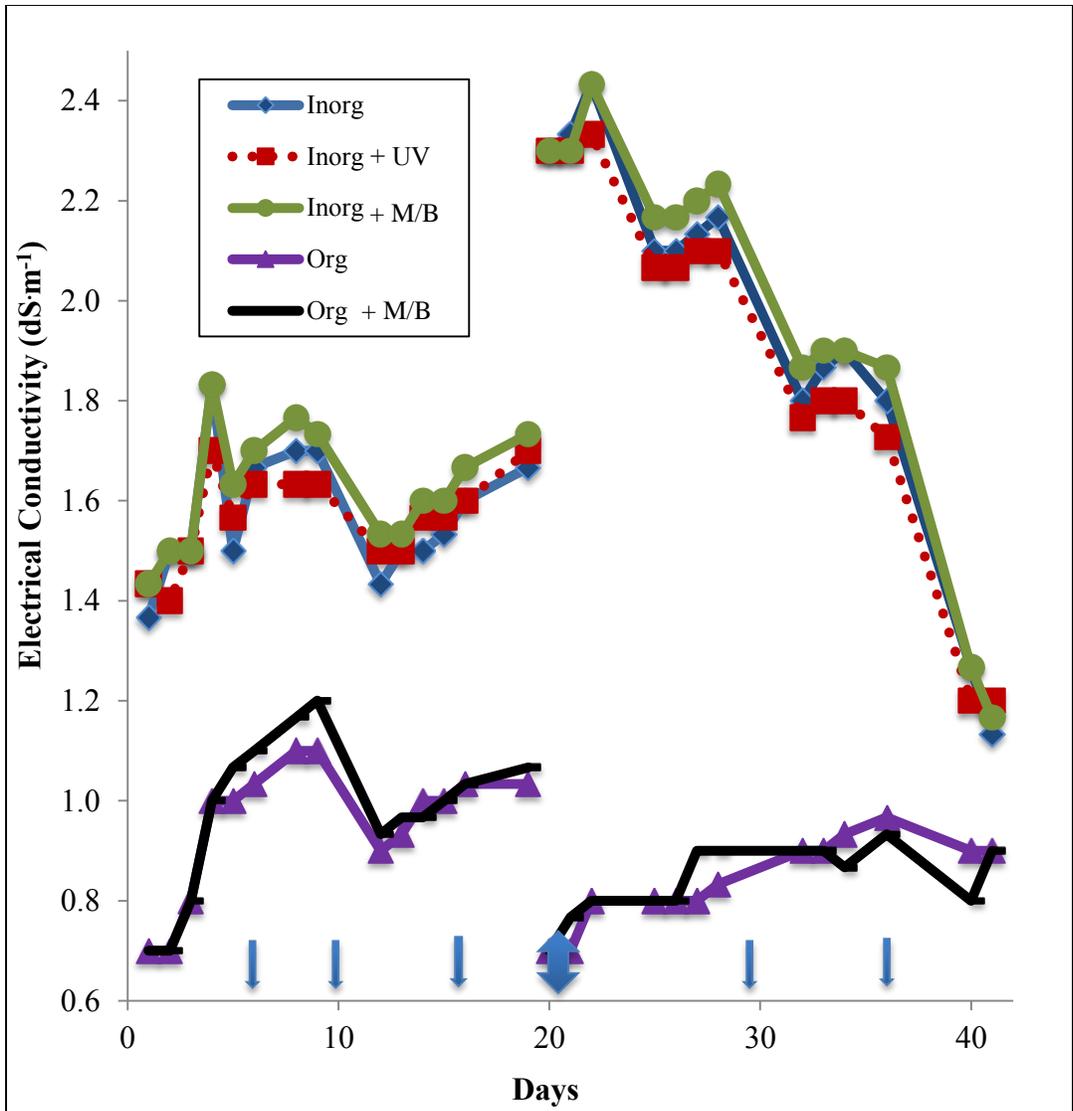
Figure 3-2. Fertilizer (Inorg or Org) and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on reservoir EC in experiment 2.

*Standard error of the least squares means estimate = 0.3; n=3

↓   = Addition of tap water "top up" to nutrient solution

↕   = application of fresh nutrient regimen.
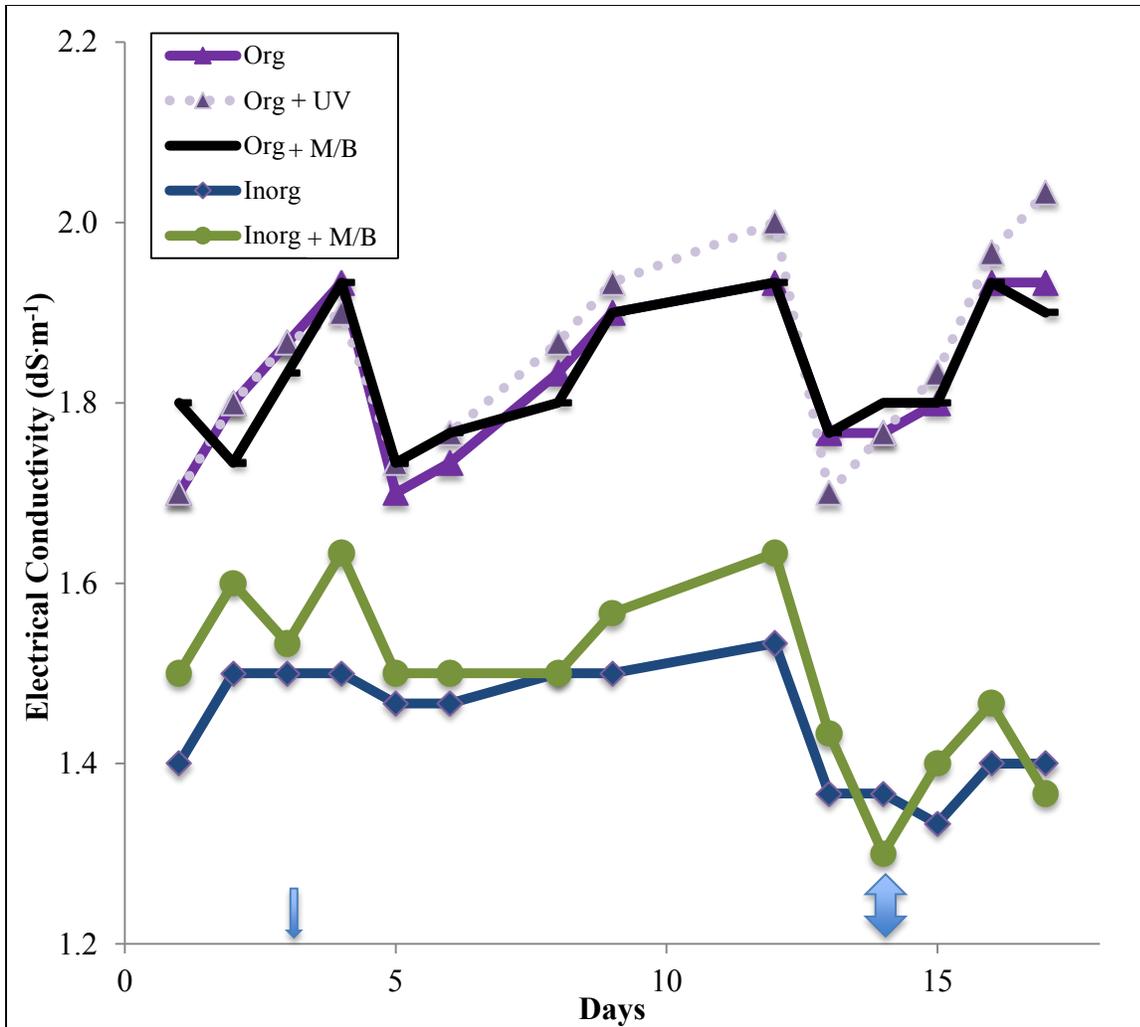
Figure 3-3. 'Low phase' fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on EC of nutrient solution in experiment 3.

Standard error of the least squares means estimate = 0.05; n=3.

⬇ = tap water "top up" to nutrient solution

⬍ = application of fresh nutrient regimen.
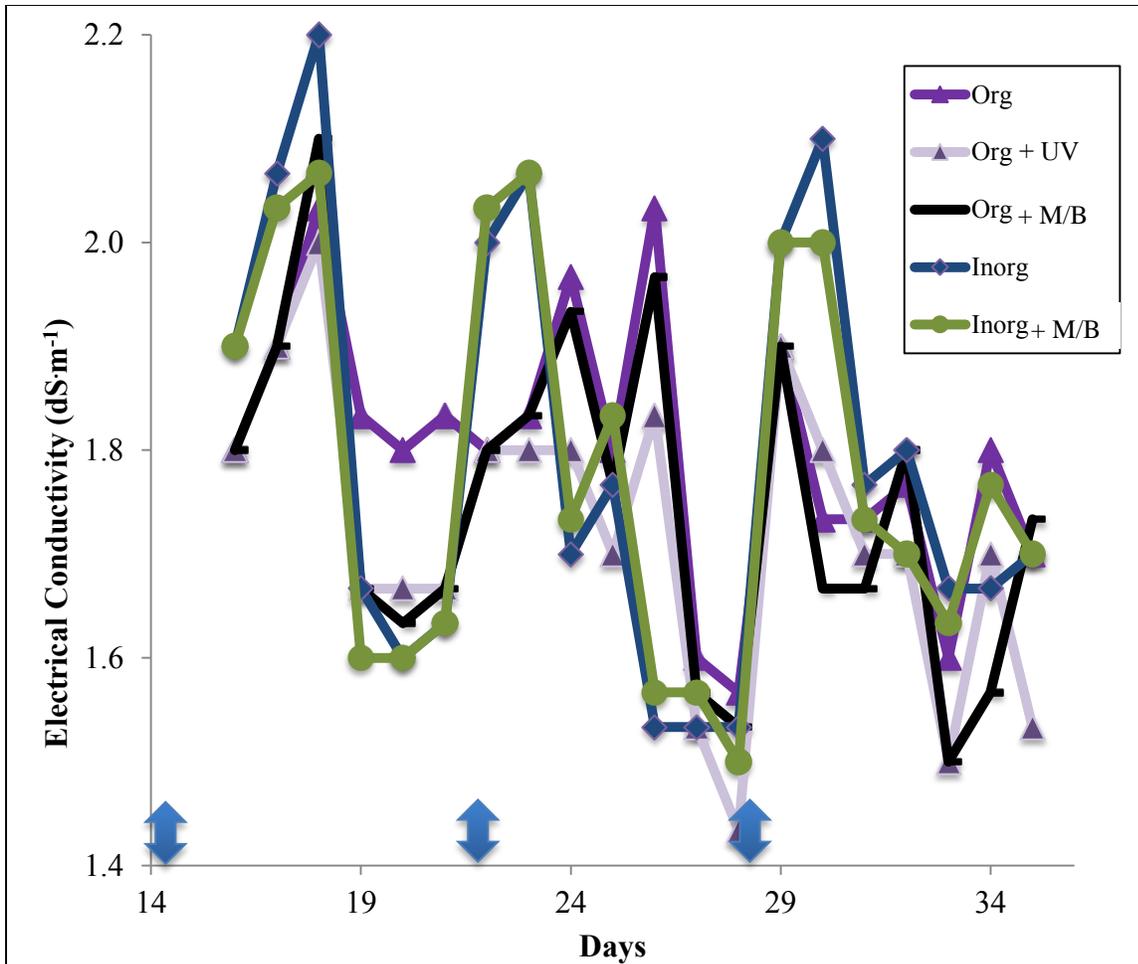
Figure 3-4. 'High phase' fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on EC of nutrient solution in experiment 3.

Standard error of the least squares means estimate = 0.04; n=3.

= application of fresh nutrient regimen.
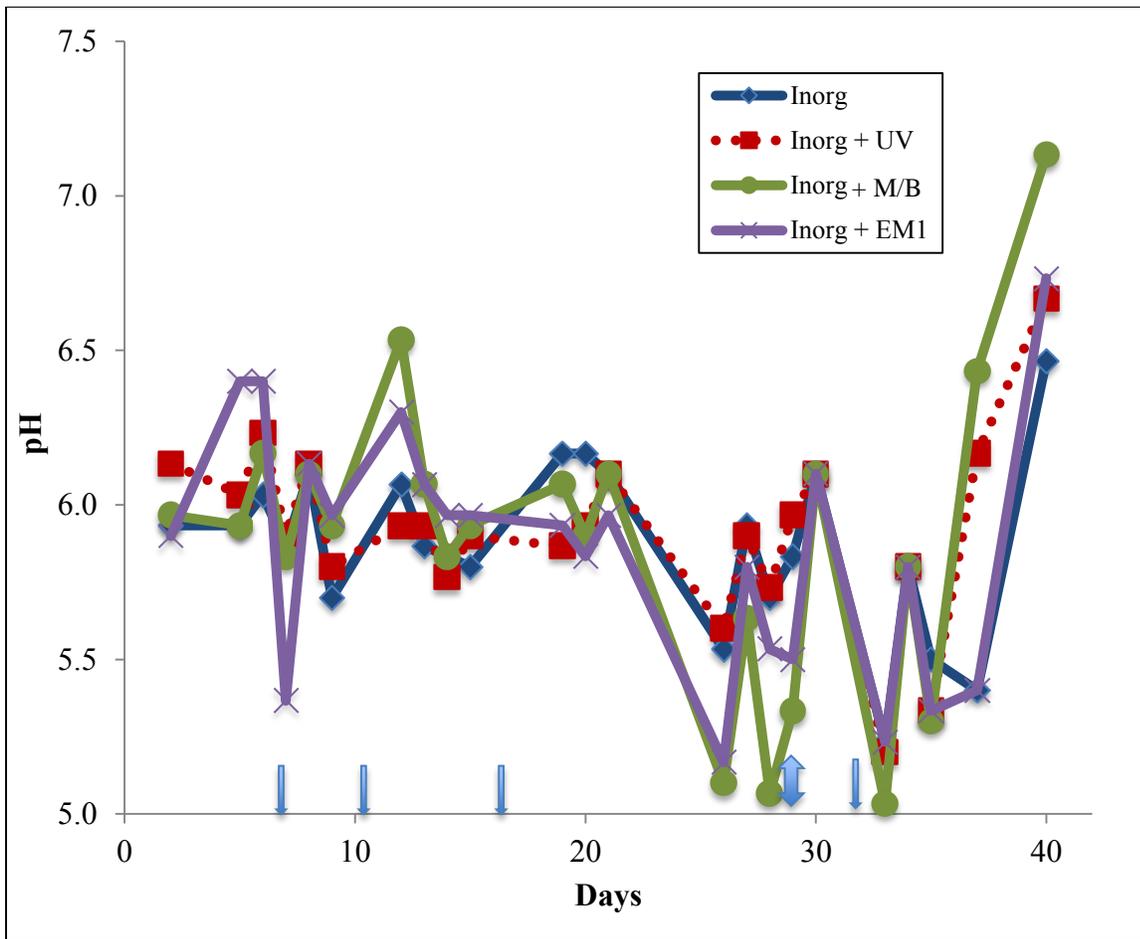
Figure 3-5. Inoculant treatment [Ultraviolet (UV), Sub Culture M/B (M/B) or EM1 (EM1)] effect on inorganic (Inorg) nutrient solution on nutrient solution pH in experiment 1.

Standard error of the least squares means estimate = 0.1; n=3.

↓ = tap water "top up" to nutrient solution

↕ = application of fresh nutrient regimen.
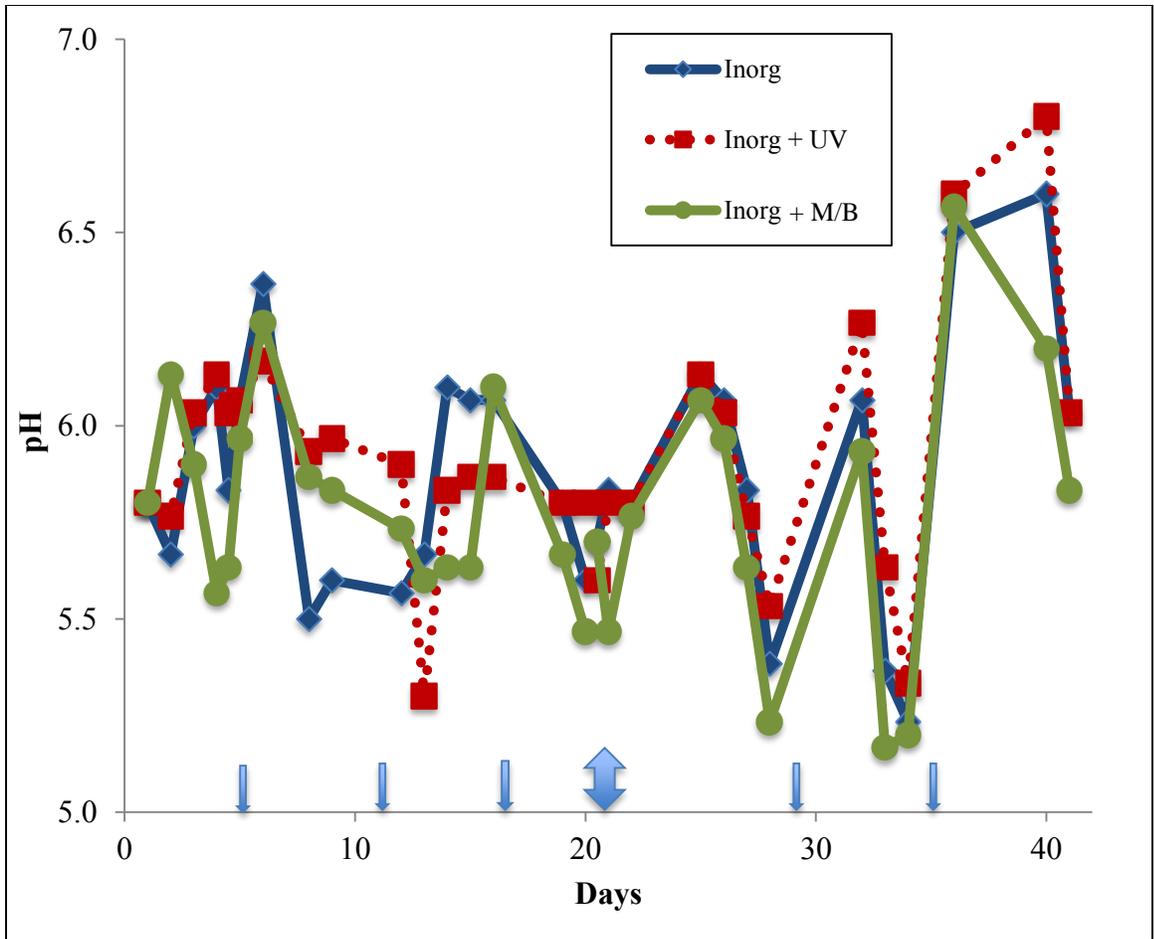
Figure 3-6. Inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on nutrient solution pH in inorganic nutrient (Inorg) regimens in experiment 2.

Standard error of the least squares means estimate = 0.12; n=3.

↓ = tap water "top up" to nutrient solution

⇕ = application of fresh nutrient regimen.
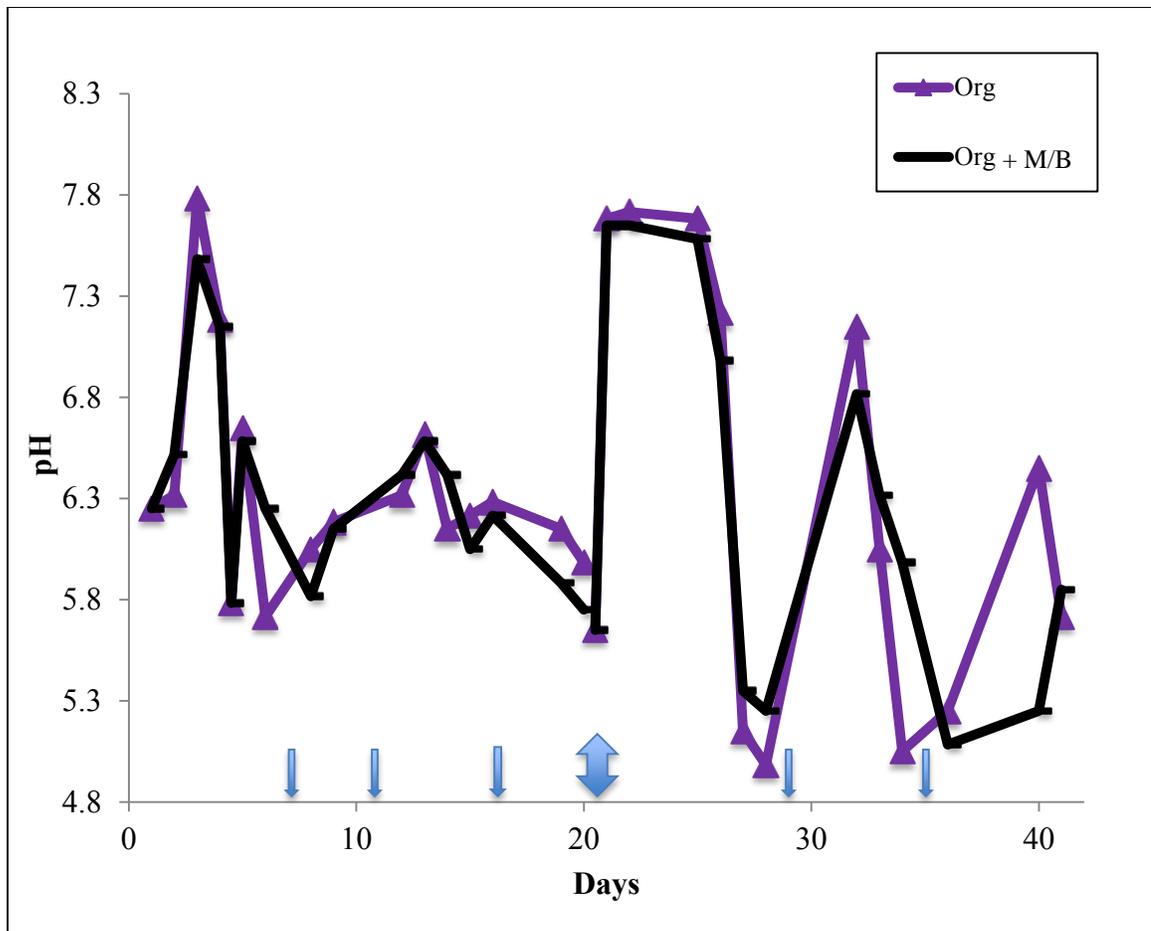
Figure 3-7. Organic fertilizer (Org) and inoculant treatment [Sub Culture M/B (M/B)] effect on nutrient solution pH in experiment 2.

