

**Fertilizers Affect Water and Substrate EC, pH,
and Nutritional Concentrations for *Nelumbo* Production**

by

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Abstract

Sacred Lotus (*Nelumbo nucifera* Gaertn.) is an aquatic, herbaceous perennial considered to be one of the most valuable plants in the world. Each part of lotus is consumed throughout Asia for food or used for medicinal purposes.

Effects of fertilizer type (conventional, organic, or no fertilizer), fertility rate, and water depth on water and substrate electrical conductivity (EC), pH, nitrate-nitrogen, and ammonium-nitrogen concentration were evaluated in greenhouse and outdoor studies to determine effect on potential lotus growth. All fertilizers influenced water and substrate EC, pH, and nutritional concentration. According to substrate analysis, EC rates were above recommended levels. Both organic treatments resulted in high sodium levels and the organic Nature Safe treatment resulted in higher levels of most macronutrients by termination of all studies.

Results indicated increased water volumes led to reduced nutrient concentration and availability. All measured parameters decreased with increased water depths due to greater water volume and dilution factors and researchers determined a water depth of 15.2 cm (6 in) resulted in satisfactory EC levels for lotus production. There would be no additional benefit in maintaining shallower or greater depths. EC is a strong factor influencing lotus growth and with shallower depths, EC could rise close to toxic levels as was revealed in the organic Medina Growin' Green treatment.

Toxic EC level of $1.0 \text{ mS}\cdot\text{cm}^{-1}$ was surpassed with increasing rates to $1.3 \text{ kg}\cdot\text{m}^{-3}\text{N}$ among both conventional and organic treatments. Under greenhouse conditions with moderate temperatures, researchers determined $0.6 \text{ kg}\cdot\text{m}^{-3}\text{N}$ was a potentially acceptable rate to target for the fertilizers tested for outdoor production. The rate resulted in toxic levels of soluble salts for some fertilizers and required removal and replacement of plants, substrate, and fertilizer; adjusting the rate to $0.4 \text{ kg}\cdot\text{m}^{-3}\text{N}$. A rate of $0.44 \text{ kg}\cdot\text{m}^{-3}\text{N}$ resulted in acceptable EC levels for all fertilizers trialed and tested. More research needs to be conducted to determine the interactions, cause and effect of the many variables on specific fertilizer nutrient release to target a satisfactory level to maximize growth while minimizing any potential crop damage due to an increase in EC to toxic levels.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Organic Agriculture Production: Past, Present and Future

Prior to the Industrial Revolution, agriculture was by definition primarily organic because agricultural chemicals were not yet available (Jordan, 2004). With the advent of the revolution, industrial farming did make agriculture easier, yet it also created environmental problems which ultimately led to the uprising against agricultural practices that had the potential to harm not only nature but also human health, stimulating the impetus for creating modern organic farming (Jordan, 2004).

Local, state, and federal agencies are requiring growers to confront environmental issues such as surface and ground water contamination, pesticide usage, and energy consumption (Bailey, 1998; Weiler et al., 1999). One option growers have to reduce or prevent the discharge of pollutants into the environment is by growing crops organically, a method of growing that made its way into the U.S. from England and Germany in 1938 as the topsoil blew off of the High Plains during the Dust Bowl (Worster, 1979; Fromartz, 2006). By 1941, J.I. Rodale made “organic” a household name through his magazine *Organic Farming and Gardening*. Early organic farming advocates such as Rodale envisioned reducing the use of chemical inputs in farming and restoring soil

fertility in the face of deteriorating soil conditions (Dimitri and Oberholtzer, 2005). Farming, however, lost sight of the organic philosophy upon the discovery of synthetic fertilizers and pesticides following World War II (Carson, 1962; Fromartz, 2006; Jordan, 2004; Worster, 1979). Farmers found they could generate quality produce and plants more efficiently, not realizing that this efficiency had potential hidden costs which negatively affected land, water, and air (Carson, 1962; Jordan, 2004; Worster, 1979). Nearly twenty years after the chemical revolution, there was an environmental re-awakening with the release of Rachel Carson's *Silent Spring* (1962) which suggested that short term gain was at the expense of long-term tragedy.

Federal, state, and local regulations are requiring growers/farmers to adopt measures which decrease the leaching of chemicals into the environment and improve water quality (Drinkwater et al., 1995; Fromartz, 2006; Weiler et al., 1999). In support, various agencies provide funds to assist farmers in converting their operations to more environmentally friendly operations (OECD, 2003; USDA, 2005). Numerous U.S. companies are adopting organic production to conserve nonrenewable resources, decrease input costs, secure high value markets, and increase farm income (USDA, 2006).

In 1990, the U.S. Department of Agriculture (USDA) was mandated through the Organic Foods Production Act (OFPA) to develop standards for U.S. organic products (Dimitri and Oberholtzer, 2005). On 21 October 2002, USDA's National Organic Program (NOP) went into effect defining organic agriculture as "a production system that is managed in accordance with the Organic Foods Production Act (OFPA) and regulations to respond to site-specific conditions by integrating cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and

conserve biodiversity” (USDA, 2002). The International Federation of Organic Agriculture Movements (IFOAM), a grassroots organization based in Bonn, Germany whose goal is the worldwide adoption of systems based on organic agriculture principles, similarly defines organic agriculture as “dramatically reducing external outputs by refraining from the use of chemo-synthetic fertilizers, pesticides and pharmaceuticals. It allows the powerful laws of nature to increase both agricultural yields and disease resistance” (Willer and Yussefi, 2006). The regulations set forth by the USDA require certification of producers by approved agencies in order for organic products to have the approved USDA “organic” label and stamp. The USDA defines the term “organic” as “a labeling term that refers to an agricultural product produced in accordance with the Act and the regulations in this part” (USDA, 2002). Although the methods and materials growers use might vary, every aspect of production and handling must comply with the provisions of the OFPA, given a particular certified organic farm (Dimitri and Greene, 2002; USDA, 2002). At present, there are no regulations set for organic, aquatic production.

Organic farming has become one of the fastest growing segments of U.S. agriculture since the implementation of the National Organic Standards (NOP) in the 1990s (Sok and Glaser, 2001; USDA, 2005). Currently with 31 million hectares in production worldwide, organic agriculture is increasing annually by approximately 5 million hectares as farmers endeavor to supply the increasing demand for organic food products (Dimitri and Greene, 2002; Willer and Yussefi, 2006). Organic sales have grown approximately twenty percent (20%) per year since 1990, due mainly in part to consumers viewing organic foods as a means to aligning, not only their nutrition and

health, but also their environmental and social well-being (Fromartz, 2006; Jordan, 2004).

Benefits of organic production systems are becoming evident as products continue to develop throughout the world, especially in countries outside of North America and Europe. The principal growth in demand for organic products is in Europe and North America with the market value reaching nearly \$28 billion in 2004 (Willer and Yussefi, 2006). In 2009, total U.S. sales of organic products were estimated to be more than \$23 billion with approximately 37% in the edible market (Zurko, 2010). Although the benefits of organic agriculture are well researched and there has been extensive research on organic production, there is little to no research documented on the organic production of aquatic plants.

Organic Soil Amendments

The use of organic materials to improve soil conditions has escalated in recent years due to the growing worldwide interest in utilizing renewable forms of energy. The greatest benefit of incorporating organic materials into soils is the overall improvement in soil conditions such as development, maintenance, and improvement of structure (increasing water holding capacity) and encouragement of microbial activity that makes nutrients available faster (Chellemi and Lazarovits, 2002). Natural fertilizers (i.e. bone meal, blood meal, seaweed extracts) supply nutrients over a longer period than most synthetic ones and are less likely to burn plants (Gillman, 2008). Chellemi and Lazarovits (2002) found a high nitrogen containing organic fertilizer is a nutritional alternative to a high analysis mineral fertilizer. The continued use of synthetic, mineral nitrogen fertilizers tend to lower pH values and deplete soil organic matter content (Wen,

1982). In contrast, once organic fertilizers are applied, gardeners would rarely need to apply them more than once a year because of their slow release and at times, one application can last several years (Coleman, 1989; Gillman, 2008). Thus, keeping in mind that organic fertilizers originate from plants, animals, and/or minerals, they release half their nutrients within the first season and continue to slowly break down over subsequent years (Coleman, 1989; Gillman, 2008). In short, this means that various organic fertilizers are not readily water-soluble and subject to leaching; enough can be applied at one time to last a number of years (Coleman, 1989). Organic production yields long-term, sustainable benefits by way of building the soil: “A fertile soil, like an educated mind, is a cumulative process, and with care it is capable of continuous improvement” (Coleman, 1989).

There is a selection of organic fertilizers and/or soil amendments for use in organic production. Some of the more common materials are animal manure, blood meal, bone meal, feather meal, greensand, kelp meal, and dolomitic lime which is used to adjust soil pH (Greer, 2005; Kuepper and Everett, 2004). Animal manures contribute to the supply of nitrogen (N) and phosphorous (P), to the alleviation of potassium (K) deficiency, and help prevent micronutrient deficiencies (Wen, 1982). Additionally, best results can be obtained for plant growth rates when organic fertilizers are formulated to approximate the N-P-K levels of conventional fertilizers (Miles and Peet, 2002). According to Aung and others (1983), fish and its byproducts have been recognized as a fertilizer suitable for plants because of favorable crop responses. Nielsen and Thorup-Kristensen (2004) suggested that an ideal organic substrate blend should supply most of the nutrients needed for plant growth and limit the need for additional soluble nutrients.

Nonetheless, this increased utilization has taken place mainly in dry, upland crop fields where crops other than aquatic plants are grown (Hesse, 1982). The improvement of physical properties of soils may have more relevance to dry soils rather than flooded soils; yet there is limited information available. In aquatic plant production (more notably *Nelumbo* sp.), there is no evidence of research conducted on the effects of organic fertilizers and/or amendments within anaerobic environments. There is a plethora of research conducted on the effects of organic fertilizers for field or greenhouse production of vegetable crops for aerobic environments; yet there is no information available for growers who desire to grow aquatics organically.

Evolutionary history of *Nelumbo*

Sacred Lotus (*Nelumbo nucifera* Gaertn.) is an aquatic emergent angiosperm that belongs to the Nelumbaceae family and the genus *Nelumbo* Adans. (hereafter referred to as lotus). There are two species within the genus *Nelumbo*: *N. nucifera* Gaertn. and *N. lutea* (Willd.) Pers. North American native *N. lutea* (American Lotus) is distributed from eastern North America, extending south to Columbia. *N. nucifera* (Asian or Sacred Lotus) is distributed throughout Asia and Oceania, from Russia to Australia. Lotus has been cultivated for over three thousand years in the Far East where it continues to be used for medicine, food, and for cultural and religious activities (La-Ongsri, 2004; Shen-Miller, 2002). Both species of lotus prefer shallow (approximately one meter), still water with a mud bottom where its rhizomes will spread rapidly into a water depth of two and a half (2.5) meters (Cook et al., 1974; Main et al., 2006).

Lotus has a long evolutionary history and is considered one of the world's most ancient plants, known as a molecular living fossil (Qichao and Xingyan, 2005; Sanderson

and Doyle, 2001). Based on fossil records, lotus appeared in the Northern Hemisphere 135 mya (million years ago). The earliest record is assigned to the Cretaceous period (Fischer, 1996; Qichao and Xingyan, 2005). From all over the world, lotus fossils have been excavated and in North America there are thirty fossil species of the Upper Cretaceous and Tertiary age that have been assigned to *Nelumbo* (Gandolfo and Cuneo, 2005). It was thought that *Nelumbo* had only grown in the Northern Hemisphere until 2005 when a fossil of *N. puertae* from the Upper Cretaceous period was found in Patagonia, Argentina; which is the only fossil of lotus found in the Southern Hemisphere to date (Gandolfo and Cuneo, 2005). Fossil records further reveal lotus was more widely distributed in the past than today; only two species (*Nelumbo lutea* and *N. nucifera*) survived the Ice Age perhaps due to the ‘bottleneck effect’ and/or the ‘founder effect’ which can cause a species to be squeezed out of existence (Qichao and Xingyan, 2005; Tian et al., 2008; Tian, 2008).

There is an ongoing debate among researchers (and countries) on the origin of lotus. There have been suggestions that lotus originated in India, which may have come from the man who named the plant: J. Gaertner, a European botanist. ‘*Nelumbo*’ is the name of an area located south of India, in Sri Lanka, which may have led to its common name ‘East Indian Lotus’ (Qichao and Xingyan, 2005; Wang and Zhang, 2004). Additionally, India based their claim on the first Indian fossil of lotus leaf and rhizome impressions that were recorded from the Pleistocene epoch of Kashmir (1.8 to 0.01 Ma) in the Tertiary of Assam; giving them ‘evidence’ that lotus is indigenous to India (Mitra and Kapoor, 1975; Sharma and Goel, 2000). Based on the wild lotus populations in India along with iconographic and scriptural records, the history of lotus may be traced to

about 4,000 years ago (McDonald, 2004). Mitra and Kapoor (1975) state that lotus is “according to Indian thought and culture, is the ‘visible expression of creative activity from waters of Creation’ [and] is a highly valued plant in Indian Medicine.”