Standard error of the least squares means estimate = 0.08; n=3.

⬇ = tap water "top up" to nutrient solution

⬍ = application of fresh nutrient regimen.
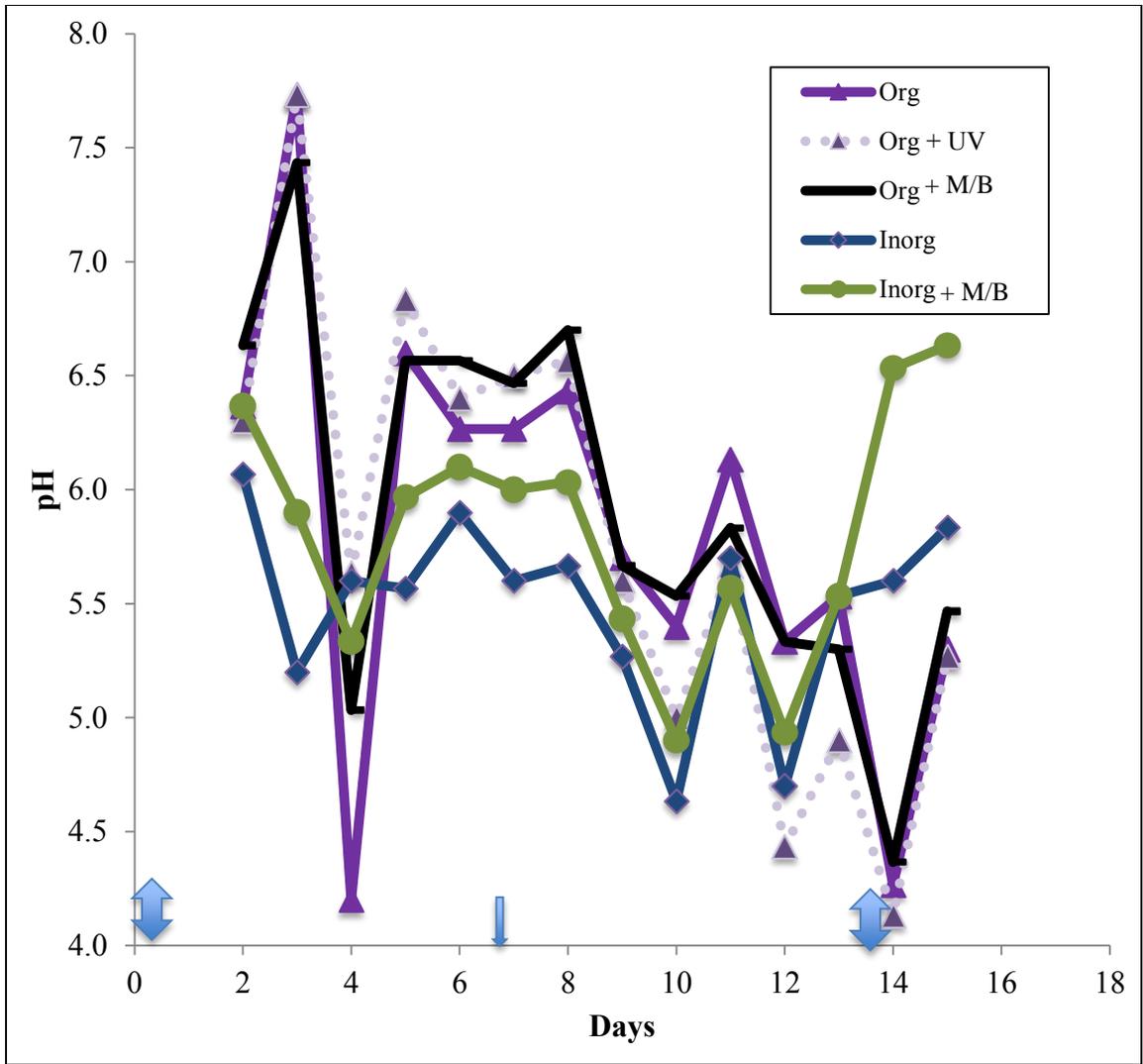
Figure 3-8. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on reservoir pH 'Low phase' nutrient regimen in experiment 3.

Standard error of the least squares means estimate = 0.12; n=3.

    = tap water "top up" to nutrient solution

    = application of fresh nutrient regimen.
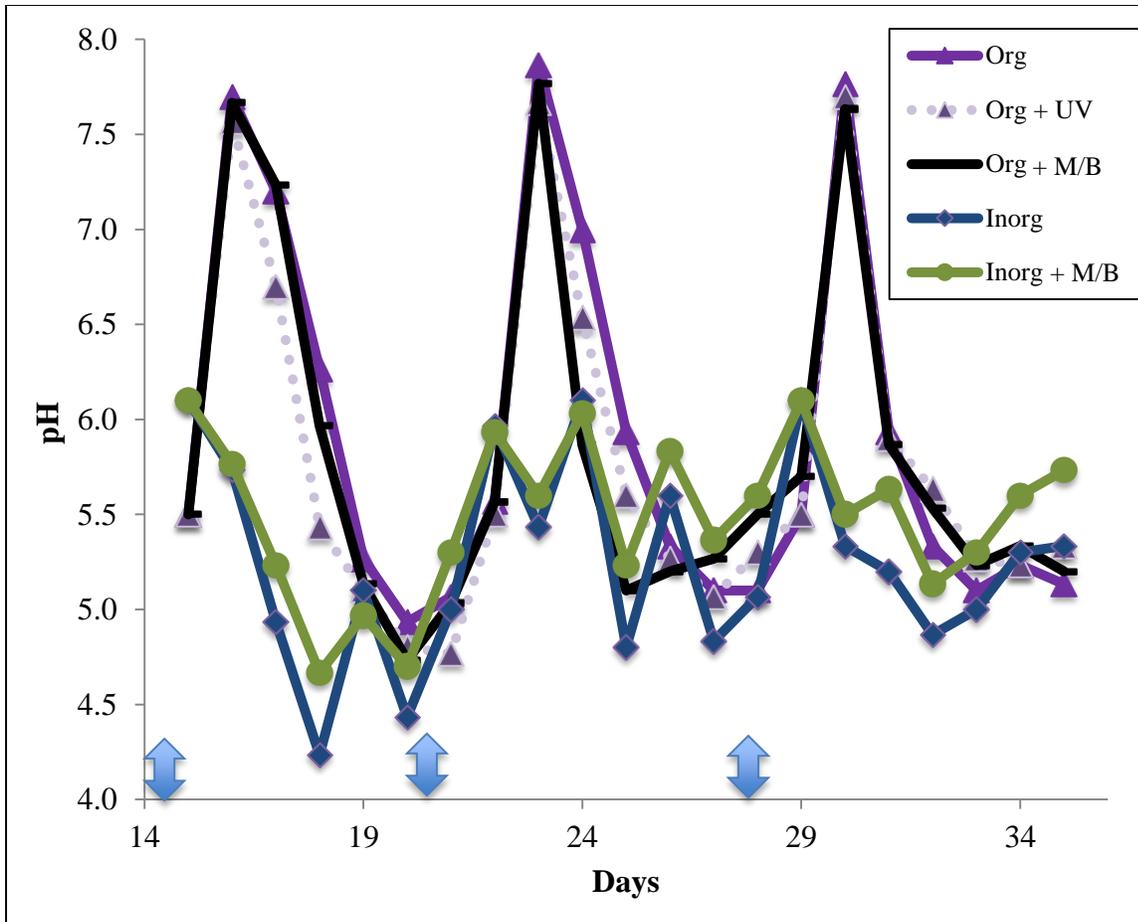
Figure 3-9. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on solution pH in the 'High phase' nutrient regimen in experiment 3.

Standard error of the least squares means estimate = 0.11; n=3.

= Application of fresh nutrient source
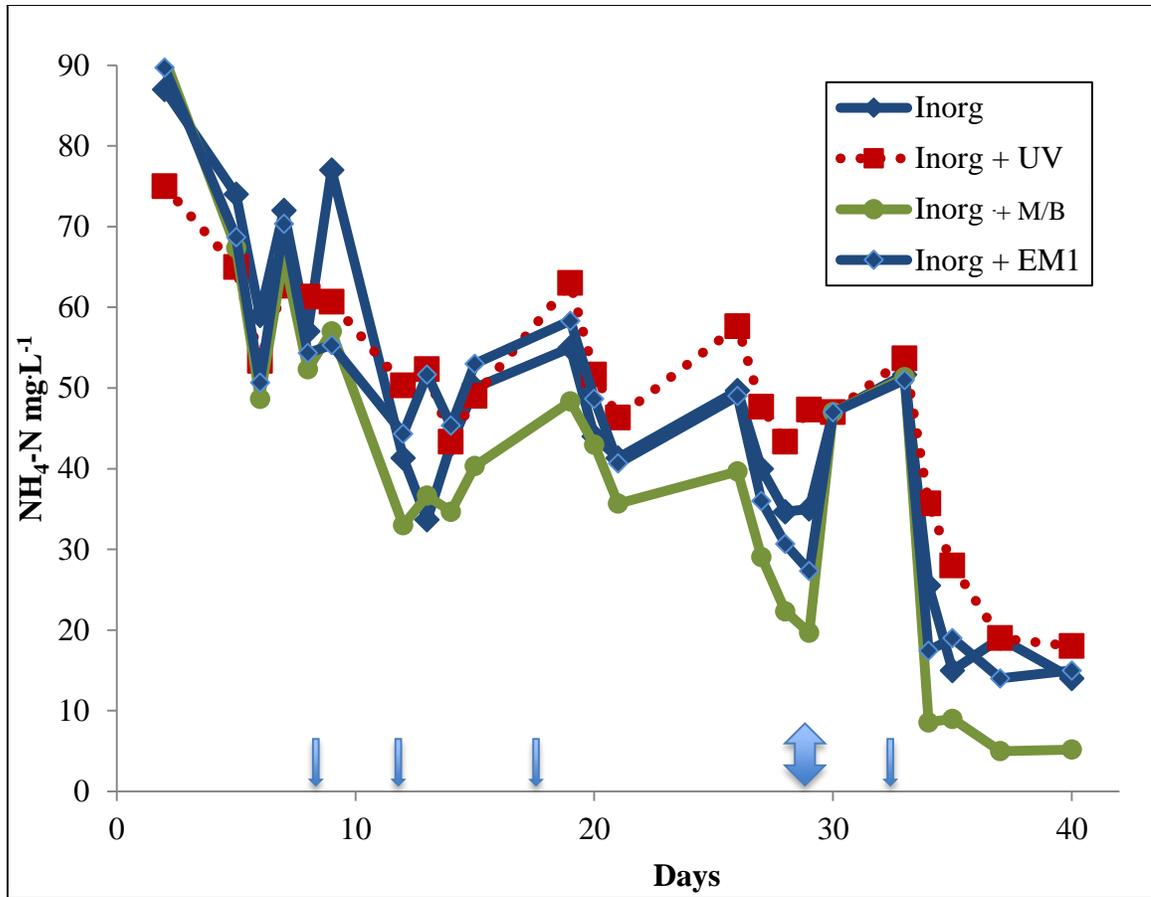
Figure 3-10. Inoculant treatment [Ultraviolet (UV), Sub Culture M/B (M/B) or EM1 (EM1)] effect on inorganic (Inorg) nutrient solution on ammonium (NH₄-N) concentrations in experiment 1.

Standard error of the least squares means estimate = 1.7; n=3

↓ = tap water "top up" to nutrient solution

⇕ = application of fresh nutrient regimen.

3-10
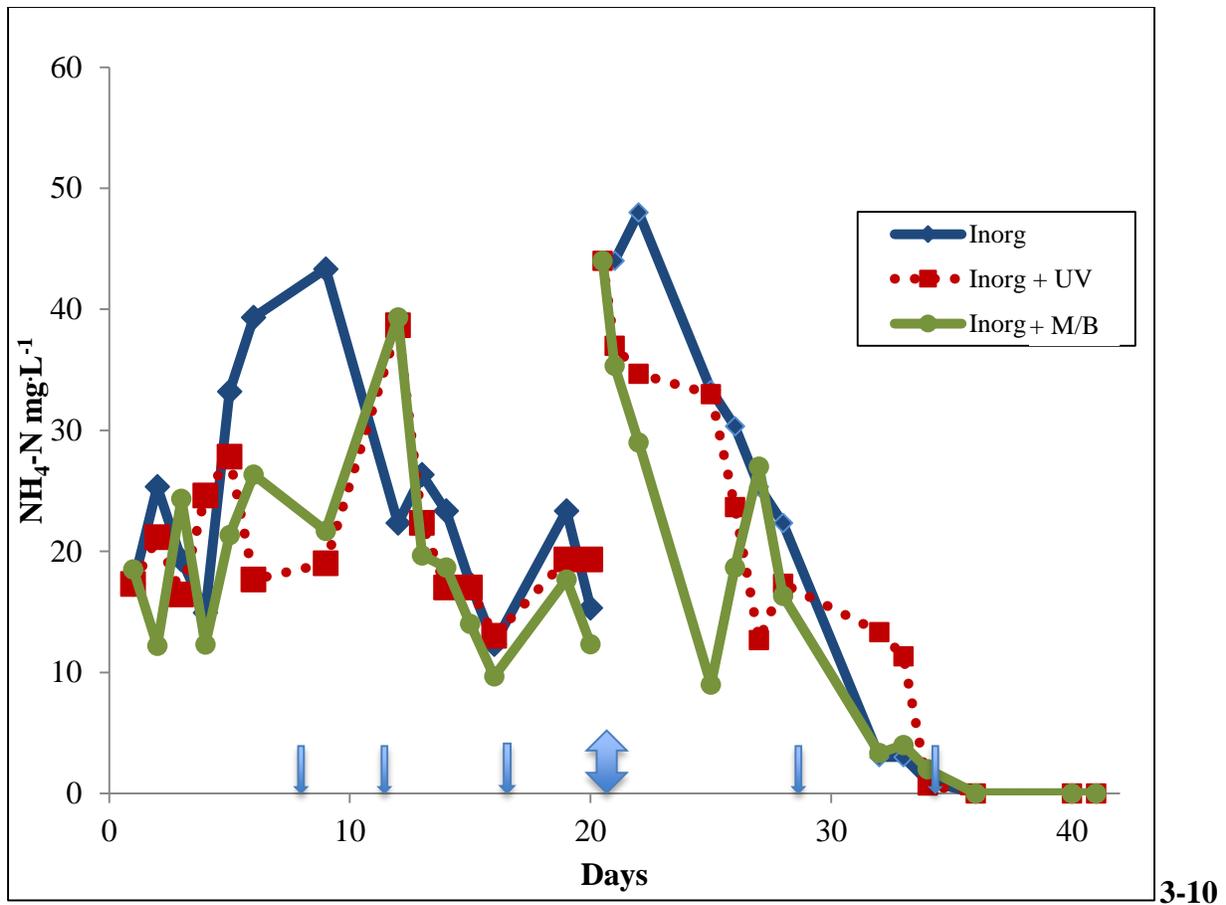
Figure 3-11. Inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on ammonium (NH$_4$-N) concentration in inorganic nutrient regimen in experiment 2. n=3

*Standard error of the least squares means estimate = 2.4

↓ = tap water "top up" to nutrient solution

⇕ = application of fresh nutrient regimen.
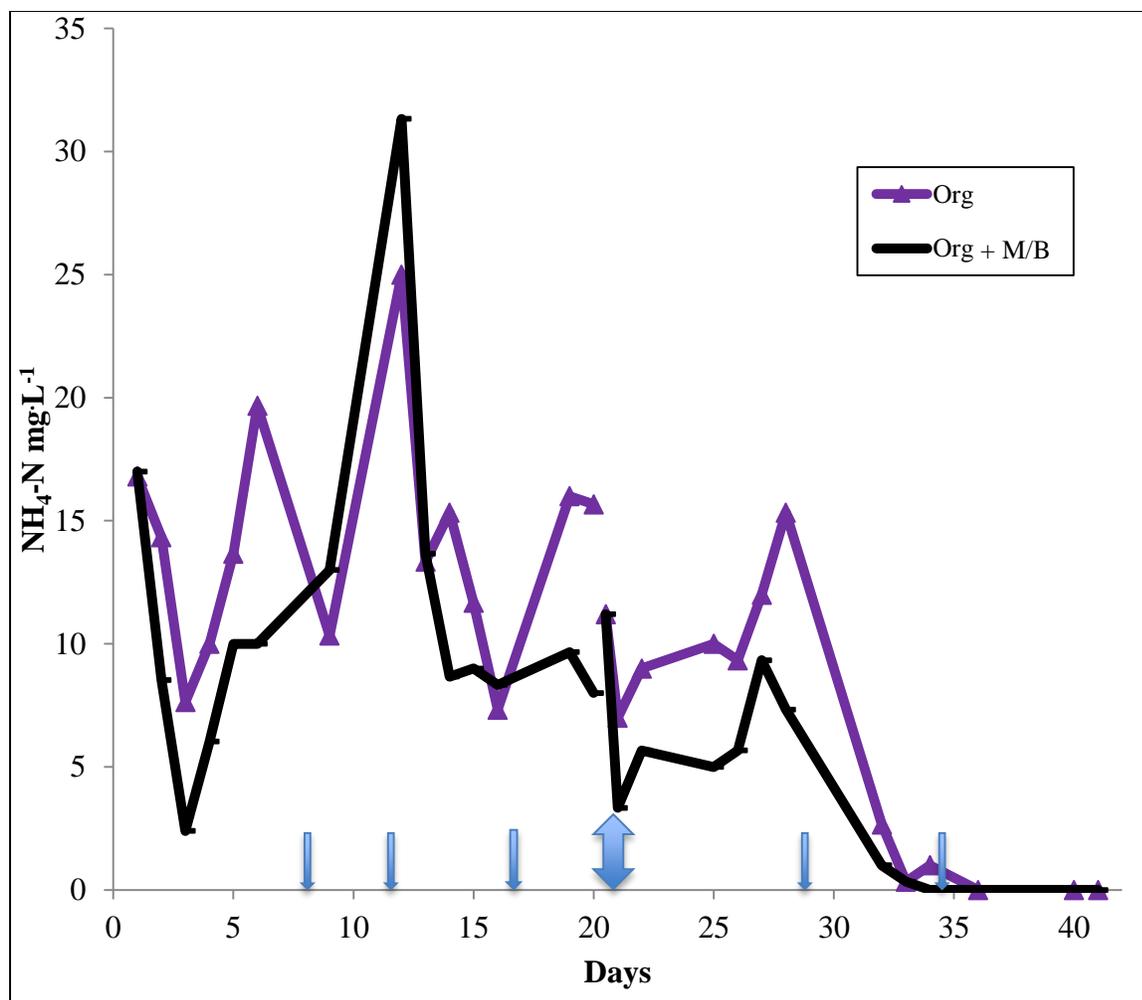
Figure 3-12. Organic fertilizer (Org) and inoculant [Sub Culture M/B (M/B)] effect on ammonium (NH_4-N) concentrations of nutrient solution in experiment 2.

Standard error of the least squares means estimate = 2.1; n=3.

↓   = tap water "top up" to nutrient solution

⇕   = application of fresh nutrient regimen.
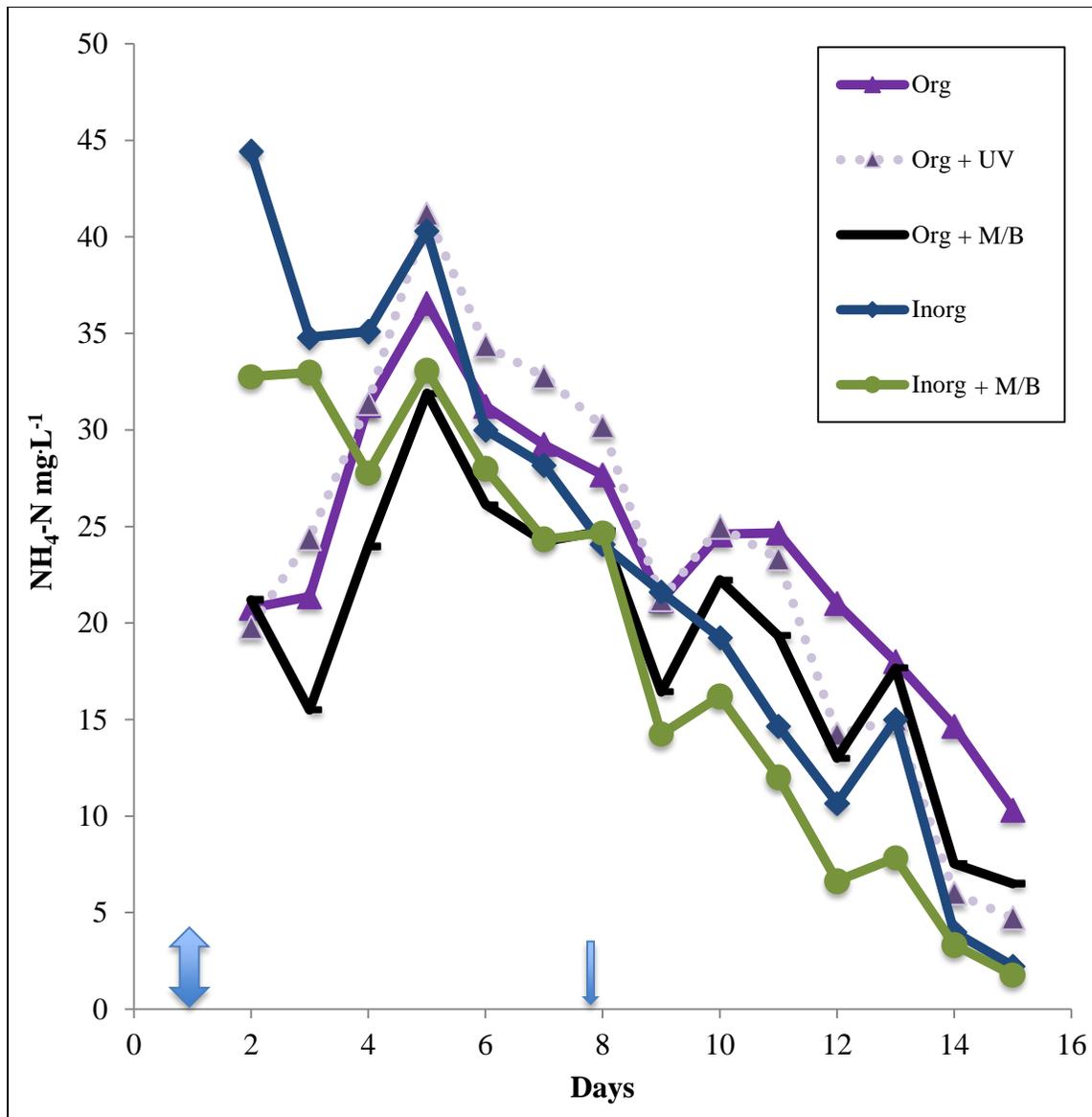
Figure 3-13. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on ammonium (NH^4-N) concentrations of the nutrient solution in 'low phase' nutrient regimen in experiment 3.