Nonetheless, when a fossilized lotus leaf was excavated from Linqu, Shandong, China proved to be much older, (approximately 15 mya), it was concluded that lotus was not originally native to India but to China (Wang and Zhang, 2004). In 1973, lotus pollen fossils more than 7,000 years old were found among cultural relics excavated from the monument ‘Hemudu Culture’ in Yuyao, Zhejiang, China (Tian, 2008). According to archaeological studies and C- 14 analysis, China has quite a rich history related to lotus for at least 7,000 years and has cultivated it for more than 3,000 years (Guo, 2009; Qichao and Xingyan, 2005; Tian, 2008; Xeuming, 1987).

Historically, many researchers have considered *Nelumbo* to be closely related to *Nymphaea* (i.e. water lilies). This taxonomic classification was based primarily on similarities in floral and vegetative morphology, as well as in habitat (Stevens, 2001). Doubt first arose regarding its classification in the early 20th century, when York (1904) stated that the “systematic position of [*Nelumbo* in] the Nymphaeaceae has again become a prominent question” by studying its embryo and floral structure. Convincing evidence came to light when Les and others (1991) compared serology suggesting that *Nelumbo* be removed from Nymphaeales and be recognized as its own, distinct order; supported by contrasting alkaloid chemistry between *Nelumbo* and other Nymphaeaceae members. Another important difference between a lotus and water lily is the triaperturate pollen of *Nelumbo*, which differs greatly from the monoaperturate pollen grains of *Nymphaea*; thereby placing *Nelumbo* in its own family and subclass/order (Fig. 1) (Friis

et al., 2001; Kreunen and Osborn, 1999; Stevens, 2001). In addition, *Nelumbo* leaves can be distinguished from genera in the Nymphaeaceae family as they are peltate and the latter have a single, characteristic ‘Pac-Man’ like notch from the edge into the center of the lily pad.

Molecular studies to date have placed lotus among the clade eudicots, closely related to *Platanus*, even though there is no consensus on its exact taxonomic position (Banks et al., 2006; Hayes et al., 2000; Kreunen and Osborn, 1999). *Nelumbo* is currently recognized (and isolated) within its own family, Nelumbonaceae, and is among several, quite distinct, families in the clade eudicot, order Proteales; having trees and shrubs (Platanaceae and Proteaceae) as its closest living relatives (Hayes et al., 2000). Many leaf fossils from the Southern Hemisphere that are ‘platanoid’ in appearance explains why the Platanaceae and Proteaceae are sister taxa (Stevens, 2001). Although flowers of these taxa appear different, both consist of perianth, stamens, and fleshy structures (Stevens, 2001).

Eudicots comprise about 75% of extant angiosperm species and Proteales forms a polychotomy with Sabiaceae, followed by a polychotomy with Buxaceae and Trochodendraceae (Friis et al., 2001). Fossil pollen records indicate that eudicots appeared 125 Ma, shortly after the origin of angiosperms themselves and the origin of the order Proteales is clearly ancient, evident within the mid-Cretaceous period (~100 Ma) and both *Platanus* and *Nelumbo* can be thought of as living fossils (Sanderson and Doyle, 2001; Stevens, 2001). The divergence of both Nelumbonaceae and Platanaceae is suggested to have happened 121-115 Ma, based on using molecular and fossil data (Stevens, 2001). Of interest are the current family distributions: Platanaceae and

Nelumbonaceae from the Northern Hemisphere and Proteaceae from the Southern Hemisphere.

Platanaceae is a family of flowering plants and more notably known as the 'plane tree family' and common in the Northern Hemisphere. This family consists of only one living genus *Platanus* and according to Dirr (1998) contains only four species: *P. occidentalis* L. (American Plane tree or Buttonwood), *P. orientalis* L. (Oriental Plane tree), *P. racemosa* Nutt. (California Sycamore), and *P. wrightii* S. Wats. (Arizona Sycamore). *P. occidentalis* and *P. orientalis* are the parents of hybrid *Platanus x acerifolia* (Ait.) Willd., the infamous London Plane tree one can find planted all over urban areas due to its ability to withstand pollution and severe pruning (Dirr, 1998; Russell and Cutler, 2003). Plane trees/Sycamores are one of the largest of all deciduous temperate trees which can exceed heights of 30 m (100 ft), have great spreading canopies, and trunk girths of six m (20 ft) (Russell and Cutler, 2003).

The family Proteaceae consists of nearly eighty flowering genera and about 2000 species and is mainly restricted to the Southern Hemisphere. Well known genera include: *Protea*, *Banksia*, *Embothrium*, *Grevillea*, *Hakea*, *Dryandra* and *Macadamia*. Most of these species are found in Australia and South Africa, yet it does extend throughout most of the Southern Hemisphere and into SE Asia. Many species produce long-lasting, showy flowers utilized in the cut flower industry and portray thick, waxy/hairy leaves which are adaptations for water retention (Russell and Cutler, 2003). This family is regarded as a classic 'Gondwanic Group': A group that originated well before the fragmentation of the ancient supercontinent Gondwanaland (120 Ma) which molecular dating confirmed its placement (Barker et al., 2007).

Anatomically speaking, lotus does not visually resemble any of the families that it is deemed to be close relatives with. Furthermore, it is believed that these three families have been classified in the order Proteales due to each having such distinct characteristics that they cannot be placed elsewhere. Yet, lotus flowers do share some biological characteristics of higher land plants such as their blooms, pollination, and fertilization (Qichao and Xingyan, 2005).

Lotus cultivars are categorized into three groups based on utilization and strongest feature: flower, seed, and rhizome lotus (Nguyen, 2001; Qichao and Xingyan, 2005). All lotus cultivars have ornamental values; yet, seed lotus is bred for high yield and quality for seeds; rhizome lotus is bred for rhizome quality and yield, not flowers. Each and every plant part of lotus is consumed throughout Asia for food or used for medicinal purposes, including rhizome, nodes, runners, seed, young shoot, leaf, stalk, petal, stamen, and pericarp (or fruit receptacle, seedpod) (Nguyen, 2001; Qichao and Xingyan, 2005; Wang and Zhang, 2004). ‘Ornamental’ lotus cultivars have smaller, swollen rhizomes and low starch content compared to ‘rhizome’ cultivars; thus are not used for vegetable production (Tian, 2008).