Standard error of the least squares means estimate = 1.6; n=3.

↓ = tap water "top up" to nutrient solution

↕ = application of fresh nutrient regimen.
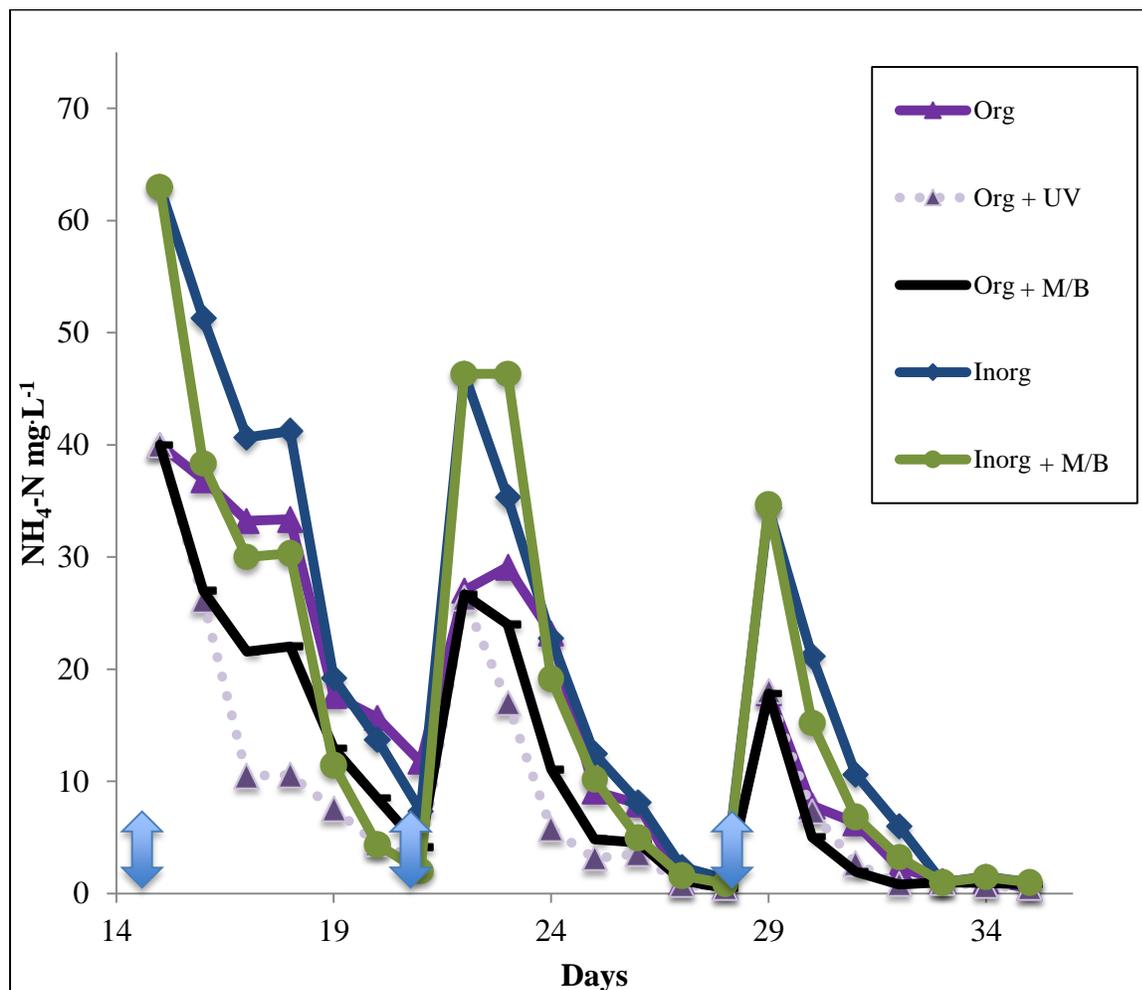
Figure 3-14. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on ammonium (NH^4-N) concentrations of the nutrient solution in 'high phase' nutrient regimen in experiment 3. Standard error of the least squares means estimate = 1.9; n=3.

    ↓   = tap water "top up" to nutrient solution

    ⇕   = application of fresh nutrient regimen.
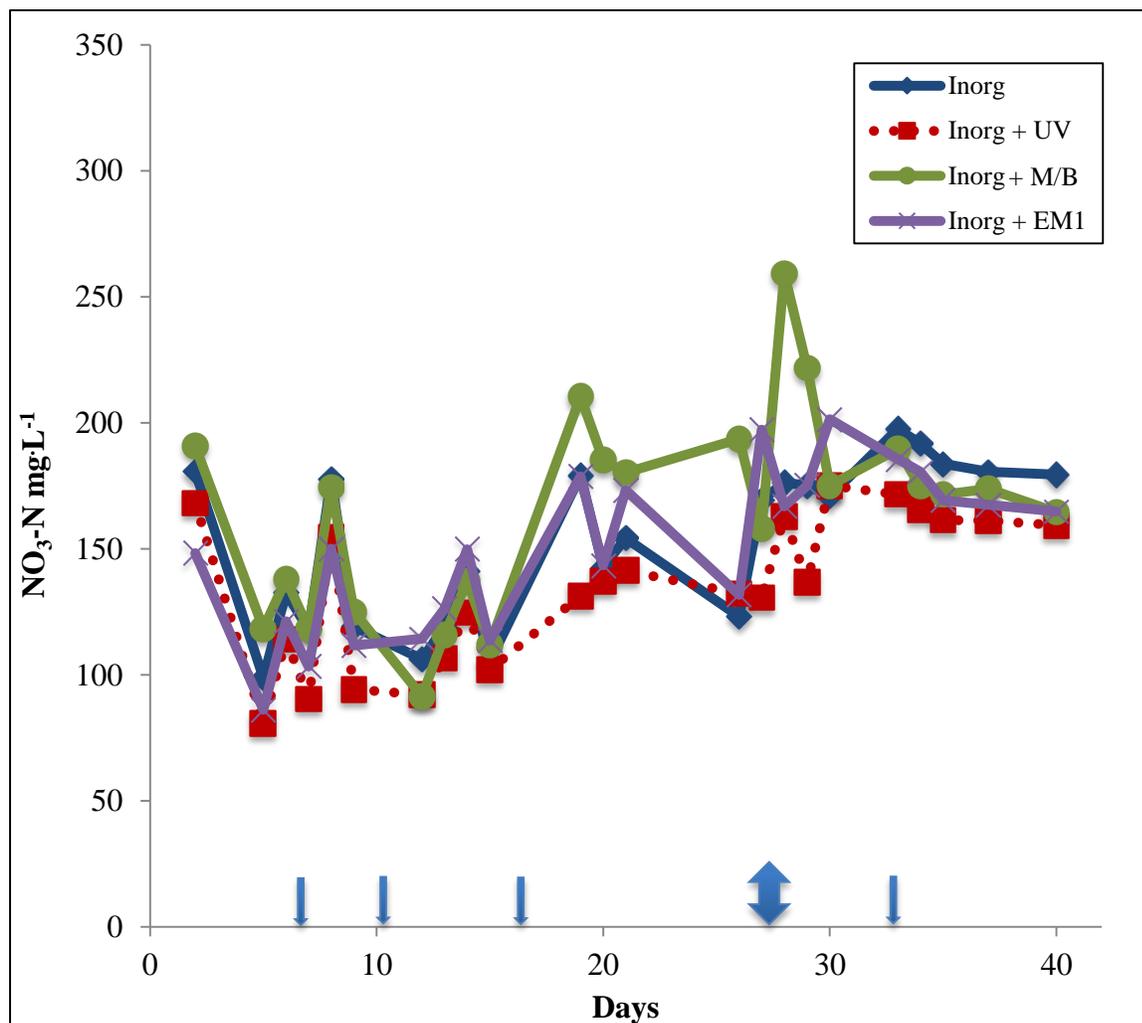
Figure 3-15. Inoculant treatment [Ultraviolet (UV), Sub Culture M/B (M/B) or EM1 (EM1)] effect on inorganic (Inorg) nutrient solution nitrate (NO_3-N) concentration in experiment 1.

Standard error of the least squares means estimate = 6.7; n=3.

↓   =   tap water "top up" to nutrient solution

↕   = application of fresh nutrient regimen.
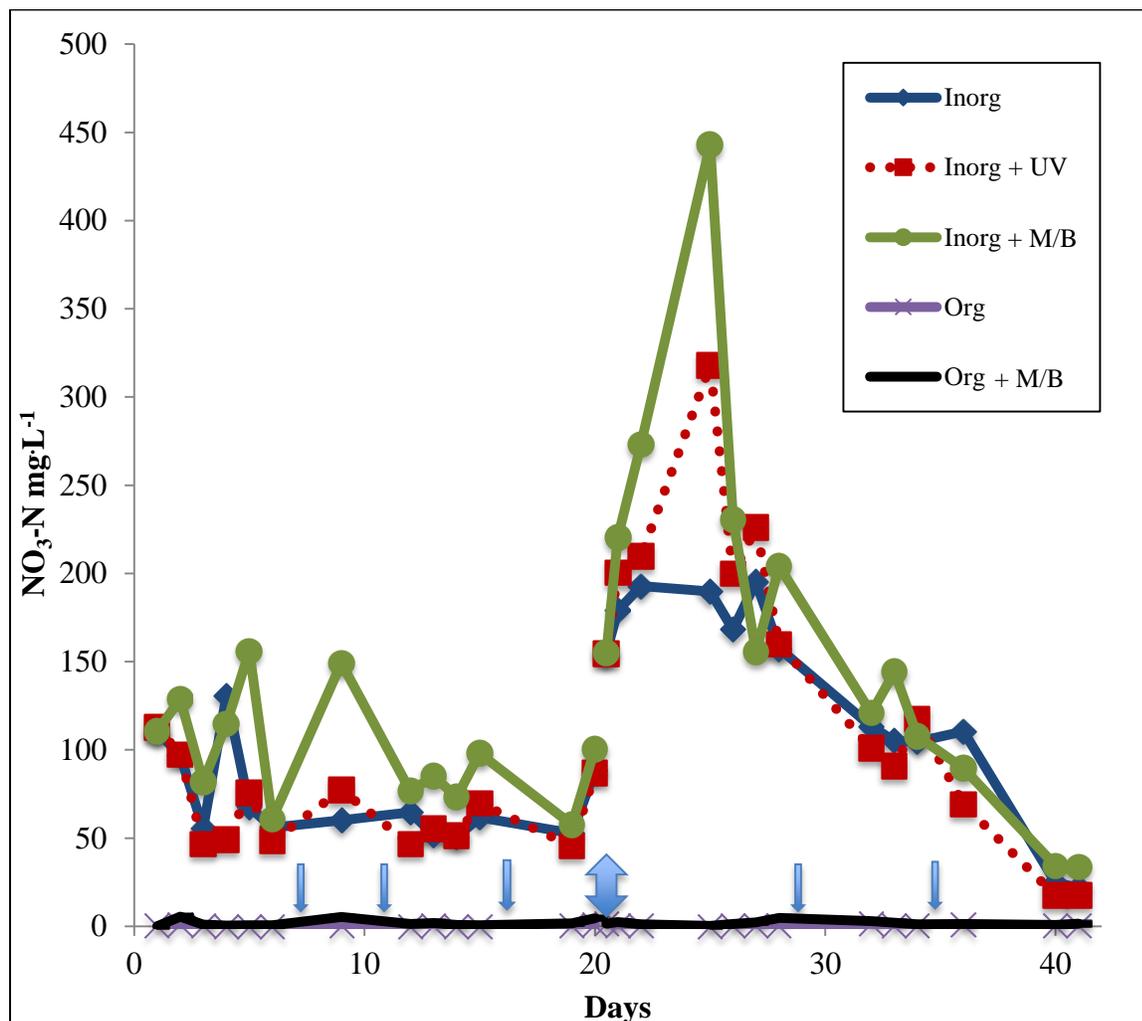
Figure 3-16. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on nitrate (NO₃-N) concentrations of the nutrient solution in experiment 2.

Standard error of the least squares means estimate = 6.7; n=3.

↓ = tap water "top up" to nutrient solution

⇕ = application of fresh nutrient regimen.
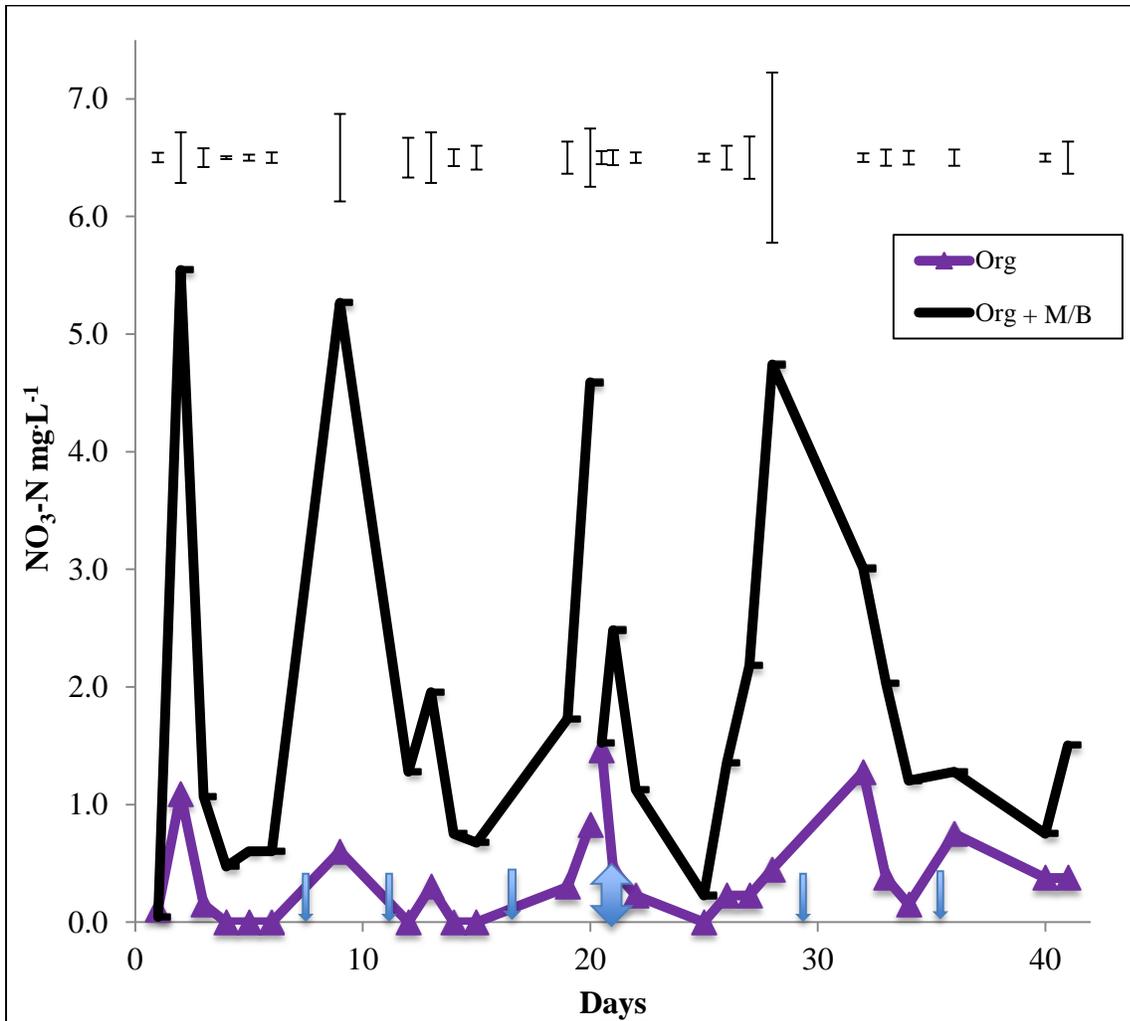
Figure 3-17. Organic fertilizer (Org) and inoculant [Sub Culture M/B (M/B)] effect on nitrate (NO_3-N) concentrations in experiment 2.

Bars indicate a 'by date' standard error analysis of the least squared means; n=3.

↓ = tap water "top up" to nutrient solution

⇕ = application of fresh nutrient regimen.
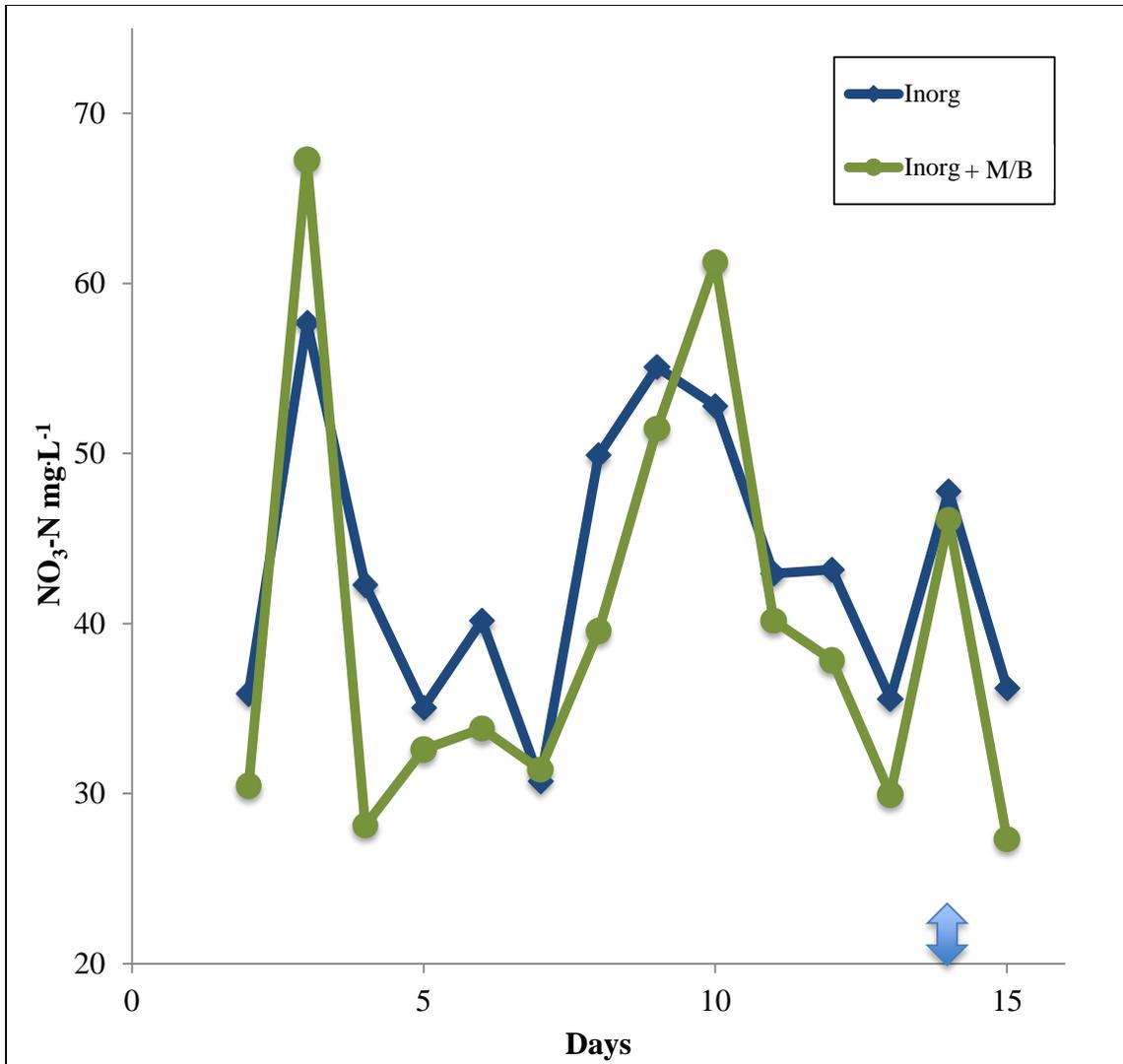
Figure 3-18. Inorganic fertilizer (Inorg) and inoculant [Sub Culture M/B (M/B)] effect on nitrate (NO$_3$-N) concentrations of 'low phase' nutrient regimen inorganic treatments in experiment 3.

Standard error of the least squares means estimate = 5.0; n=3.

⬍ = application of fresh nutrient regimen.
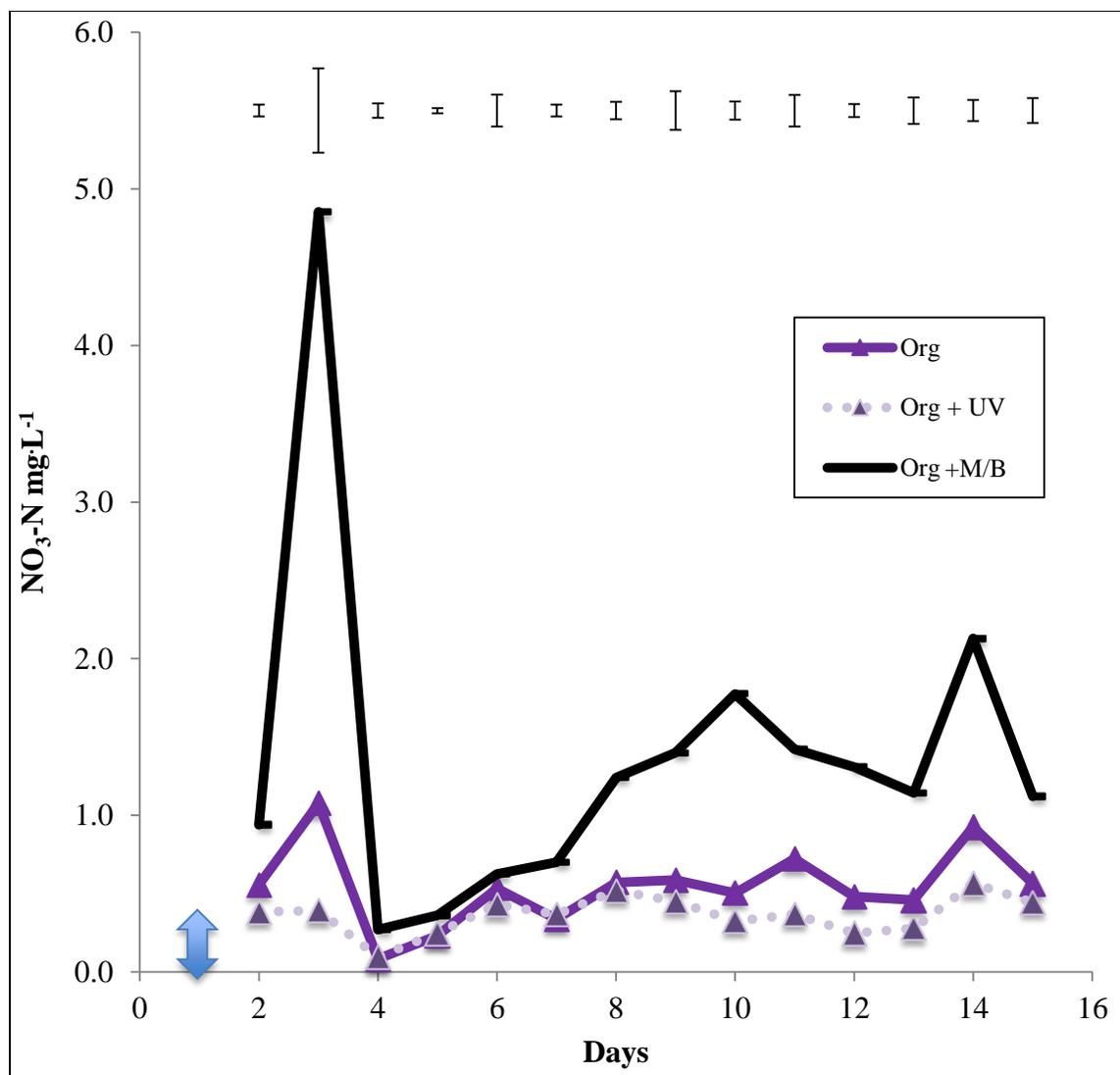
Figure 3-19. Nitrate (NO$_3$-N) concentrations of 'low phase' organic treatments (Org) and inoculant treatment [Sub Culture M/B (M/B)] effect in experiment 3.

Bars indicate a 'by date' standard error analysis of the least squared means; n=3.

↕ = application of fresh nutrient regimen.
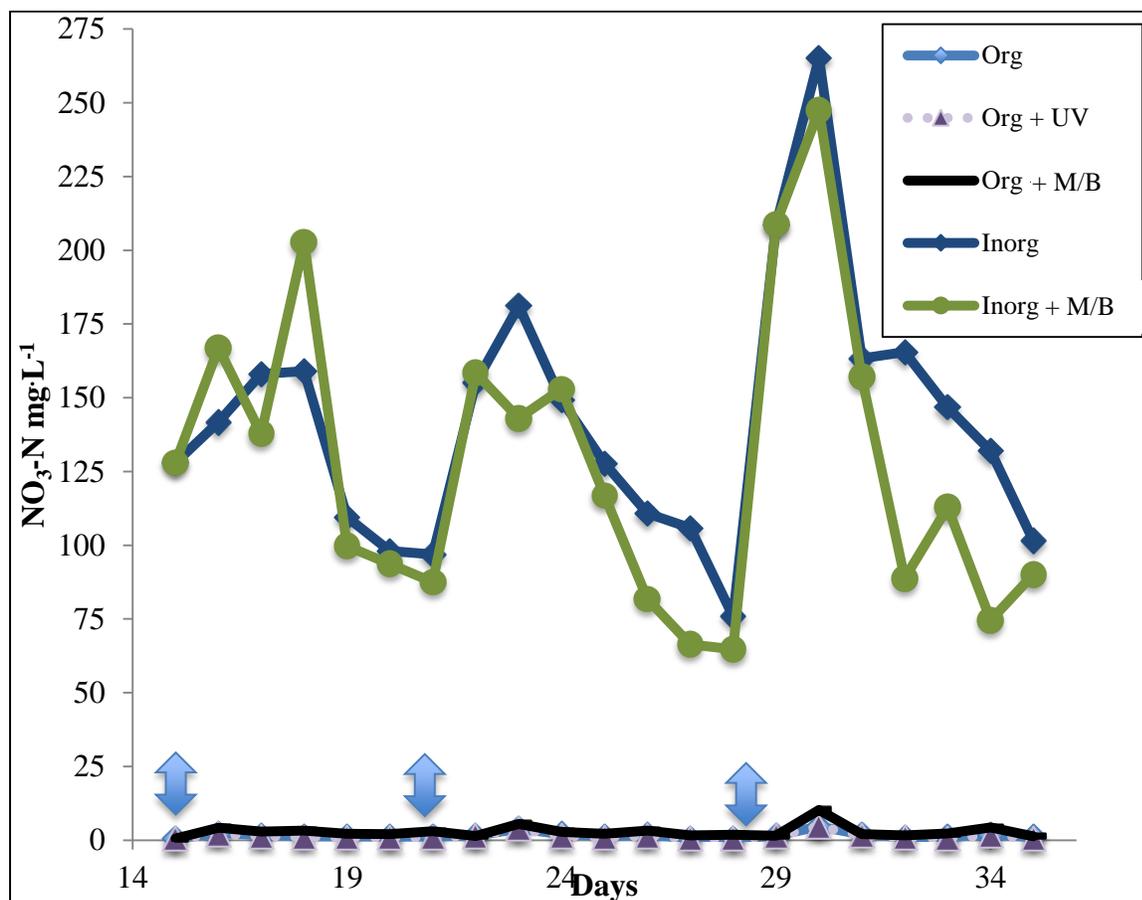
Figure 3-20. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on nitrate (NO_3-N) concentrations of the 'high phase' nutrient solution in experiment 3.

Standard error of the least squares means estimate = 4.5; n=3.

= application of fresh nutrient regimen.
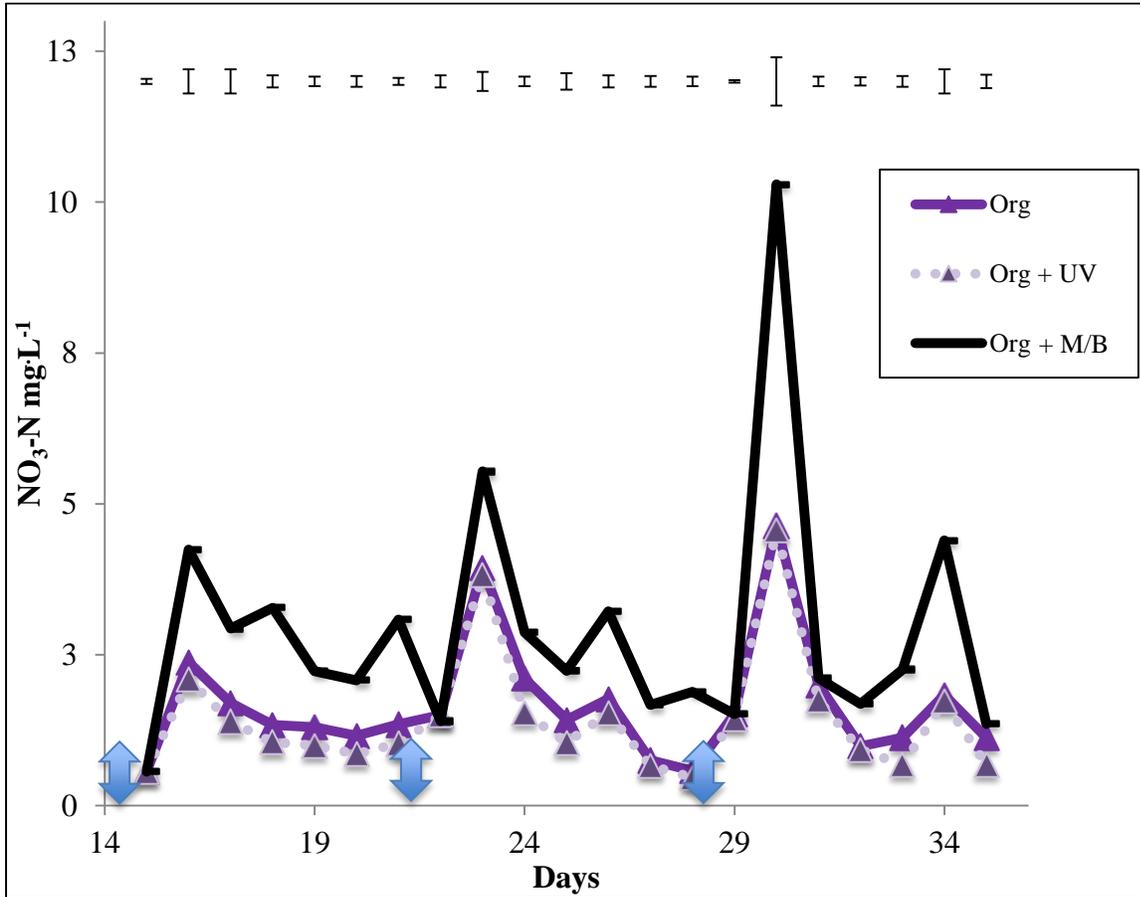
Figure 3-21. Organic fertilizer (Org) and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect and inoculant effect on nitrate (NO_3-N) concentrations of the 'high phase' nutrient regimen in experiment 3.

Bars indicate a 'by date' standard error analysis of the least squared means; n=3.

= application of fresh nutrient regimen.
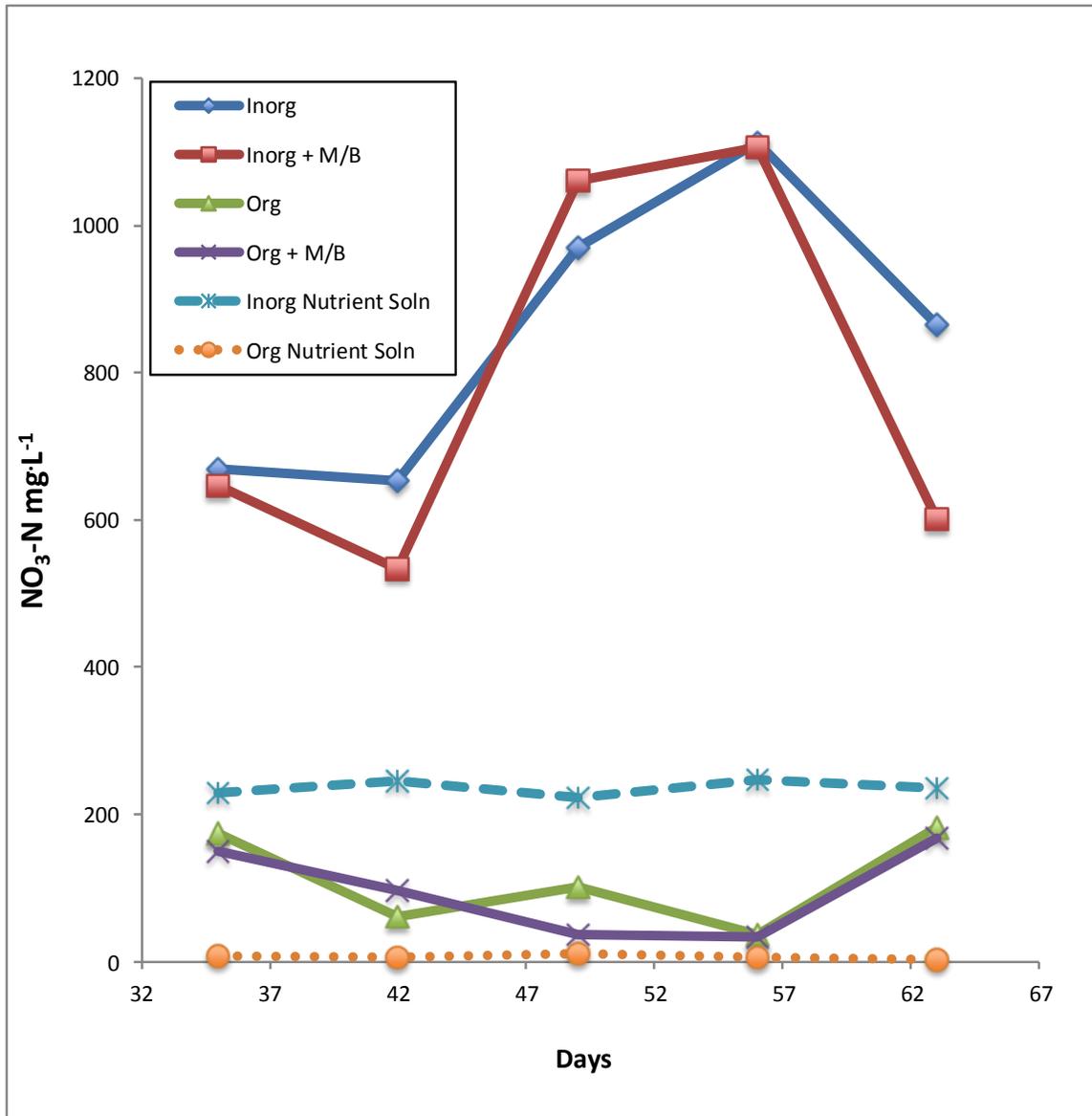
Figure 3-22. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on petiole nitrate (NO_3-N) concentrations of butterhead lettuce and nutrient solution (Inorg or Org Nutrient Soln) nitrate in the experiment 4.

Standard error of the least squares means estimate petiole NO_3 = 14.6; n=3.

Standard error of the least square means estimate of nutrient solution NO_3 = 1.9; n=3.
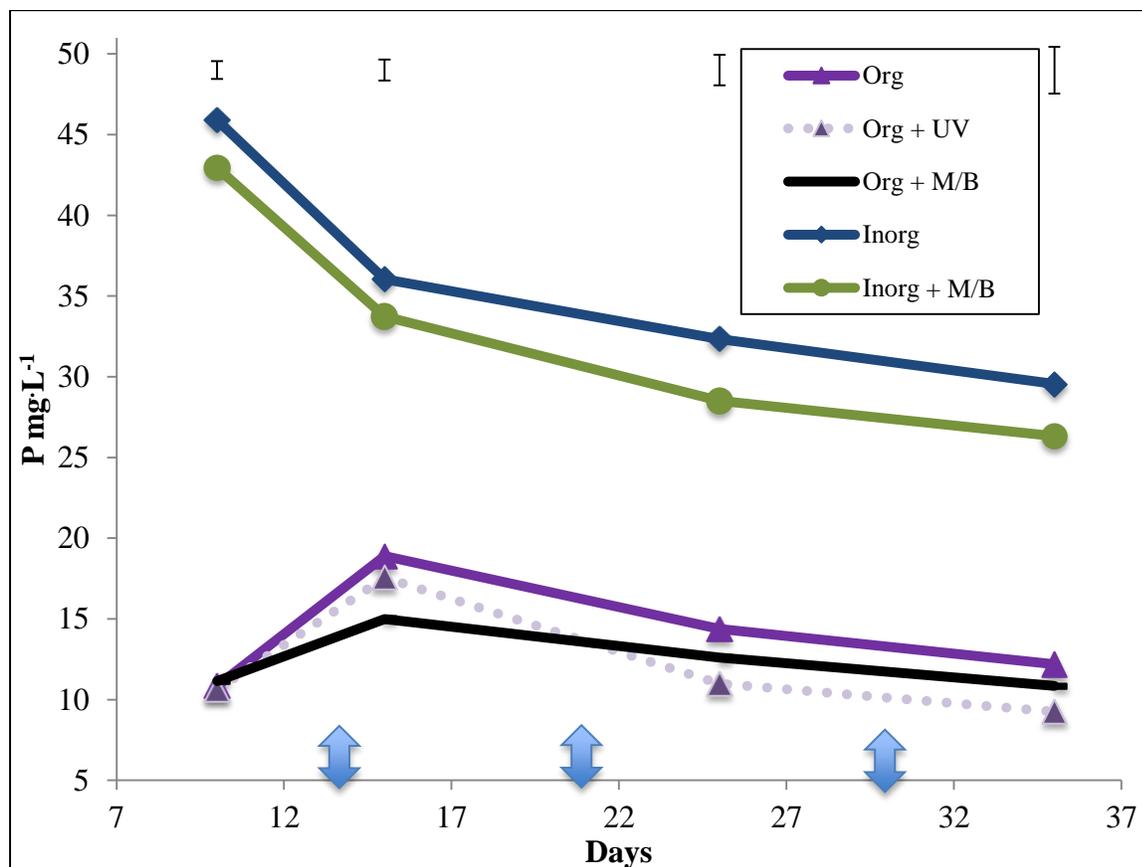
Figure 3-23. Fertilizer [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on P concentrations in experiment 3. Bars indicate a 'by date' standard error analysis of the least squared means; n=3.

= application of fresh nutrient regimen.
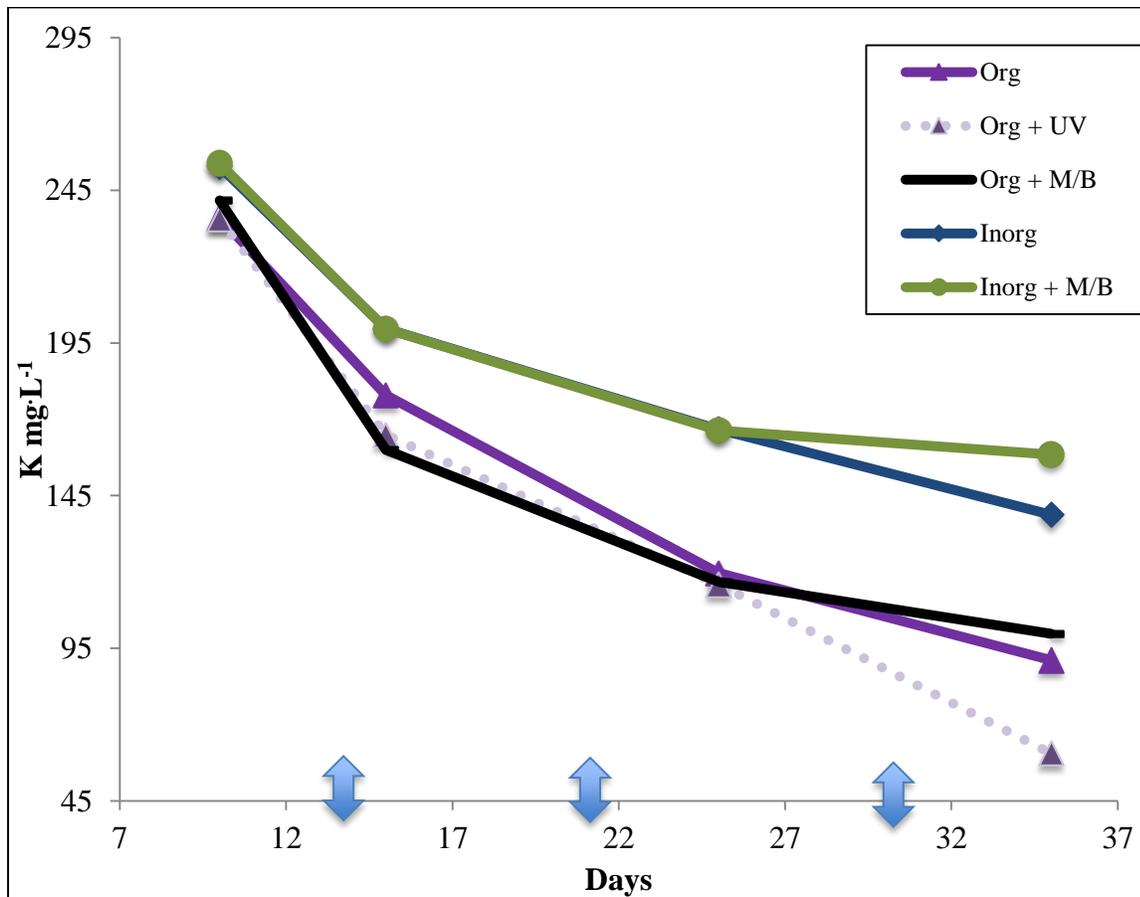
Figure 3-24. Fertilizer [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on K concentration of nutrient solution in experiment 3.

Standard error of the least squares means estimate = 15.1; n=3.