Lotus is among the world of geophytes: corms, tubers and rhizomes which are classified as underground organs that are modified from parts of the stem (Masuda et al., 2007). Rhizomes are defined as “an underground stem that grows horizontally and, through branching, acts as an agent of vegetative propagation...serving as organs for perennation” (Tootill and Blackmore, 1984). They differ from tubers because a tuber lasts for one year only (i.e. acting as annual such as potato, *Solanum tuberosum* L.) and rhizomes are generally perennial in nature, such as a bearded iris, *Iris germanica* L.

(Tootill and Blackmore, 1984). Lotus is an herbaceous, aquatic perennial with rhizomes that elongate with two types of emerging leaves: floating and upright (standing) leaves (Nohara and Kimura, 1997). As with many perennials, lotus is affected by photoperiod: Short day-length promotes rhizome enlargement and inhibits leaf production (entering dormancy) with decreased temperature (Shen-Miller et al., 2002).

Lotus has several unique morphological traits including its ability to grow in anaerobic conditions. Lotus rhizomes extend and creep throughout anaerobic sediments, portraying a special adaptability to grow in such conditions (Matthews and Seymour, 2006). This adaptability resides in lotus' two-way gas transport system, which may carry oxygen rich air down to the rhizome and exude the excess air back to the atmosphere via the leaves (Mevi-Schutz and Grosse, 1988). Blaylock and Seymour (2000) state that gas canals are essential for convective and diffusive aeration of rhizomes growing in anoxic sediments. The thermo-osmotic gas transport is linked to the temperature difference between the lacunar air of the leaves and surrounding atmosphere (Mevi-Schutz and Grosse, 1988). Mevi-Schutz and Grosse (1988) concluded that lotus achieved gas transport in two separate ways: Air absorbed by the lamina is driven downwards to the rhizome; improving the oxygen supply of the underground organs, and gas from the rhizome streams in the opposite direction of the central plate (Vogel, 2004). The adaxial side of the leaf of *Nelumbo* has two distinct regions in terms of gas exchange characteristics. Across the expanse of the lamina, air enters the leaf and escapes back to the atmosphere through the highly porous region at the center of the lamina (Dacey, 1987). Air is then channeled through gas canals from the leaves throughout the petioles and rhizomes. Through one of two petiolar canal pairs, air flows from a leaf to a

rhizome, joining with the lowermost of three canal pairs in the rhizome through a chamber in the node (Tian, 2008). The lowermost canal pair links these nodal chambers along the length of a rhizome, allowing air from a node to flow both forward, toward a growing shoot, and backward, toward preceding leaves (Matthews and Seymour, 2006).

As unique a characteristic is as a two-way gas transport system, thermogenesis is also an uncommon phenomenon and has been known to occur in lotus flowers for over a century; temperature elevations up to 10°C (50°F) can occur above that of air (Seymour and Schultze-Motel, 1998). Yet, the physiological regulation of flower temperature is far rarer, only occurring within three genera, lotus included (Seymour and Schultze-Motel, 1998). Thermoregulation occurs at the cellular level and may be linked to the cyanide-insensitive respiratory pathway found in several aroids and the North American Lotus (*Nelumbo lutea* Willd.) (Seymour and Schultze-Motel, 1998). This process is to benefit insects that are needed for the lotus to be pollinated by creating a stable environment which enhances their ability to eat, mate, and prepare for flight (Watling et al., 2008).

An evolutionary achievement that has secured its rightful place as a living fossil is how lotus seed is able to persist through centuries and still be able to germinate (Qichao and Xingyan, 2005; Wang and Zhang, 2004; Vogel and Hadacek, 2004). Seeds have germinated after 1000 years or longer (Hayes et al., 2000; Shen-Miller et al., 2002; Qichao and Xingyan, 2005; Wang and Zhang, 2004). Holder of the world's record for the longest-term seed viability is a lotus seed excavated from Xipaozi, Liaoning, China (Shen-Miller et al., 2002). Metabolic activities in germinated ancient lotus seeds have been investigated by Maeda et al. (1996). Shen-Miller (2002) stated that their long-term

viability may reside within a capability to repair cellular damage that is not well understood in plant biology.

Nelumbo nucifera is well-known all over the world for not only its utilization in religious ceremonies but also for being a food staple for numerous Asian countries. Lotus' diverse nature and international popularity as a vegetable, ornamental, and/or medicinal would suggest that there may be opportunities for growing this plant locally. Its status as a living fossil symbolizes its worldly significance and stature and as an ancient prayer still heard in Tibet today: Om mani padme hum, which translates "Oh, the jewel [is] in the heart of the lotus" (Slocum and Robinson, 1996).

Economic importance of *Nelumbo*

Due to its remarkable historic, economic, and scientific diversity, lotus is considered to be one of the most valuable plants in the world. Currently, lotus grown in Europe and the U.S. are mainly used for ornamental purposes rather than for food (Nguyen and Hicks, 2004). Moreover, lotus has been grown in the Imperial Valley in California and Yamaguchi (1990) indicated that it could be successfully grown in the southeastern U.S. Lotus is cultivated for its edible rhizomes in several different countries: China, India, Japan, Korea, and the U.S. [Hawaii] (Hanelt, 2001). Recently, the crop has become a non-traditional vegetable for the export market in countries such as Mexico (Rogers and Redding, 2003) and Australia (Nguyen, 2001).

N. lutea was once utilized by several Native American tribes as a food staple. The rhizomes are rich in starch and when baked, they become sweet and mealy, somewhat like a sweet potato. The starchy rhizomes are roasted, pickled, dried, fried as chips, and/or used for starch production. Additionally, the acorn-like seeds are an

oriental delicacy and are eaten raw, roasted, boiled, pickled, candied, or ground as meal. The fruit is an enlarged receptacle containing many embedded seeds that contain up to nineteen percent (19%) protein and edible oil can be extracted from them (Magness et al., 1971; Sayre, 2004).

Lotus is an economically important aquatic plant prized not only for its ornamental/edible appeal, but also as a source of herbal medicine with strong antipyretic, cooling, astringent, and demulcent properties (Han et al., 2007). In Bueng Boraphet, the largest freshwater swamp and lake in Thailand, lotus leaves are used for flavoring and wrapping material while the seeds and rhizomes are utilized for table food (Jintanugool and Round, n.d.). In Tamilnadu, India, the dried petals of lotus are mixed with water and made into a paste to treat snake bites (Sandhya et al., 2006).