= application of fresh nutrient regimen.
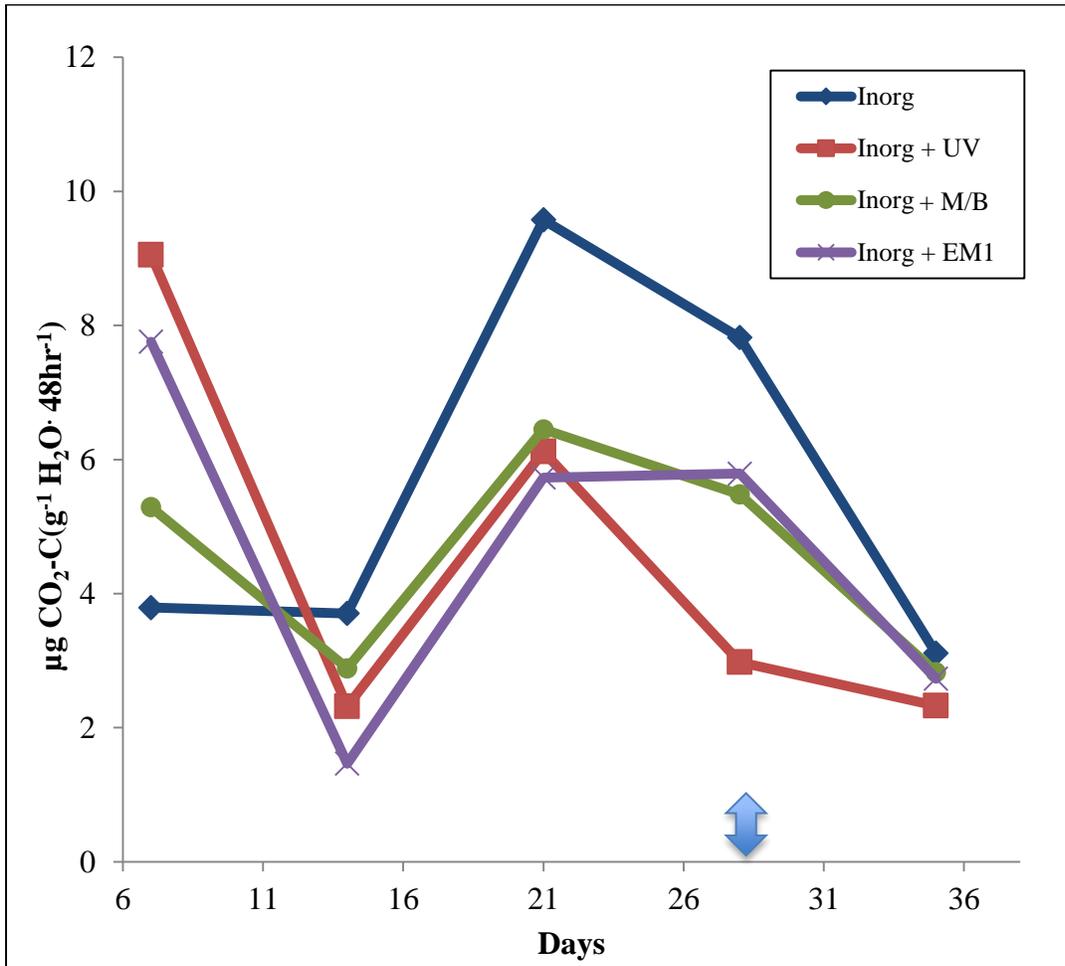
Figure 3-25. Inoculant treatment [Ultraviolet (UV), Sub Culture M/B (M/B) or EM1 (EM1)] effect on inorganic (Inorg) nutrient solution CO_2-C respiration from microbial activity in experiment 1.

Standard error of the least squares means estimate = 2.7; n=3.

= application of fresh nutrient regimen.
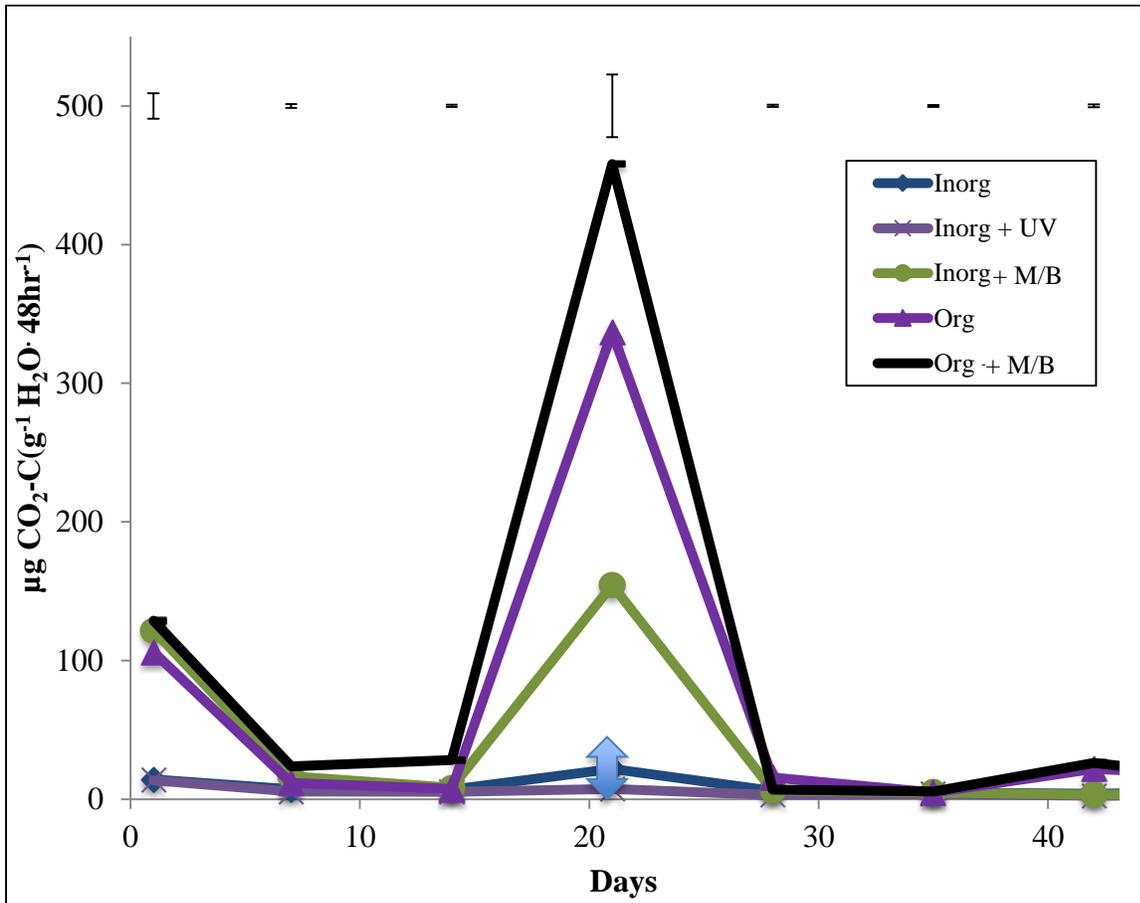
Figure 3-26. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on CO_2-C respiration from microbial activity of the nutrient solution in experiment 2.

Bars indicate a 'by date' standard error analysis of the least squared means; n=3.

= application of fresh nutrient regimen.

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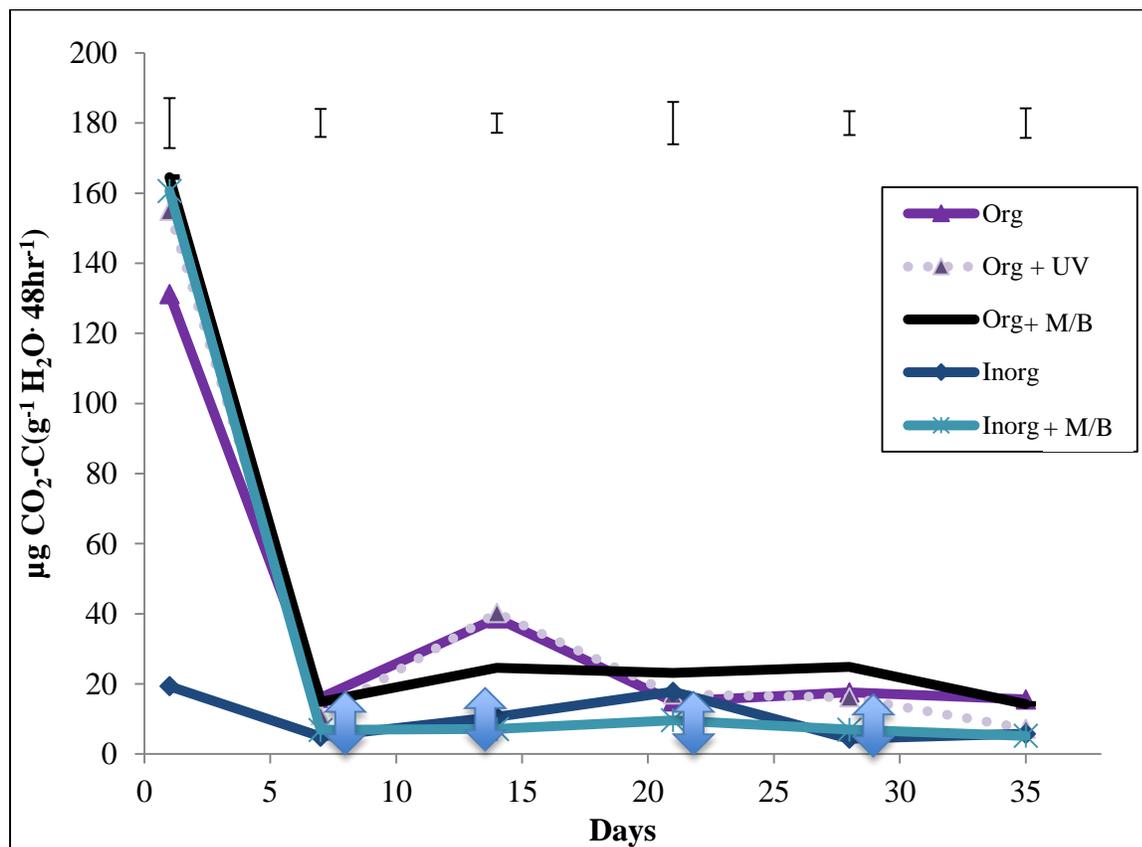
Figure 3-27. Fertilizer treatment [Inorganic (Inorg) or Organic (Org)] and inoculant treatment [Ultraviolet (UV) or Sub Culture M/B (M/B)] effect on CO_2-C respiration from microbial activity of the nutrient solution in experiment 3.

Bars indicate a 'by date' standard error analysis of the least squared means; n=3.

= application of fresh nutrient regimen.
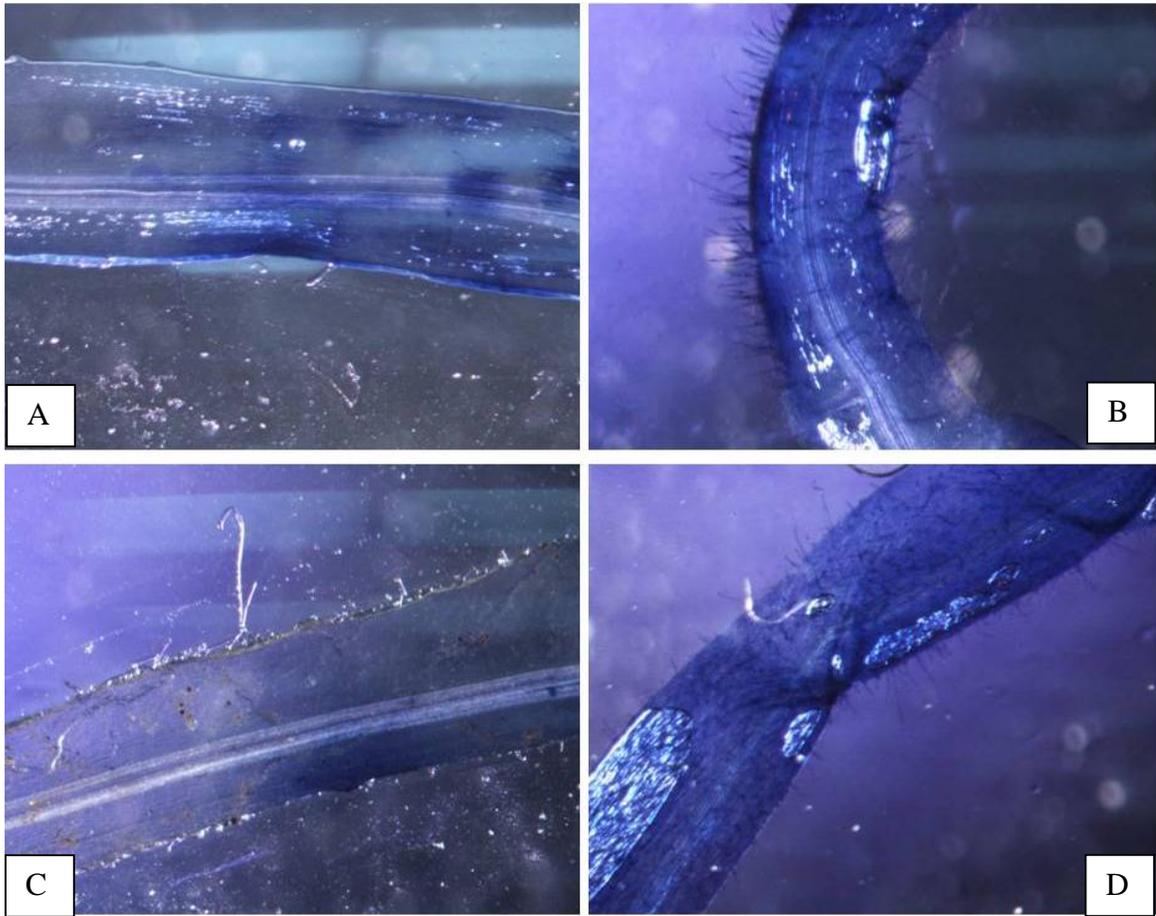
Figure 3-28. Cross sectional image of root samples from experiment 4. A: Inorganic nutrient; B: Organic nutrient; C: Inorganic + M/B inoculant; D: Organic + M/B inoculant.
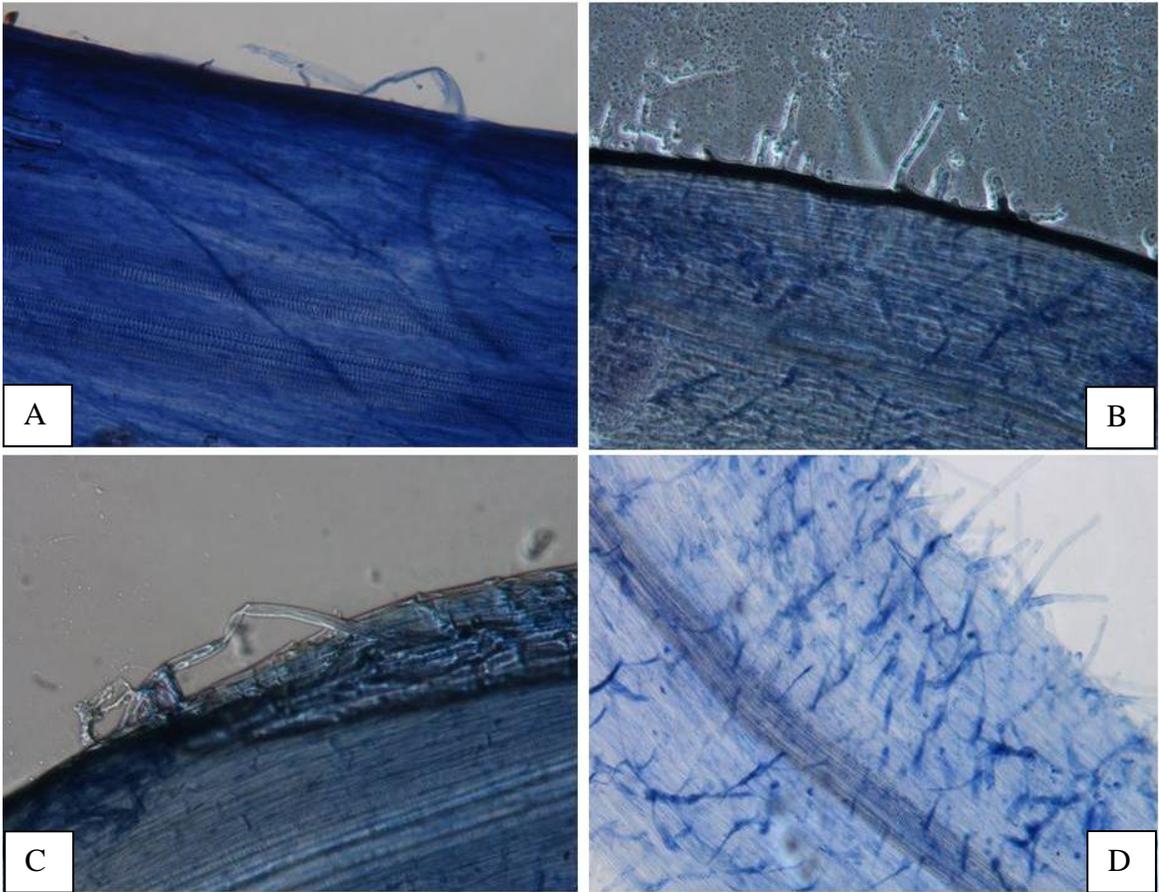
Figure 3-29. Cross sectional microscopic slides of root samples from experiment 4.
A: Inorganic nutrient; B: Organic nutrient; C: Inorganic + M/B inoculant; D: Organic + M/B inoculant.