Globally, lotus rhizomes are one of the most popular vegetables due to its crisp texture, attractive color and abundant nutrients. Lotus is rich in nutrients including starch, sugars, proteins, lipids, vitamins, minerals, alkaloids, flavonoids, and other medicinal chemicals. Chemical and nutritional compositions in lotus organs or tissues (rhizomes, nodes, seeds, embryos, petals, stamens, pollens, seedpods, leaves and petioles) have been widely reported and new compounds are continuously found. A popular method of marketing and adding value to agricultural products is classifying them as functional foods. Functional foods are those that provide health benefits beyond basic nutrition. The Nutritional Business Journal reported that sales of functional foods in the U.S. grew from \$11.3 billion in 1995 to \$18.5 billion in 2001. There has been significant research that indicates that consumers are willing to pay a premium for functional foods, given that they are expected to reduce healthcare costs in the long run.

A study by Sloan (2000) found that about 66% of grocery shoppers indicated that their purchase decisions were based on their desire to reduce the risk of or manage a specific health concern. Maynard and Franklin (2003) found that a significant segment of consumers were willing to pay a premium of \$0.35 per gallon of milk above additional processing costs for “cancer-fighting” dairy products. Markosyan and others (2007) conducted a study on willingness to pay for apples enriched with antioxidants and found that consumers were on average willing to pay a 6% premium for these products. Lusk and others (2008) found that consumers were willing to pay a higher premium for grass-fed beef products when they were informed of the health benefits (higher levels of CLAs, Omega 3, fatty acids, and Vitamin E) as compared to grain fed products. Considering the taste, nutrient, and antioxidant levels found within lotus rhizomes, perhaps consumers would be more than willing to adopt them into everyday diets.

Markets

Lotus rhizomes are used extensively in China and Japan, sold fresh whole, or sliced into pieces and frozen or canned. Rhizome consumption in Japan accounts for nearly 1% of all vegetables consumed annually and although the country grows its own, it still has to import 18,000 tons of lotus rhizomes each year, of which China provides 15,000 tons (Dharmananda, 2002). In 1995, Japan imported 1,347 tons of fresh and 14,887 tons of salted lotus rhizomes (Nguyen and Hicks, 2004). In Taiwan, lotus is traded in both rhizome and seed forms. Seed trade represents 5% of the entire industry but the price is twice that for rhizomes (Nguyen, 2001). Mass production and sale of container lotus is just beginning in the U.S., however it is focused on ornamental and not

vegetable (Creamer, 2008). In the U.S., despite the large Asian and Asian-American population, the potential demand for edible lotus is unknown.

There have been reports of imports from China to San Francisco. In 2001, a USDA study cleared the import of lotus rhizomes from El Salvador, Honduras, and Nicaragua (Khan and Lima, 2001) and in 2003, fresh rhizomes from Guatemala were allowed into the country (APHIS, 2003). Of the several countries in Asia where lotus is cultivated and consumed, the Japanese market seems to offer the best opportunities for export. Its market produces 70,000 tons annually valued at approximately \$800 million (Nguyen and Hicks, 2004).

Production

Lotus is quite versatile and can be grown in ponds, containers, greenhouses, and raised beds. Additionally, it is often integrated with aquaculture to increase profits. When growing under glasshouses and high tunnels, a year-round supply can be ensured for markets. Cultivar selection and cultivation techniques are location dependent. For vegetable and seed production, large size cultivars are planted in lakes and ponds whereas for ornamental lotus, small and medium size cultivars are planted in water gardens or containers. Field production plant density varies from 4,115 to 7,936 plants per hectare and in China the planting area of vegetable lotus is approximately five to seven million hectare. The total yield of edible rhizomes is about 6 million tons (Tian, 2008).

Planting and Harvesting Times

The optimal planting time of lotus is between late March and early May, depending on the local climate (Tian, 2008). The largest cultivation area of lotus resides in Wuhan, Hubei Province, China, which resides close to the latitude of Auburn, Ala.

(Wuhan: 30°35'0" N; Auburn: 32°36'35" N), suggesting the southeastern U.S. would be optimal for commercial lotus production. In 2003, the cultivation area in Hubei province was 67,300 ha and the harvest of fresh produce reached 1.97 million tons (Guo, 2009; Liu et al., 2006). The general times for harvesting rhizomes are from July-September for early season varieties and October-March for late season varieties. In China, harvesting rhizomes is done mainly by hand (preferred method) which is quite difficult and laborious (McGrath, personal observation).

Propagation

Lotus can be propagated by seeds or by underground rhizome division. Seed may survive for long periods of time due to its extremely rigid seed coat (1,000 to 2,000 years). When utilizing seed, it must be treated either physically or chemically prior to sowing for favorable germination. Plants grown from seed can finish a full life cycle (seed-to-seed) within one year with no large difference in plant growth between seed and rhizome propagation (Tian, 2008). Seed propagation is mainly used in breeding new cultivars because seeds are highly heterozygous.

Rhizomes enlarged in the previous year are typically used for commercial cultivation (Masuda et al., 2006). This is considered the most practical method, helping to ensure a harvestable, uniform crop and yield and in one growing season. The enlarged rhizome found in lotus acts as a dormant organ to aid in the survival of the plant under unfavorable circumstances. According to Ni (1987), asexual propagation allows the original characteristics of the mother plant to be preserved, flowers to be enjoyed, and the lotus rhizome to be harvested in the same season. Even though rhizomes seem to be the

preferred propagation method, apical buds or running stems are also used (Qichao and Xingyan, 2005).

Substrate Type and Depth

For container and pond production, several substrates and depths have been suggested but the information is not well documented. Lotus prefers rich, fertile soil and lake or pond bottoms containing large amounts of organic matter (La-ongsri et al., 2009; Ni, 1987). Optimal soil is a soft silt loam, free-form particulate matter (Meyer, 1930; Nguyen and Hicks, 2004). Min and others (2006) reported that lotus yield can be increased by 31% through incorporation of crop residue chips in soil. Soil level is not a factor when lotus is grown in lakes or ponds, yet it is when grown in containers as it affects growth, EC, pH, and plant nutritional content (Tian, 2008). Tian (2008) found containers filled with $\frac{1}{4}$ soil level height of the container were more efficient than $\frac{3}{4}$ soil level for lotus production which had pronounced effects on both plant height and underground fresh weight of American lotus and *N. nucifera* 'Karizma.'

Water Depth

Chen and others (2007) found lotus planted in shallow water generated higher yields and that the ideal water depth was approximately 10 to 20 cm (4 to 8 in) (Nguyen, 2001). Chomchalow (2004) reported that shallow water is ideal for lotus growth because water temperatures increase faster in shallow versus deeper water. These findings are cultivar size dependent: Small-medium lotus prefers water depths of 5 to 50 cm (2 to 20 in) while larger varieties grow well in depths of 50 to 100 cm (1.5 to 3 ft) (Tian, 2008).

Fertilizer

Lotus is known to be a consumer of fertilizer and it is recommended to be applied in four to five split applications as young plants may burn readily (Nguyen, 2001; Tian, 2008). After formation of three to five coin (floating) leaves, begin fertilizing lotus and terminate applications around 1 – 10 Aug. Tian (2008) stated that the fertilization of lotus should be dependent on water level (volume) in containers, not soil volume. Tian (2008) additionally states that the application dose should decrease slightly if the water level remains low. The use of slow-release, coated fertilizer, produces nearly the same yield of rhizomes yet saved 29% N and reduced 41% effluent N fertilizer compared with four to five split applications with conventional, water soluble fertilizer (Stroupe, 2009; Tian, 2008). Stroupe (2009) states that fertilizer tablets are the water garden industry standard, yet are quite expensive and are labor intensive to apply. In China, organic fertilizer is a major choice, although there is not any scientific information available on its effects. Although animal manure has been recommended as an incorporated fertilizer, Shen-Miller and others (2002) disagree. In their studies, manure proved fatal to young seedlings and they reported clay as an essential component for nutrient retention and as a minor nutrient source for lotus production. Slocum and Robinson (1996) suggested that one-part of well-rotted and composted cow manure be mixed with two- to three-parts topsoil. This discrepancy illustrates a need for more research on organic lotus production which serves as a foundation for this dissertation.

Temperature, pH, EC & Nitrogen

Considering that the heart of Chinese lotus production lies close to the same latitude as Auburn, one may assume that lotus performs well in warm climates. Meyer

(1930) found temperatures from 20 to 30°C (68 to 86°F) were ideal in accelerating growth whereas low temperatures, such as below 15°C (59°F), impede growth. Yang and others (2006) reported that the optimal growing temperatures are from 22 to 32°C (72 to 90°F) and when grown in full sun, lotus has improved chlorophyll content, flower number, and thicker stalks (Tian, 2008).

Lotus has the ability to tolerate a wide pH range (4.5 to 9.0), and is not affected by a range of 5.5 to 8.0 in water (Meyer, 1930). Whereas Nguyen (2001) reported an apt electrical conductivity (EC) range for vegetative growth as 2.8 to 3.1 mS·cm⁻¹, Tian (2008) reported that an EC less than 0.5 mS·cm⁻¹ is ideal and should not exceed 1.0 mS·cm⁻¹, even for large plants. Plants become hungry when EC drops off to 0.15 – 0.2 mS·cm⁻¹. Suitable EC ranges are dependent on growing conditions, season, plant size, and temperature.

The nitrogen cycle (Fig. 2) is one of the most important and studied chemical cycles in wetlands and its cycling fluxes among interacting groups of compartments where N resides (i.e. inorganic/organic N, detritus, microbial assemblages) (Baldwin et al., 2000; Dodds et al., 2004; Mitsch and Gosselink, 2007). Furthermore, nitrogen is often a limiting nutrient within flooded soils (either in rice paddies or in wetland environments) and once introduced via agricultural run-off, can cause pollution and anoxic/anaerobic conditions (Dodds et al., 2004; Essington and Carpenter, 2000; Ford and Bormans, 2000). Different reactions can occur under oxic and anoxic conditions; the anoxic zone is at the very bottom with the oxic zone residing between the anoxic and the water layer.

Considering we are dealing with lentic systems (when discussing lotus) and due to its reduced flow, there is little porosity within the sediment and with the high organic matter load results in sediments that are devoid of oxygen (Baldwin et al., 2000). This facilitates anaerobic processes such as denitrification where dissimilatory nitrate reduces to ammonia (Baldwin et al., 2000; Ford and Bormans, 2000; Hamilton, 2000). Due to lake morphology, nutrient losses are reduced and can enhance nutrient cycling and enhance primary production (Essington and Carpenter, 2000).

Whereas in lentic systems its morphology can enhance nutrient cycling via bacteria and microbiota, lotic systems have a down stream vector, deemed a downward 'spiral' (Essington and Carpenter, 2000). Organic matter degradation can be influenced by local conditions such as current velocity, substratum, and channel morphology. With moving water, it supplies oxygen, enhancing organic decomposition. Winter floods, causing the streams to swell and flow, will deliver more ammonia than nitrate to estuaries (reverse happening in summer) (Ford and Bormans, 2000). N loss rate declines rapidly with increasing channel size (Ford and Bormans, 2000). Within lotic systems (or watersheds), minerals will be carried by the watershed to the mouth and accumulate there, disturbing the natural mineral balance; meaning that watersheds can serve as sinks for nutrients (Rosenblatt et al., 2001). This can cause eutrophication where plant growth is accelerated by the additional material.

Nitrification and denitrification are highly temperature dependent in freshwater systems (Ford and Bormans, 2000). Organic and conventional slow-release fertilizers are temperature dependent as well. Slow-release conventional fertilizers are coated with urea which enables them to break down much slower (4-6 months on average) whereas

organic fertilizers release/break down slowly in the soil, they break down a bit faster within aquatic environments due to dissolving since they do not have that protective urea coating.

The organic fertilizers researched within anaerobic environments at Auburn's Plant Science Research Center (PSRC) have displayed eutrophication qualities by forming algal blooms on the surface of the water; which may lead to depleted oxygen. If a large influx of nitrogen enters a lentic system, the N will be taken up by algae. This algal bloom will eventually die off, creating anoxic conditions within the water column system (Skagen et al., 2008). The leftover oxygen will be required to fully decompose the algae, which would make the lentic system completely devoid of oxygen, resulting in fish kills. However, if there were macrophytes (such as lotus) being grown within them, there would be no signs of eutrophication – as is currently observed in the actively growing lotus pots in the same facility.