Appendix A - Soilless Substrate Data
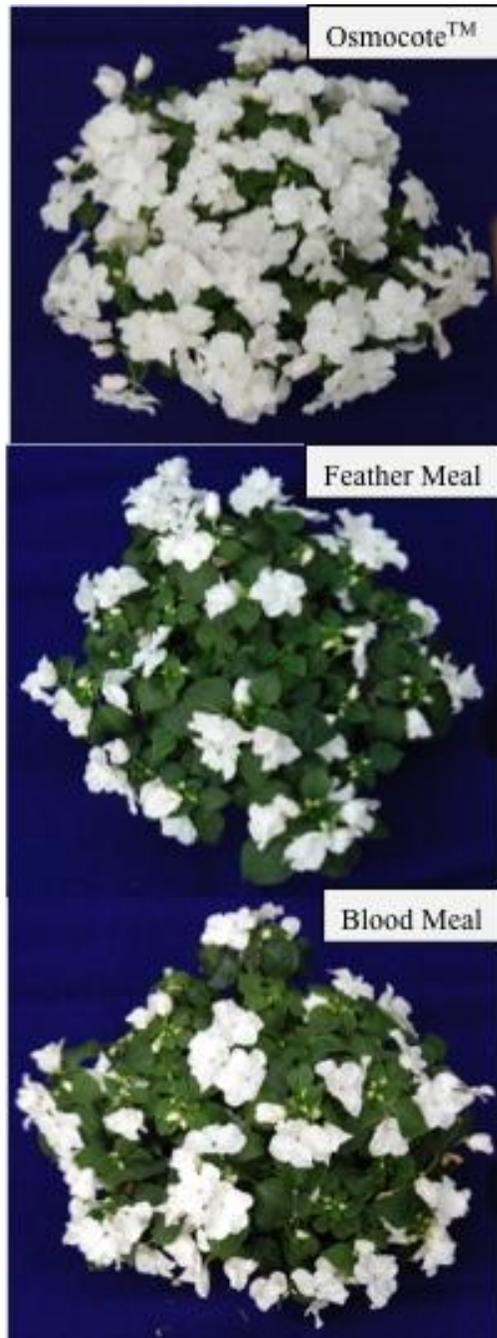
Figure A-1. Fertilizer treatment effect on floriferousness in experiment 1
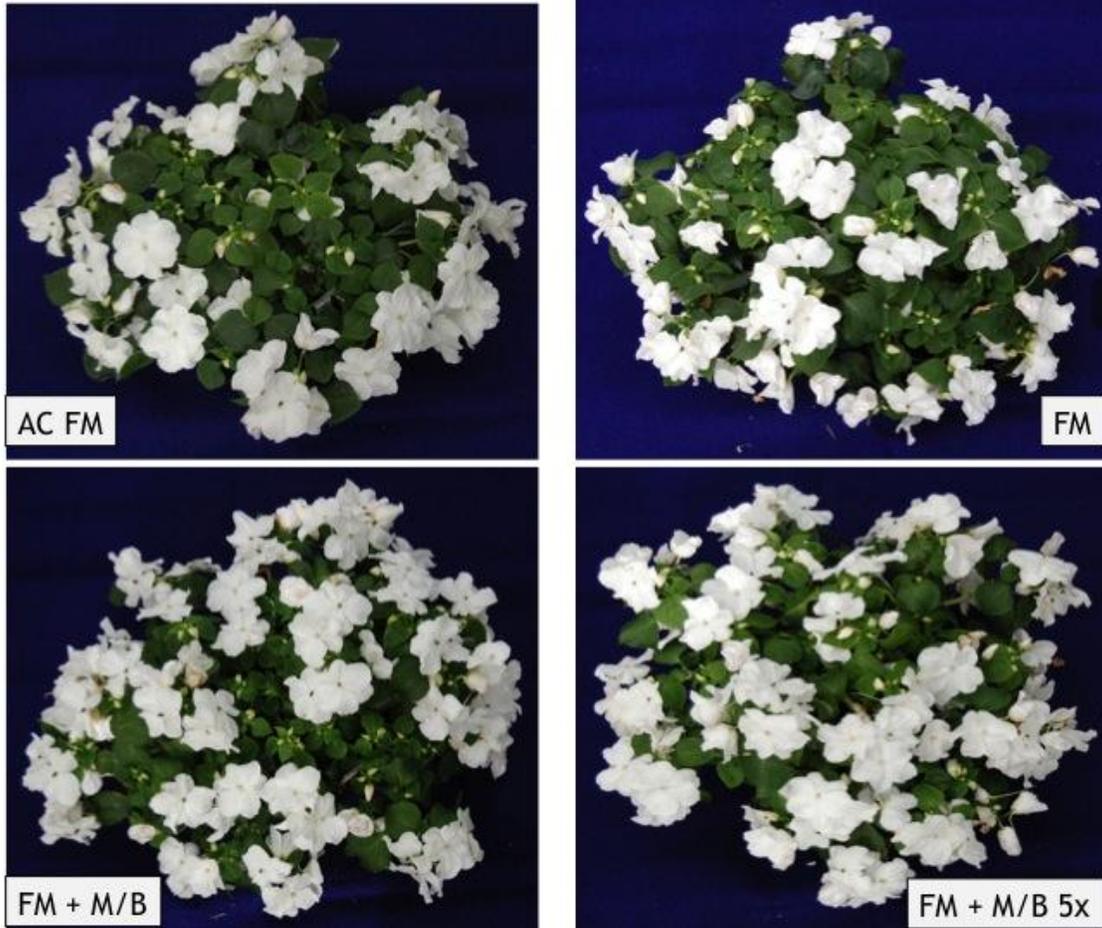
Figure A-2. Autoclave and Inoculant effect on floriferousness in feather meal treatments in experiment 1.
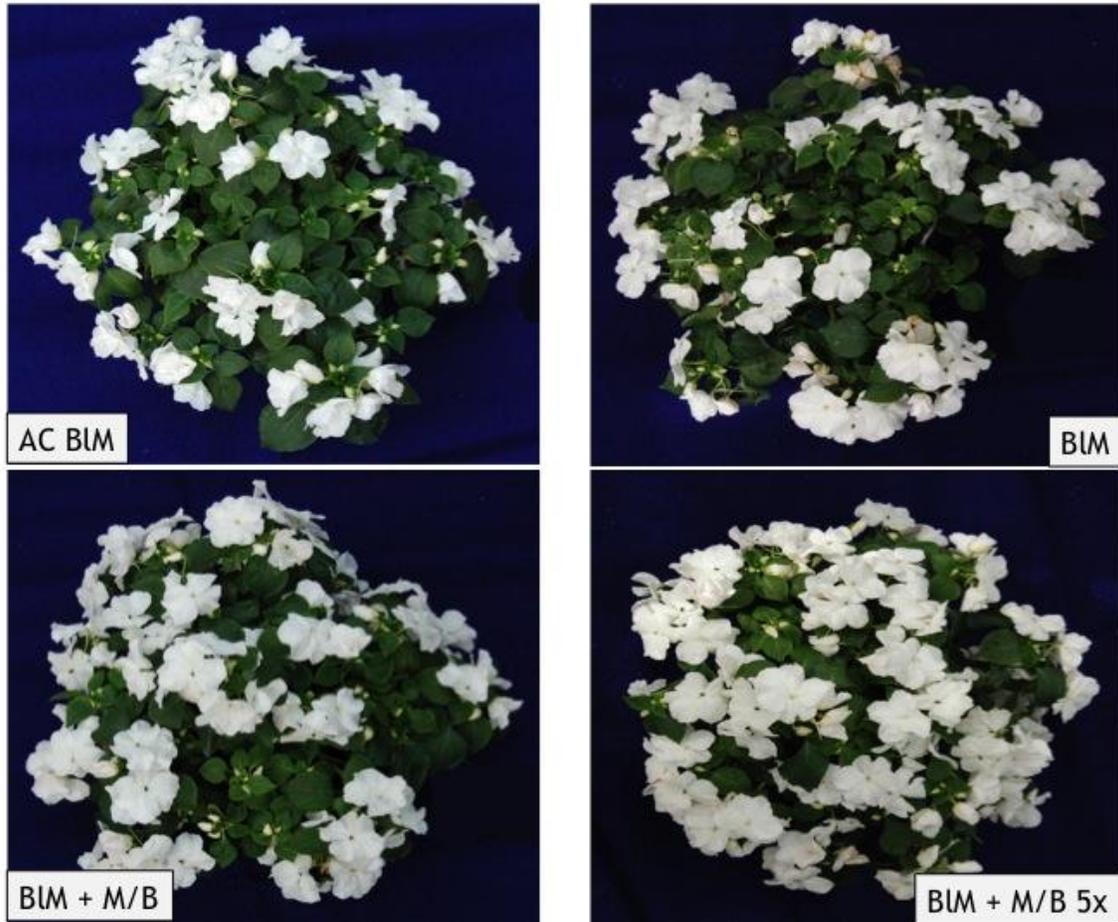
Figure A-3. Autoclave and Inoculant effect on floriferousness in blood meal treatments in experiment 1.
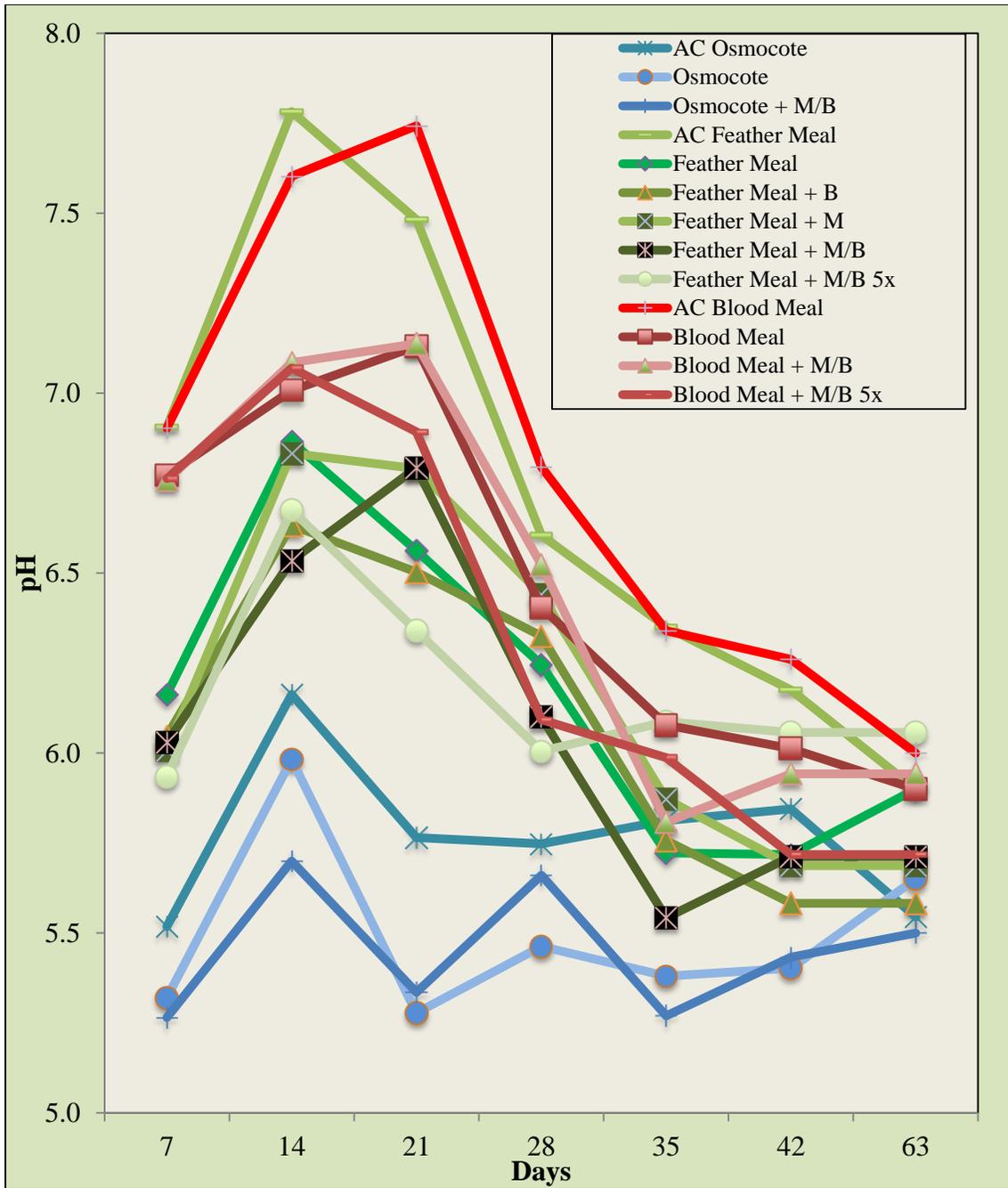
Figure A-4. Treatment effect on substrate pH in experiment 1. n=4

*Standard error of the least squares means estimate = 0.29
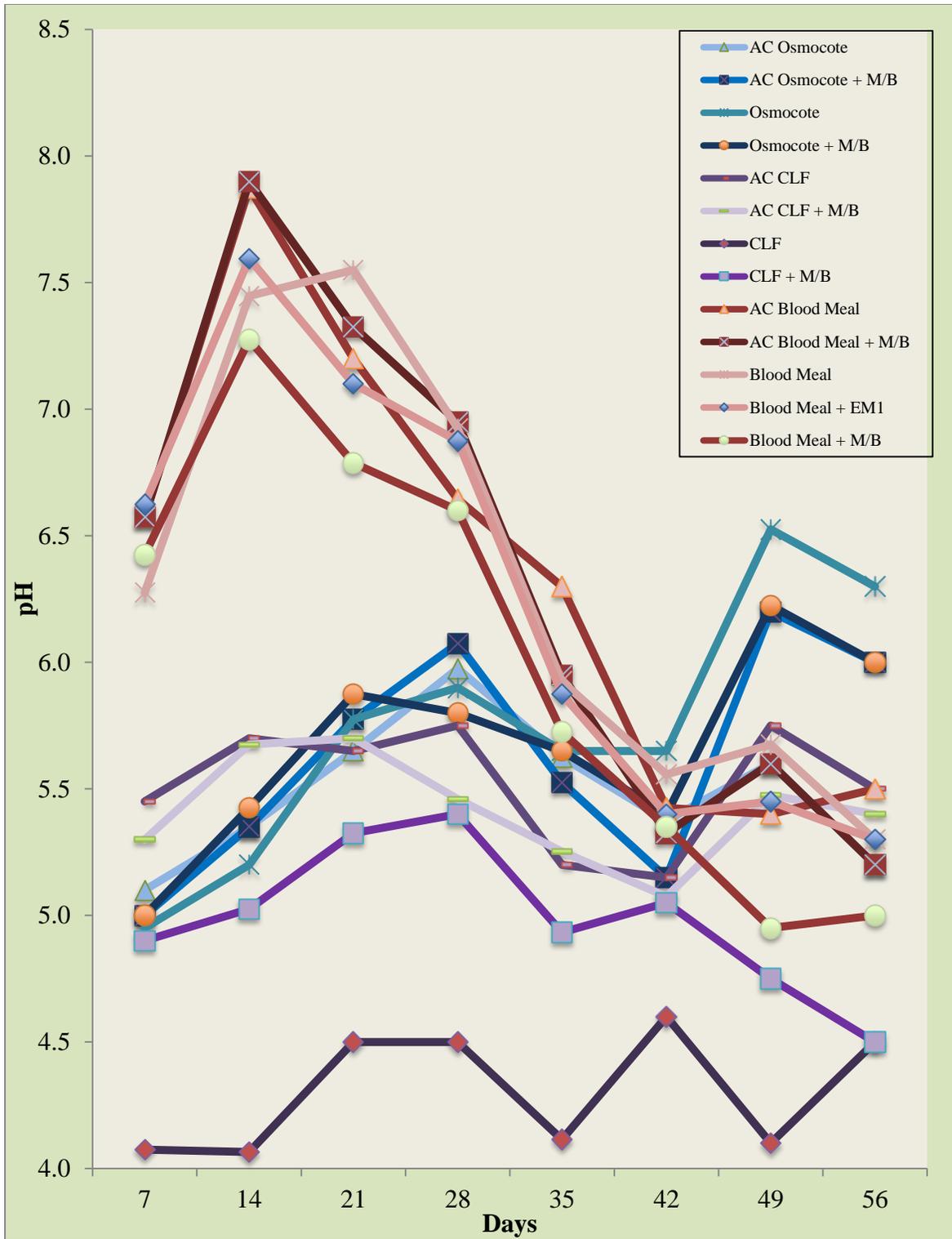
Figure A-5. Treatment effect on substrate pH in experiment 2. n=4

* Standard error of the least squares means estimate = 0.49
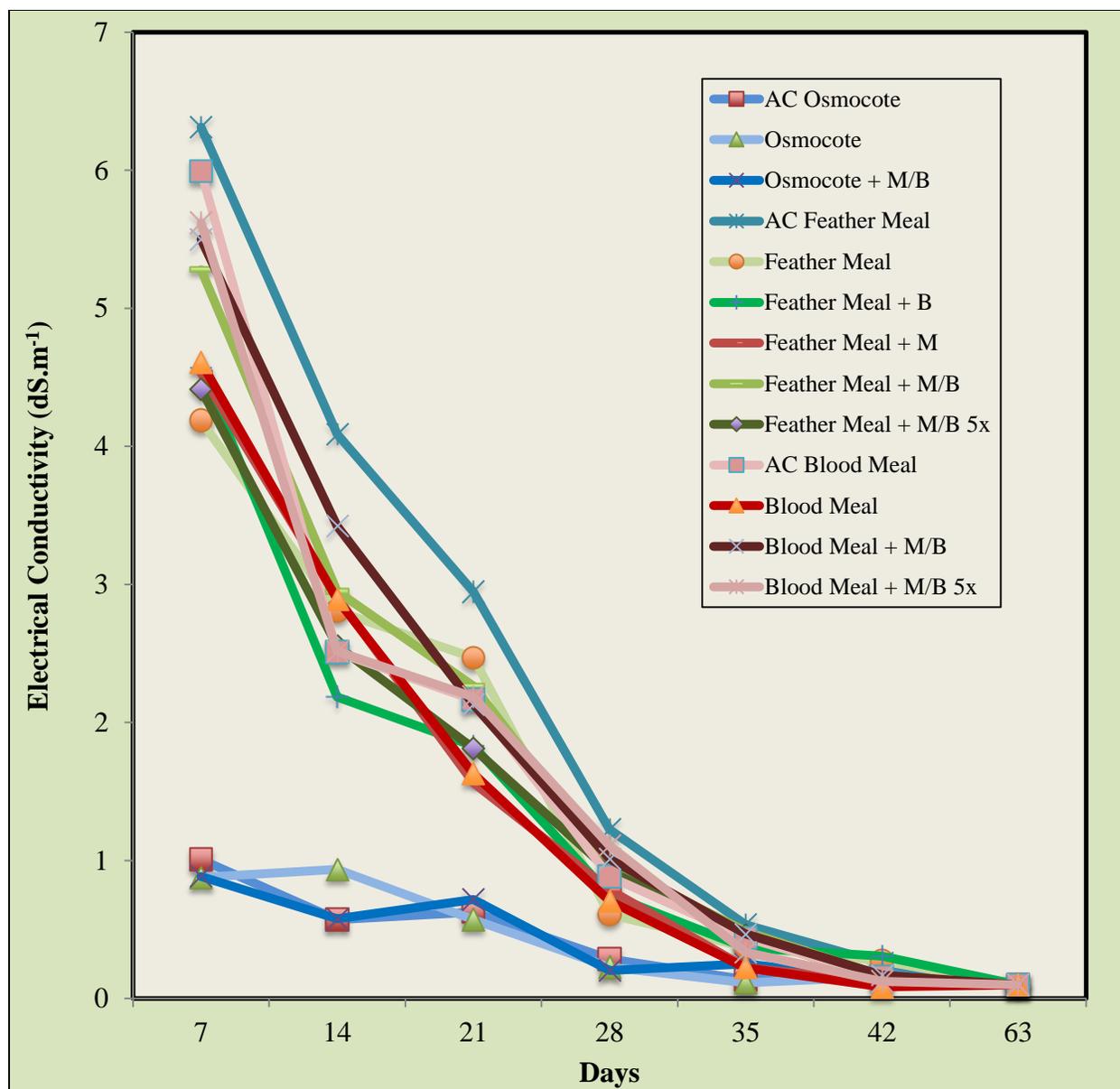
Figure A-6. Treatment effect on substrate EC in experiment 1. n=4

* Standard error of the least squares means estimate = 0.46
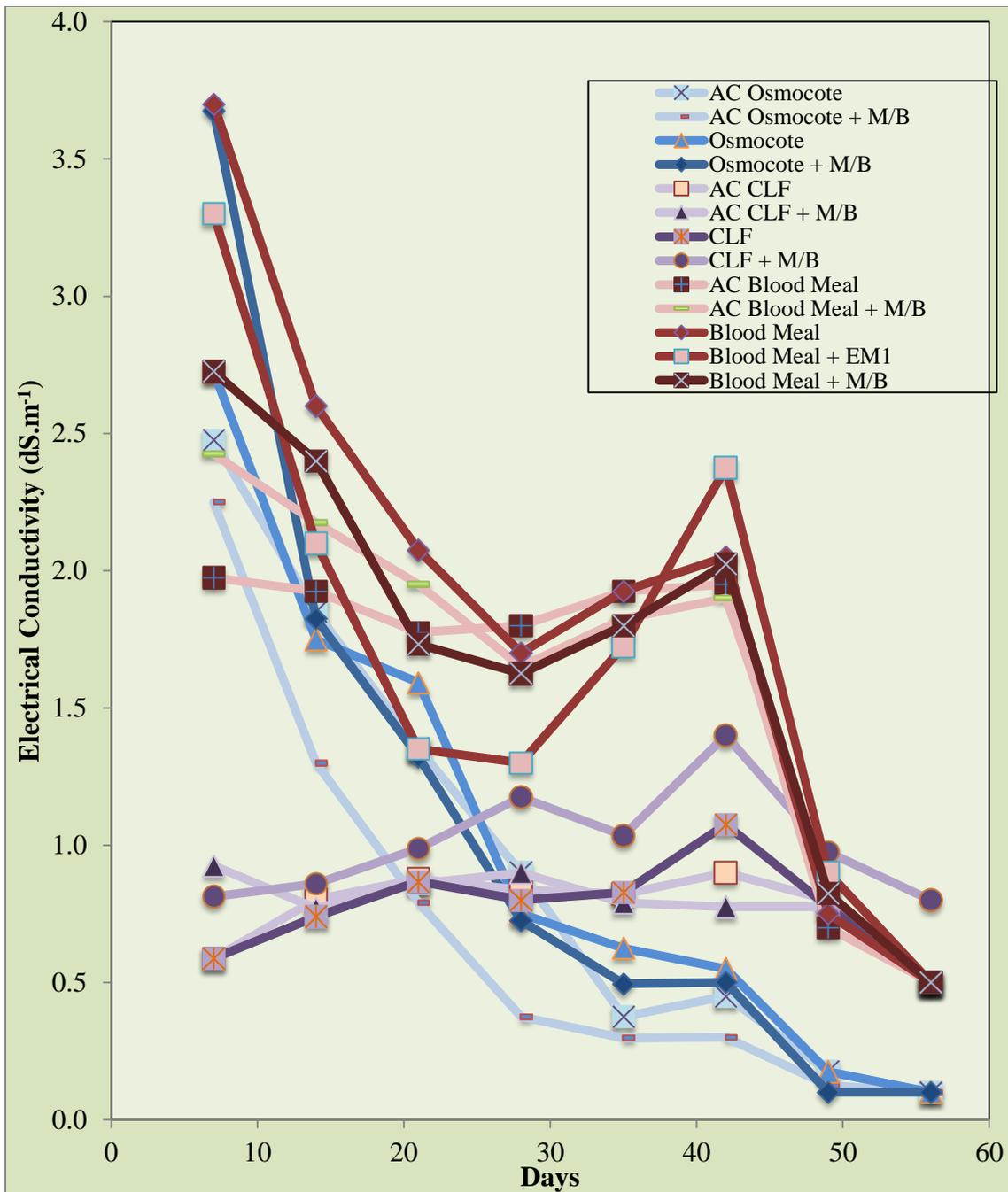
Figure A-7. Treatment effect on substrate EC in experiment 2. n=4

* Standard error of the least squares means estimate = 0.36
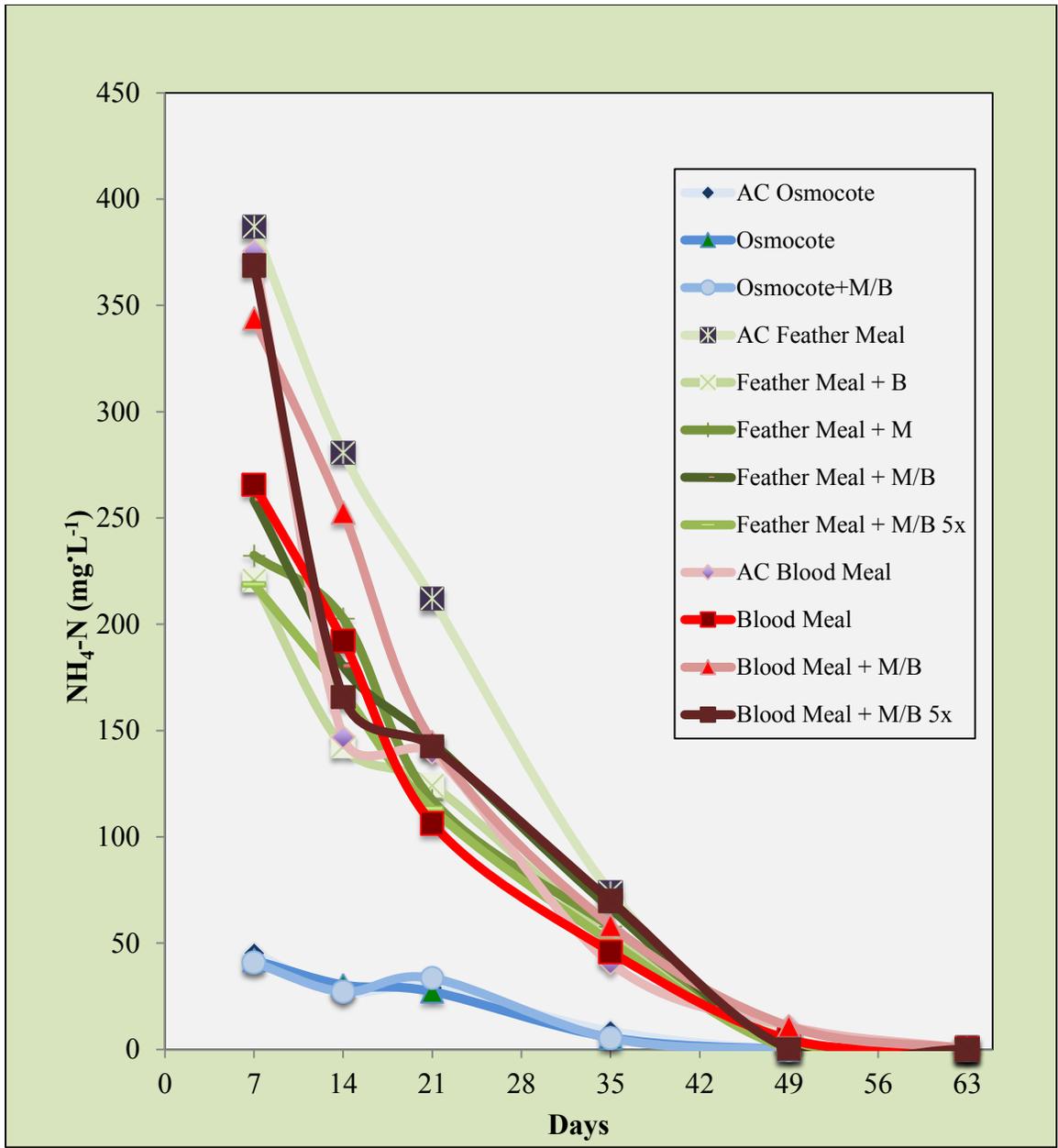
Figure A-8. Treatment effect on substrate ammonium concentrations in experiment 1. n=4

*Standard error of the least squares means estimate = 22.5
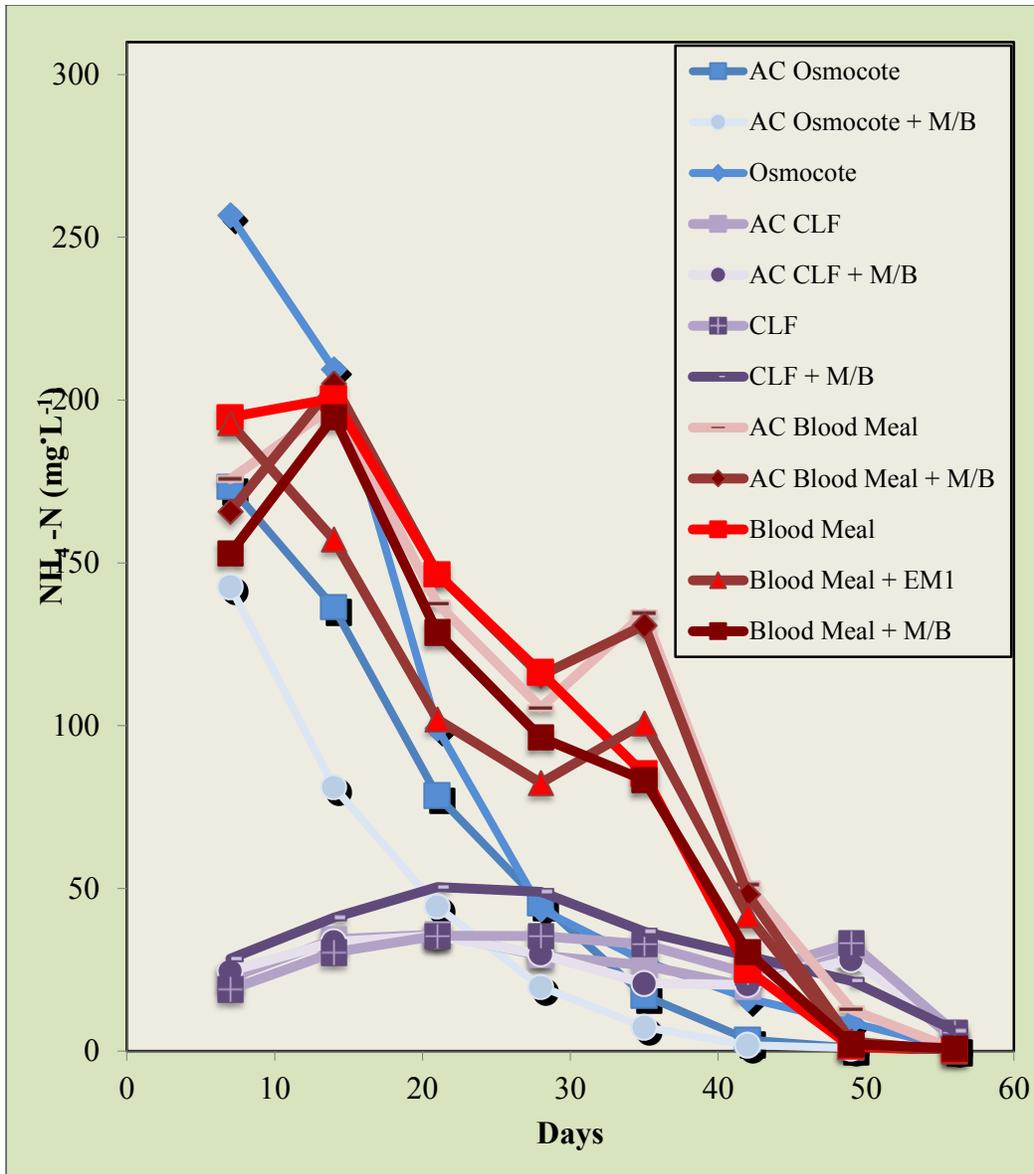
Figure A-9. Effect of treatment on substrate NH4-N concentration in experiment 2. n=
Standard error of the least squares means estimate = 16.4
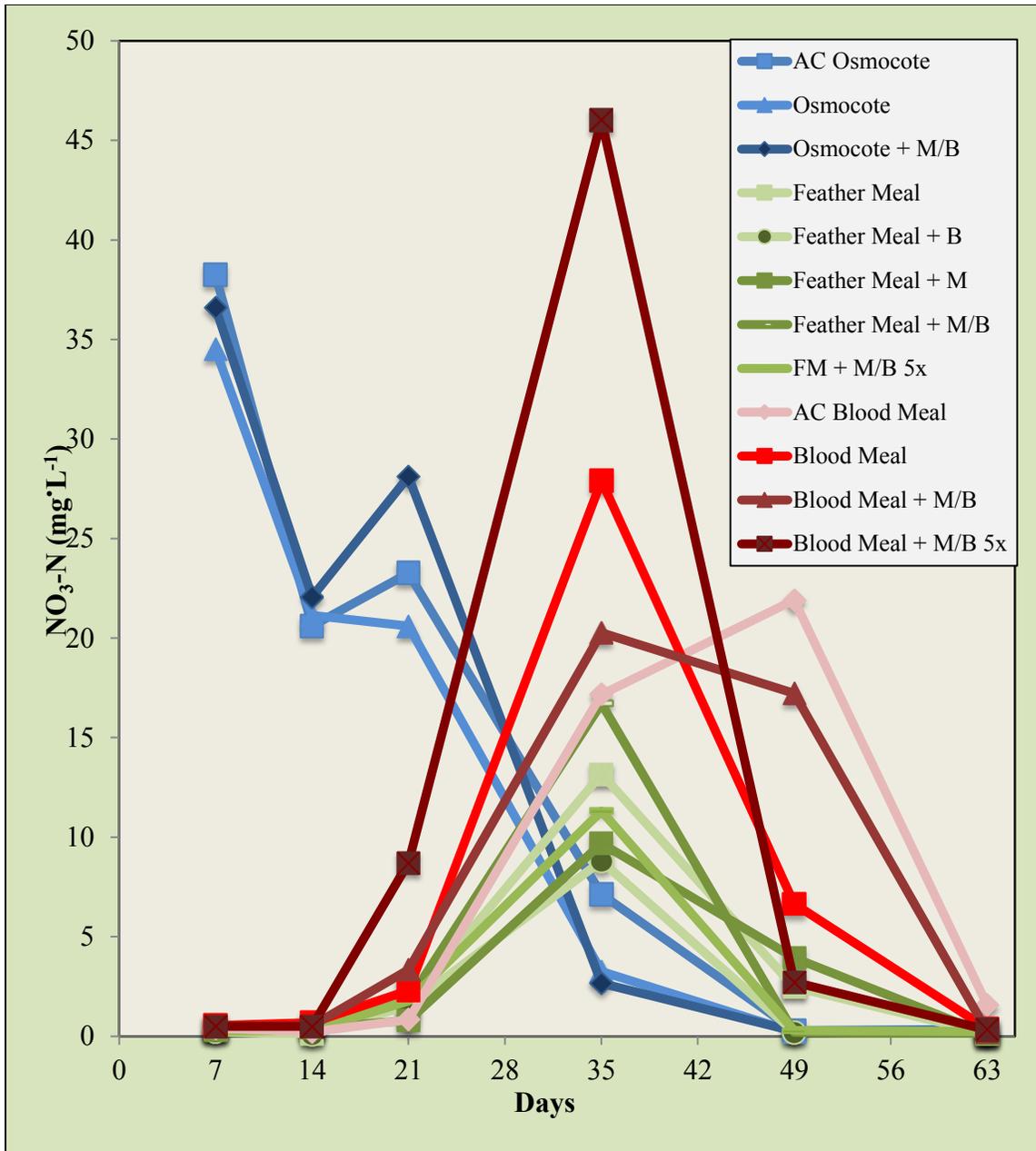
Figure A-10. Treatment effect NO$_3$-N concentrations in experiment 1. n=4

*Standard error of the least squares means estimate = 2.8
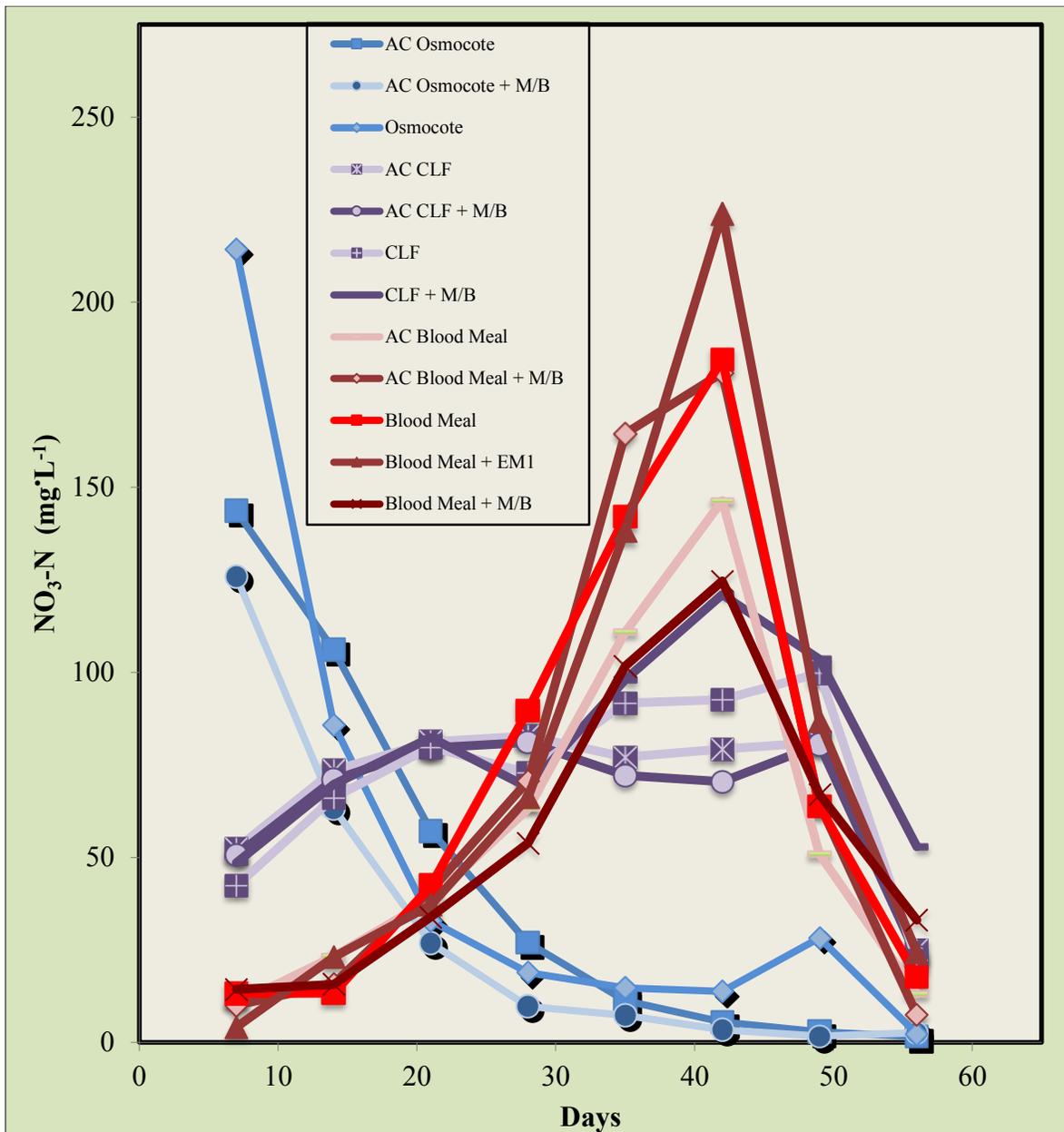
Figure A-11. Treatment effect on nitrate concentration in experiment 2. n=4

Standard error of the least squares means estimate = 18.6
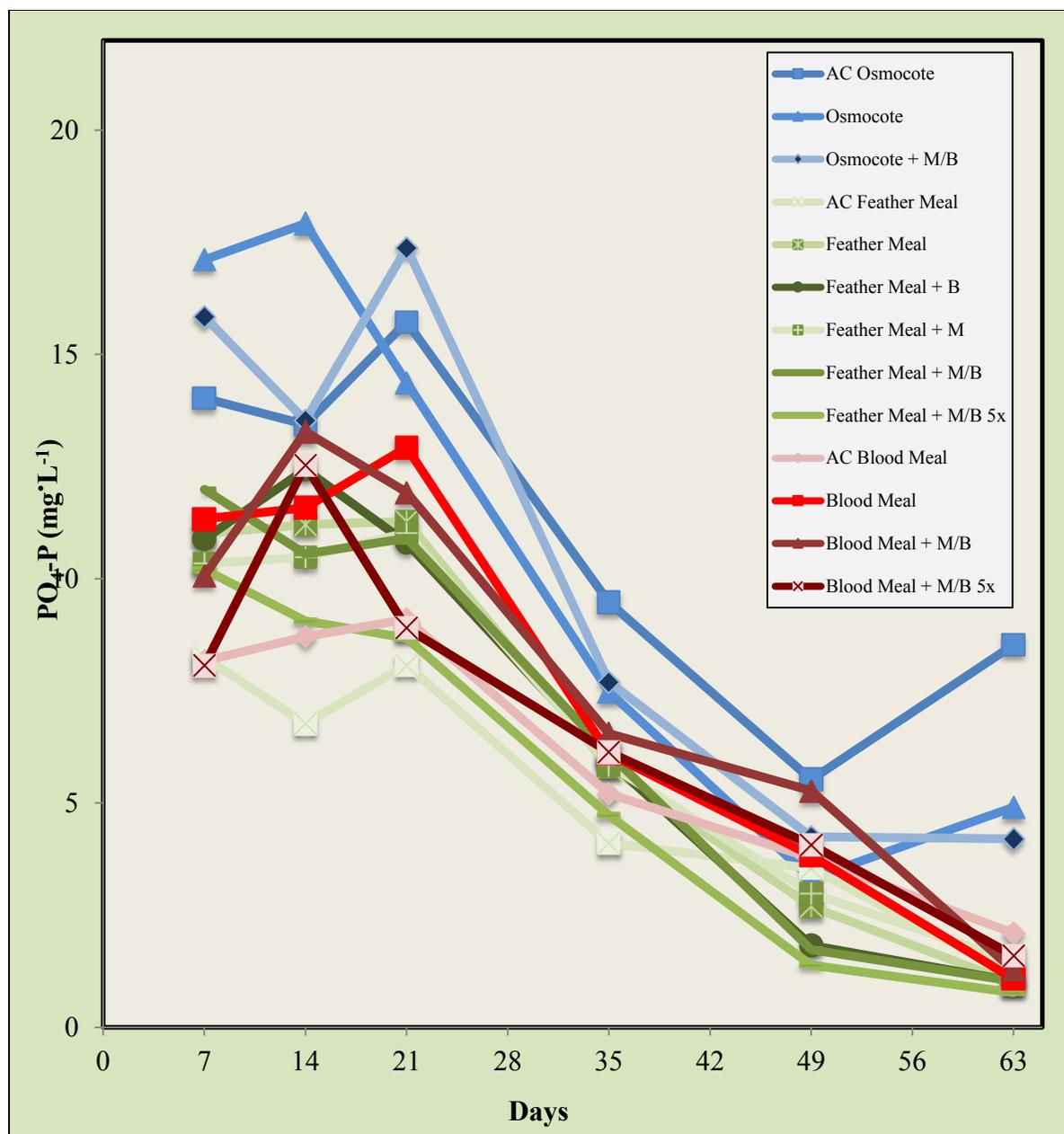
Figure A-12. Treatment effect on substrate P concentrations in experiment 1. n=4

*Standard error of the least squares means estimate = 1.4
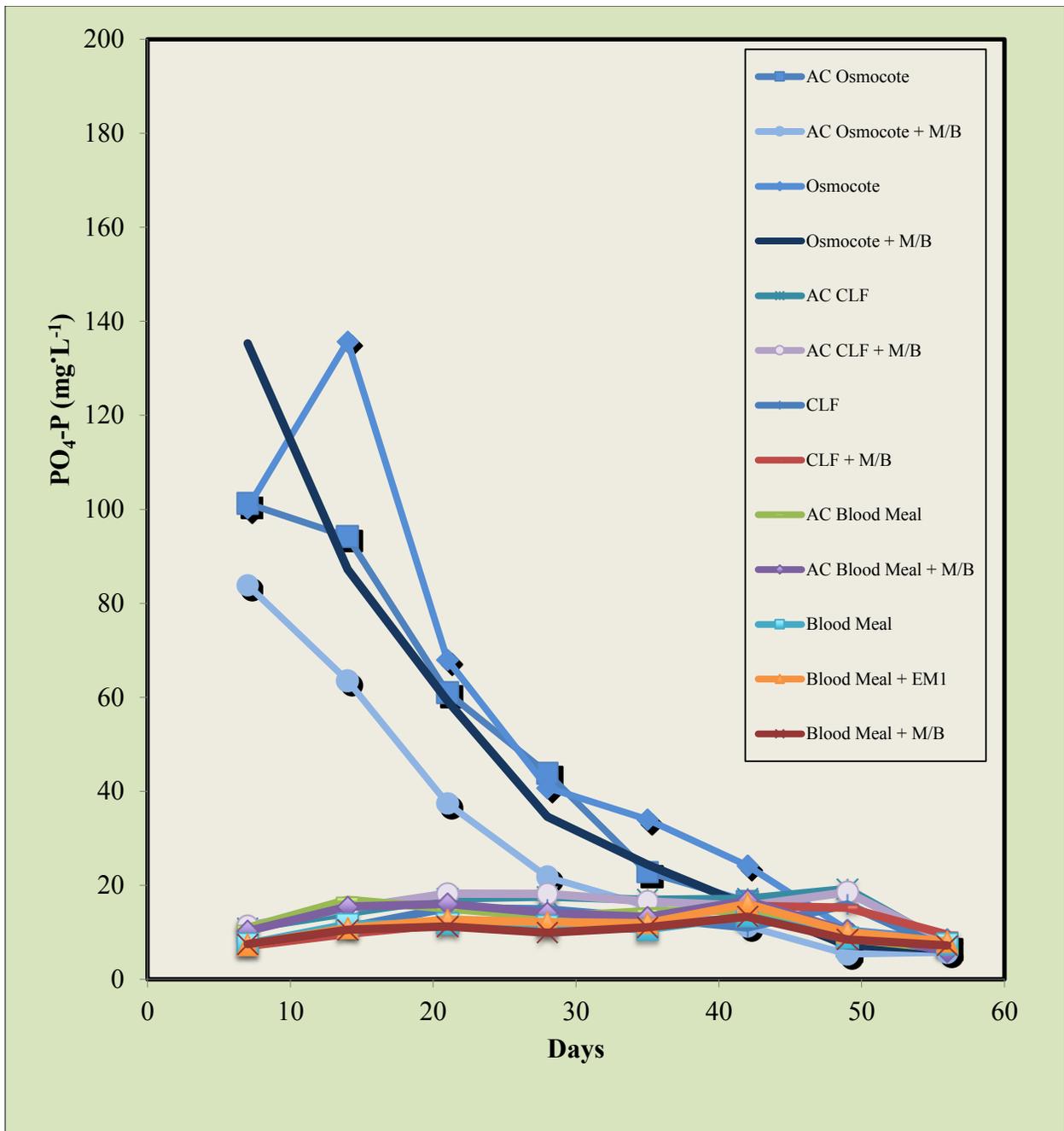
Figure A-13. Treatment effect on substrate PO4-P concentration in experiment 2. n=4

*Standard error of the least squares means estimate = 3.9
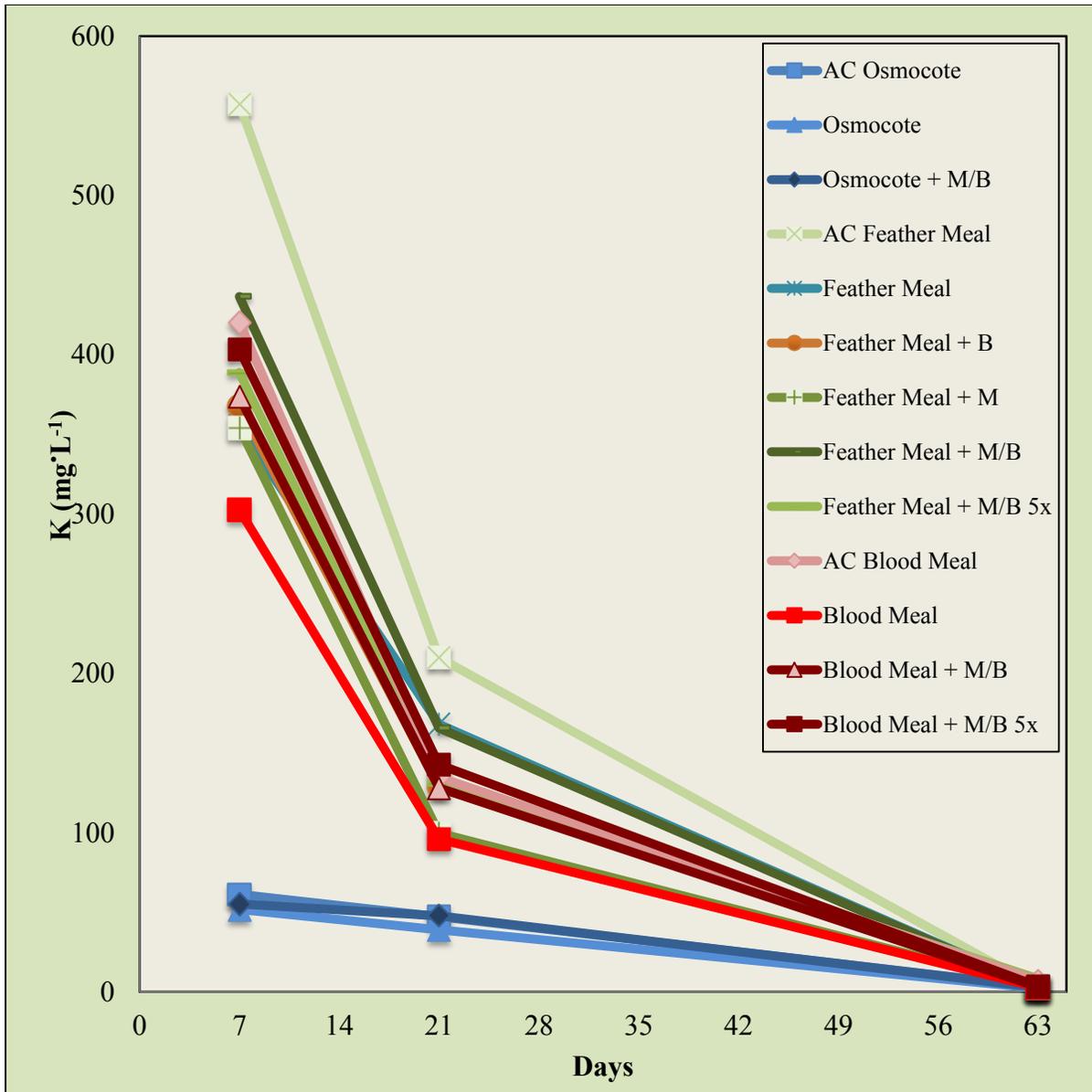
Figure A-14. Treatment effect on substrate K concentrations in the experiment 1. n=4