Within the nitrogen cycle, the bulk of nitrogen is stored as nitrogen gas in the atmosphere. This process in water/wetlands involves both aerobic and anaerobic conditions. Ammonification is when nitrogen (in the form of ammonium NH_4) is released from decaying plant and animal matter under both aerobic and anaerobic conditions and the ammonium then moves up to the aerobic layer where it is converted to nitrate (NO_3). Nitrate not taken up by plants or immobilized by adsorption onto soil particles can leach downward with percolating water to reach the groundwater supply or move with surface and subsurface flow (leading to pollution as stated previously). Nitrate can also move back to the anaerobic layer where it may be converted to nitrogen gas by denitrification, a bacterial process, and subsequently returned to the atmosphere.

Using lotus as a test/model plant, the primary objective of this research is to develop protocols for organic aquatic plant production with emphasis on using various types and rates of organic amendments and fertilizers and evaluate their effects on growth, flowering and rhizome production compared to traditional production methods. A subset of that is the development of a nutrient release curve to determine longevity of organic fertilizers in aquatic systems and to determine the optimum rate of fertilizer(s) needed to sustain production for a season. We will also evaluate how changes on environment and cultural practices, such as temperature and water depth, influence EC/fertility levels in aquatic systems. The goal is develop sustainable production practices with emphasis on soil amendments and fertilizers for organic, aquatic plant production and deliver recommendations to growers.

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Fig. 1. Scientific classification of *Nelumbo*.

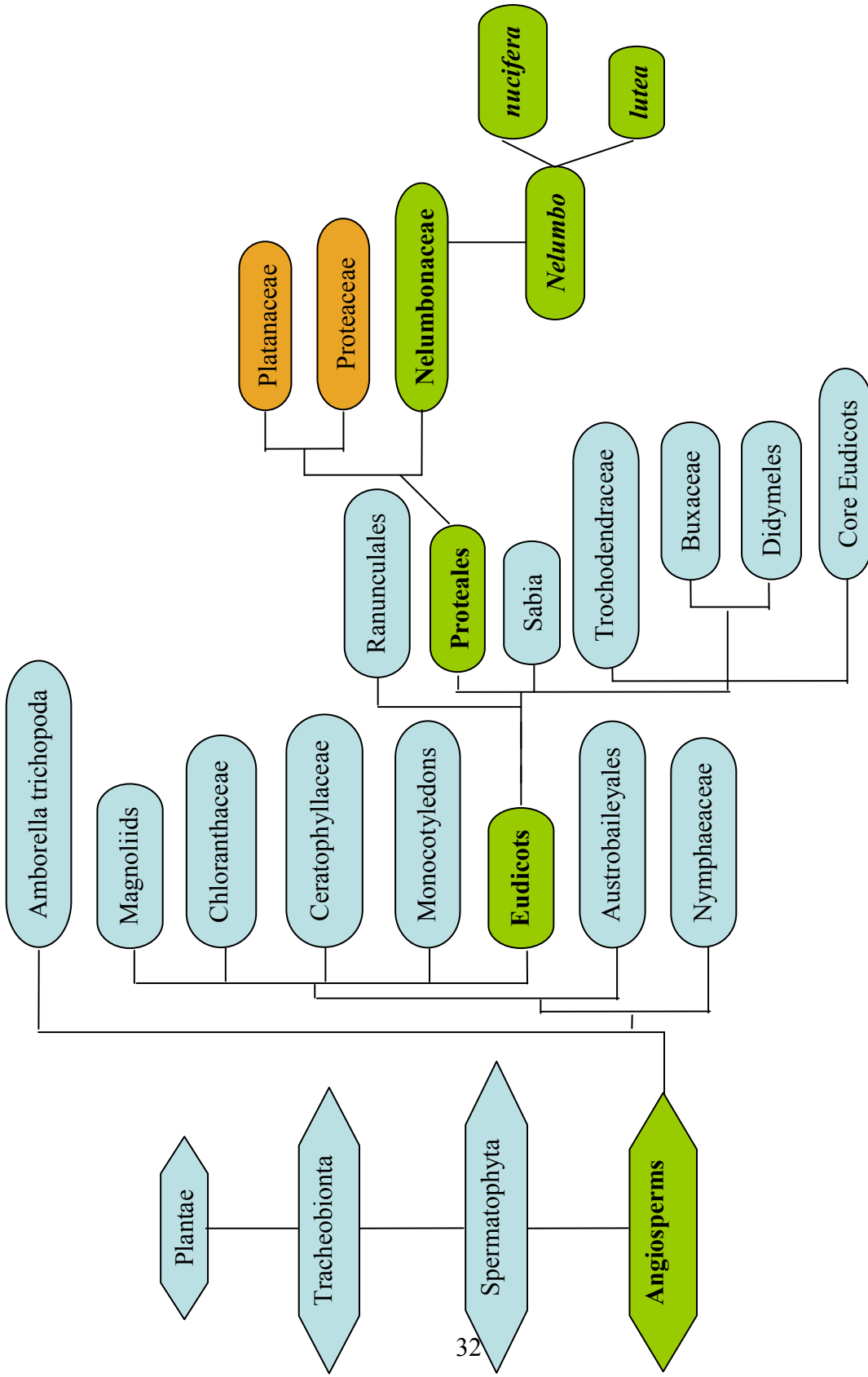
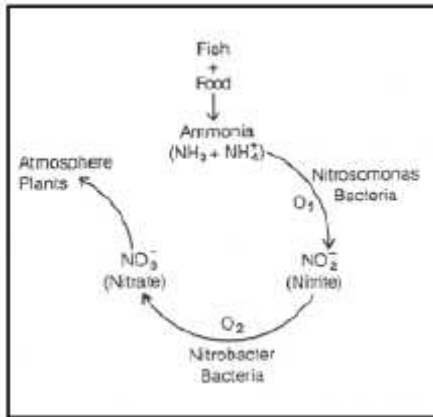


Fig. 2. The nitrogen cycle.^{zy}



- ^z Nitrifying bacteria utilize O₂ to convert ammonia (NH₄⁺, NH₃) and nitrite (NO₂⁻) into the nontoxic byproduct, nitrate (NO₃⁻), which is then used by plants or returned to the atmosphere (N₂).
- ^y Francis-Floyd, R. and C. Watson (1996)

CHAPTER II
EFFECT OF WATER DEPTH AND FERTILIZER TYPE
ON WATER AND SUBSTRATE EC, pH,
NITRATE-NITROGEN (NO₃), AMMONIA-NITROGEN (NH₃) AND
AMMONIUM-NITROGEN (NH₄)
IN A SIMULATED POND SYSTEM