*Standard error of the least squares means estimate = 19.5
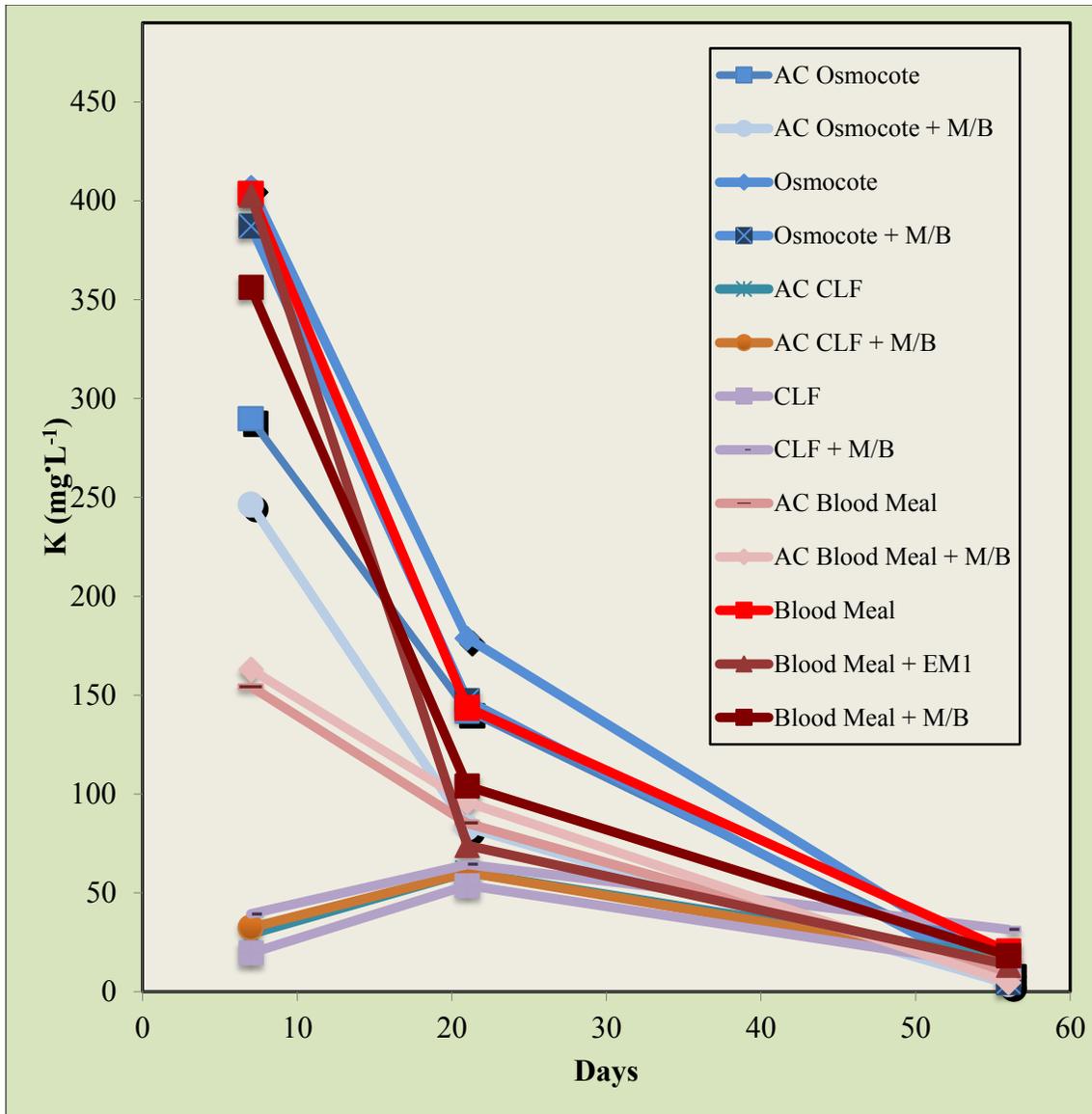
Figure A-15. Treatment effect on substrate K concentrations in experiment 2. n=4

*Standard error of the least squares means estimate = 18.6
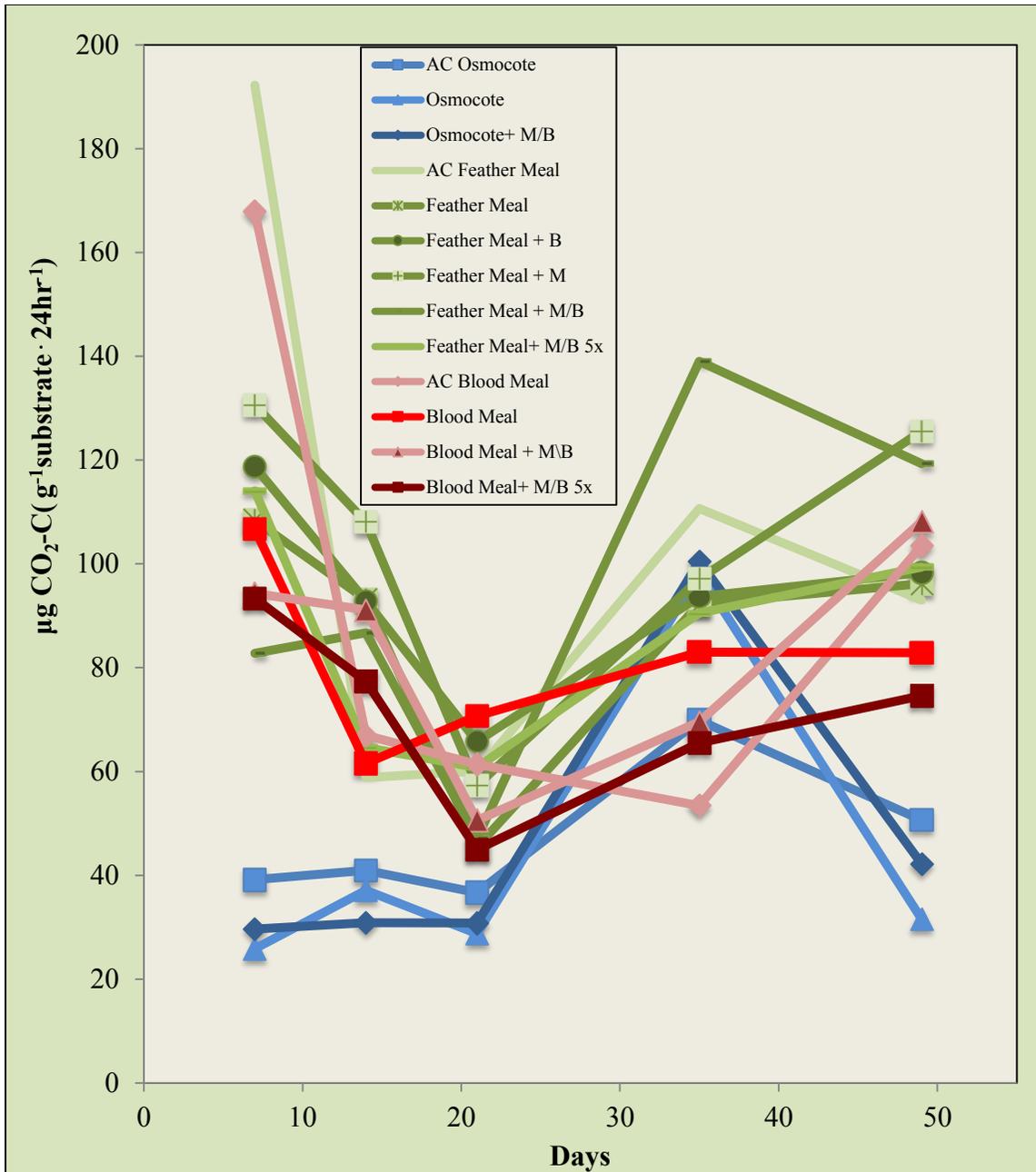
Figure A-16. Treatment effect on CO₂-C respiration from microbial activity in experiment 1. n=4

Standard error of the least squares means estimate = 26.4
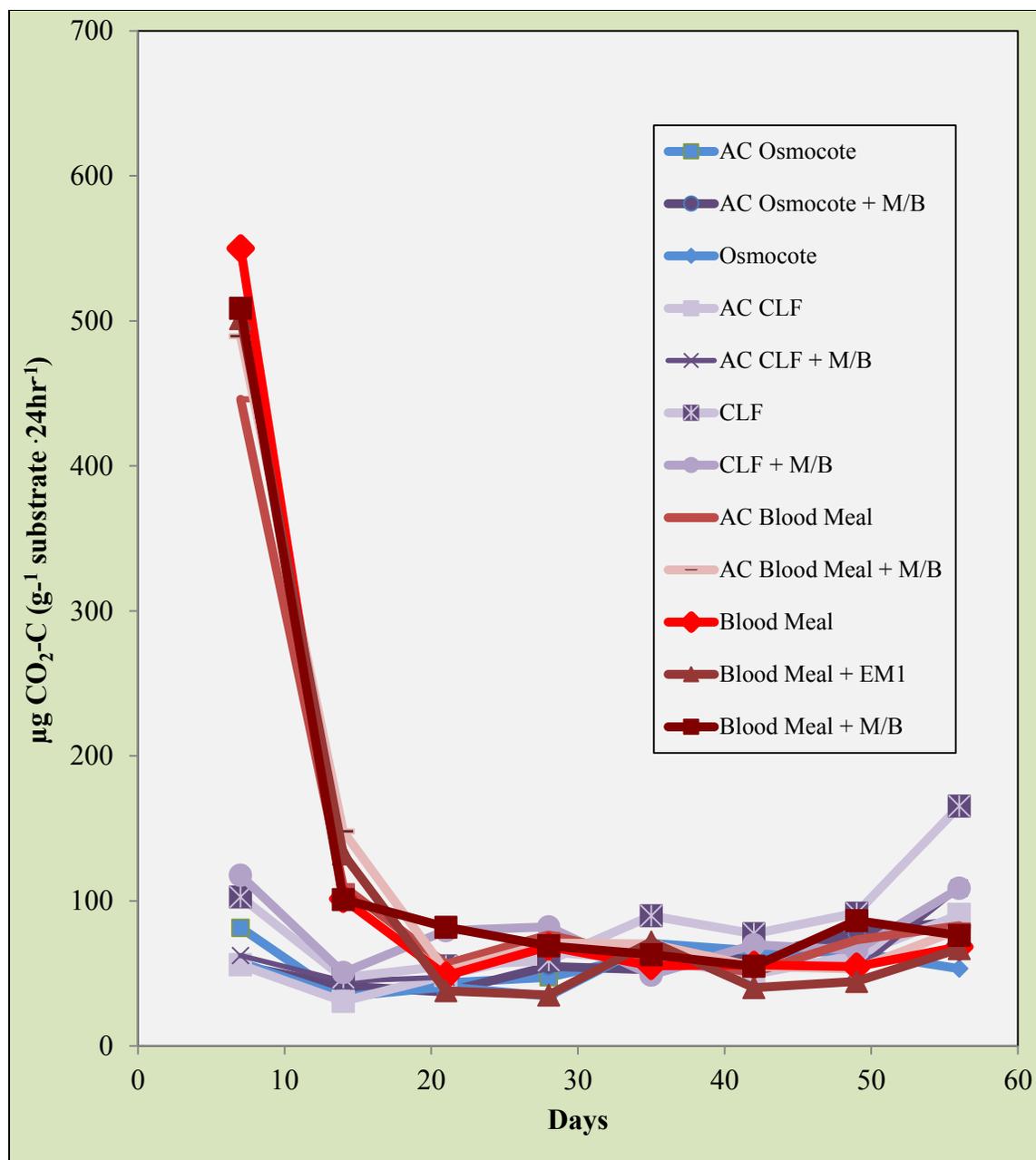
Figure A-17. Treatment effect on CO_2-C respiration from microbial activity in experiment 2. n=4

*Standard error of the least squares means estimate = 18.9
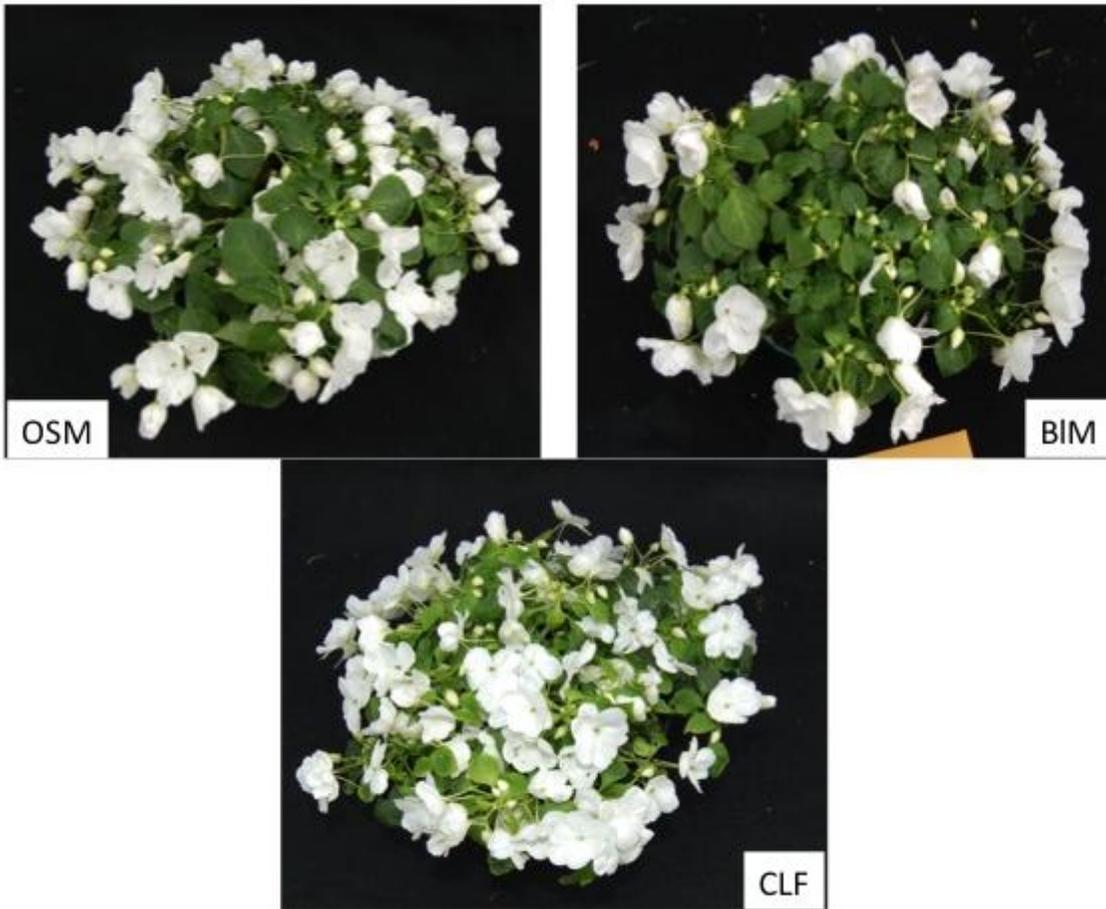
Figure A-18. Fertilizer treatment effect on floriferousness in experiment 2.
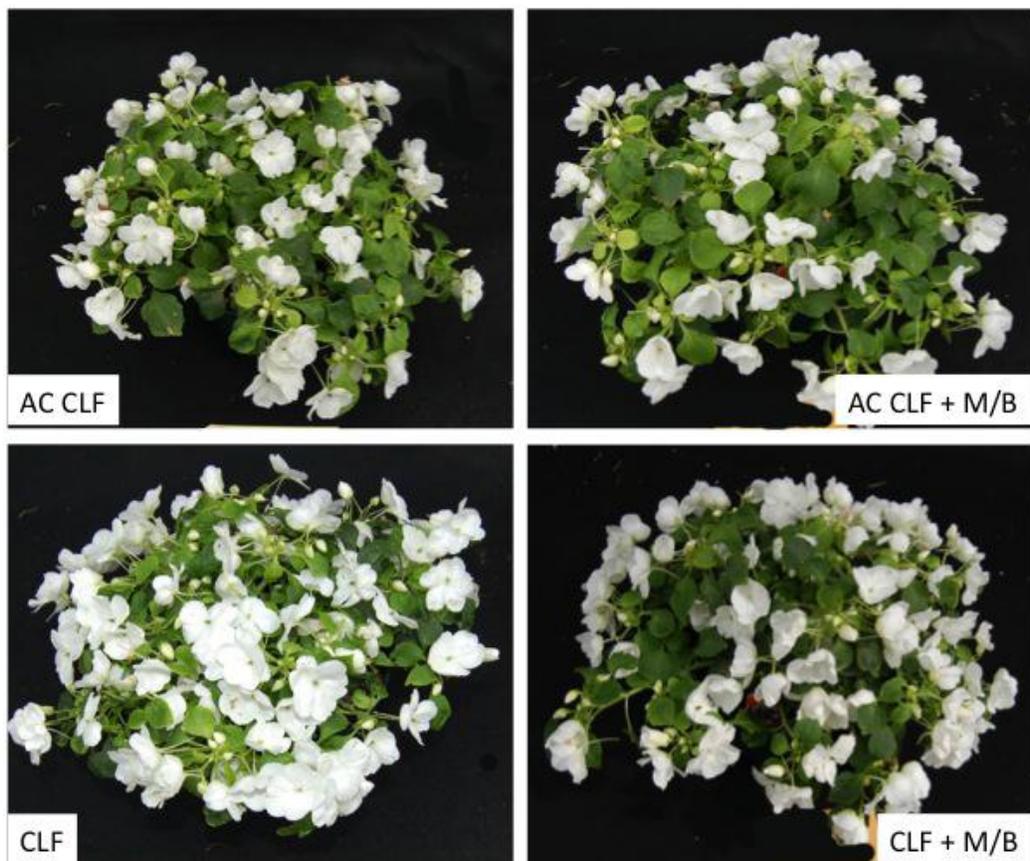
Figure A-19. Autoclave and Inoculant effect on floriferousness in CLF treatments in experiment 2.
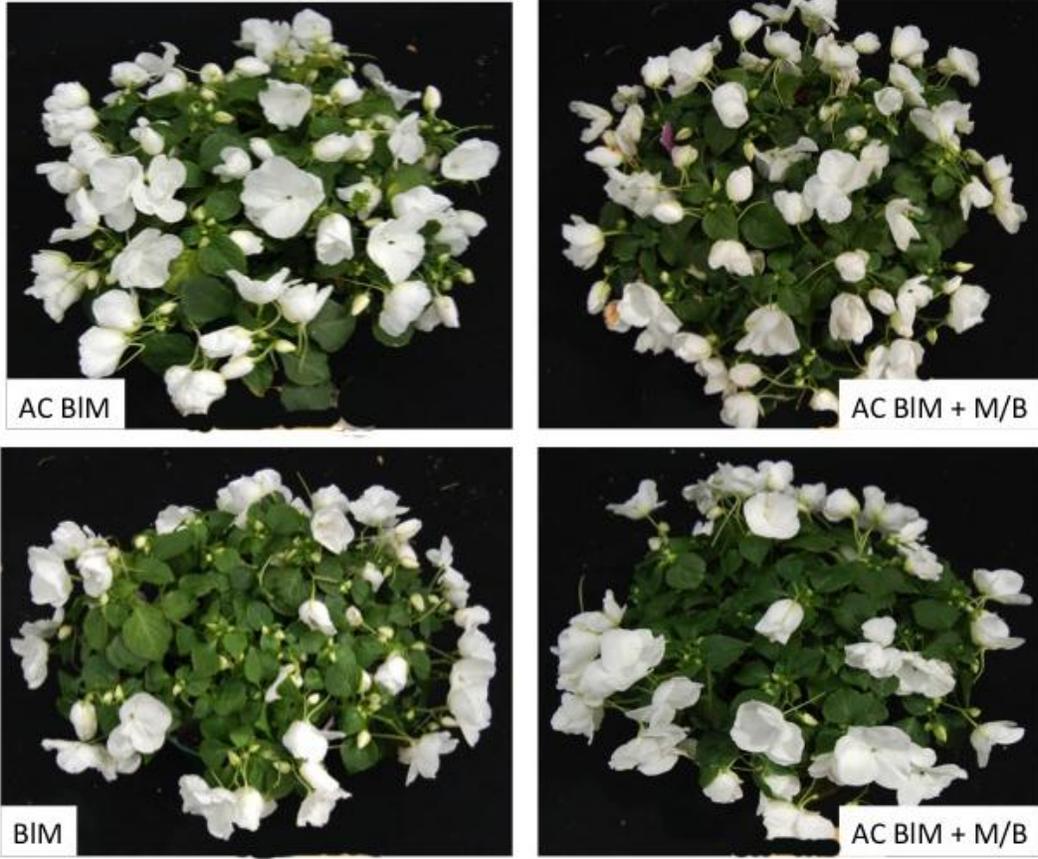
Figure A-20. Autoclave and Inoculant effect on floriferousness in blood meal treatments in experiment 2.