Abstract

A small-scale simulation pond experiment was conducted and replicated to investigate the effects of fertilizer type (conventional, organic, or no fertilizer) and water depth on water and substrate electrical conductivity (EC), pH, nitrate-nitrogen (NO₃), ammonia-nitrogen (NH₃), and ammonium-nitrogen (NH₄) concentration over a three-month period to determine potential water depths and fertilizer types conducive for lotus (*Nelumbo* sp.) growth. Fertilization influenced water and substrate EC, pH, NO₃, NH₃, and NH₄ concentrations which decreased with greater water depths (due to greater water volume and dilution factors) in both replications (runs). All measured parameters among all fertilizer treatments and within all water depths increased linearly over time, with exception of pH and NH₄ in Run 2. Water and substrate EC, pH, NO₃, NH₃, and NH₄ were higher among organic fertilizer treatments and within lower depths. EC is a strong factor influencing lotus growth. With greater temperatures and lower water depths, EC

could rise close to a toxic level ($1.0 \text{ mS}\cdot\text{cm}^{-1}$) as was seen in Run 2 among organic Medina Growin' Green (MG) treatment.

Introduction

Nelumbo nucifera (lotus) is a rhizomatous herbaceous, aquatic perennial. Growing points of the rhizomes produce two types of peltate leaves: Floating and upright (standing) leaves (Nohara and Kimura, 1997). Although all lotus cultivars display ornamental characteristics, there are three types of lotus grown for market: Seed, rhizome, and ornamental. Seed lotus is bred for high yield and quality for seeds; rhizome lotus is bred for rhizome quality and yield, not flowers. Each part of lotus plants are consumed throughout Asia and other parts of the world for food or used for medicinal purposes, including rhizome, nodes, runners, seed, young shoot, leaf, stalk, petal, stamen, and pericarp (or fruit receptacle, seedpod) (Nguyen, 2001; Qichao and Xingyan, 2005; Wang and Zhang, 2004).

Currently, lotus grown in Europe and the U.S. are mainly used for ornamental purposes rather than for food (Creamer, 2008; Nguyen and Hicks, 2004). In the U.S., despite the large Asian and Asian-American population, the potential demand for edible lotus is unknown. Lotus is revered globally for not only its religious significance but also as a vegetable due to its crisp texture, attractive color and high nutritional content. Lotus offers an abundance of nutrients including starch, sugars, proteins, lipids, vitamins, minerals, alkaloids, flavonoids, and other medicinal chemicals.

Lotus is quite versatile and can be grown in ponds, containers, greenhouses, and raised beds. Soil level is not a factor when lotus is grown in lakes or ponds, yet it is when grown in containers as it affects growth, EC, pH, and plant nutritional content (Tian,

2008). Chen and others (2007) found lotus planted in shallow water generated higher yields and that the ideal water depth was approximately 10 to 20 cm (4 to 8 in) (Nguyen, 2001). For container and pond production, several substrates and water depths have been suggested but the information is not well documented.

In China, organic fertilizer is a major choice, although there is no scientific information available on its effects. Nguyen (2001) and Tian (2008) recommended applying fertilizer in four to five split applications as young plants may burn readily. Stroupe (2009) found that fertilizer tablets are the water garden industry standard, yet are quite expensive and are labor intensive to apply. Although animal manure has been recommended as an incorporated fertilizer, Shen-Miller and others (2002) disagree. In their studies, manure proved fatal to young seedlings. Shen-Miller and others (2002) further reported clay as an essential component for nutrient retention and as a minor nutrient source for lotus production. In addition, Tian (2008) stated that the fertilization of lotus should be dependent on water depth (volume) in containers, not soil volume since interactions may exist between factors including fertilizer rate, water depth, EC, pH, and temperature. Water depth and nutrient availability are important factors that influence the growth of wetland plants (Anderson and Mitsch, 2005; Xie et al., 2009). The objective of this study is to investigate how selected water depths and fertilizers influence water EC, pH, and nutrient concentration.

Materials and Methods

A small-scale simulation pond experiment was conducted to compare the effect of fertilizer type (conventional, organic, or no fertilizer) and water depth on water EC, pH, nitrate-nitrogen (NO_3), and ammonia-nitrogen (NH_3), and ammonium-nitrogen (NH_4)

concentration over a three month period. All research was conducted in a glass greenhouse with computer controlled evaporative cooling pads and fans with XLS 15 Firebreak shade cloth (Svensson, Kinna, Sweden) in Auburn, AL at lat. 32.6°N. Temperature set-points were 29°C day and 27°C night with ambient light.

Experiment utilized a 1:1 (v:v) Marvyn sandy clay loam: pine bark substrate. Physical properties of the soil and nutritional analysis of individual and combined components were determined by the Soil Testing Laboratory at Auburn University (Table 1 – 3).

Forty-eight, [68.1 L (18 gal), 59.4 cm (L) x 46.7 cm (W) x 39.1 cm (D) (23.4 in x 18.4 in x 15.4 in)] storage tubs (Rubbermaid®, Atlanta, GA) were filled to ¼ container depth (10.2 cm, 26.5 L) with substrate. Tubs were modified using basic schedule 40 PVC plumbing parts to include a drain. Prior to addition of substrate, a 5.1 cm (2 in) hole was centered 7.6 cm (3 in) from tub bottom on one short side of each tub and cut with a 5.1 cm (2 in) arbored hole saw drill bit. The slip socket end of a 3.8 cm x 2.5 cm (1.5 in x 1 in) bushing and a 1.9 cm x 2.5 cm (0.75 in x 1 in) threaded male adapter were cemented together using PVC primer and cement with a 3.2 cm (1.25 in) piece of aquarium filter sleeve (Spectrum Brands, Madison, WI) glued in between the PVC pieces (Fig. 1). Filter was inserted to prevent substrate loss through the drain when sampling leachate. Epoxy putty was used to seal around the drains both inside and outside of each tub (Fig. 2). Each drain was fitted with nylon water shut off valves (Gilmour®, Somerset, PA). Leaks were minimal, but those that occurred were patched with epoxy putty; silicone did not work.

After tubs were filled with substrate, fertilizer treatments were applied and hand blended at their recommended label rate of $0.6 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ ($1 \text{ lb}\cdot\text{yd}^{-3} \text{ N}$). Four fertilizer types were used: Conventional controlled-release fertilizer, Harrell's Polyon 16N-2.6P-9.9K (hereafter referred to as Conv) (Harrell's LLC, Lakeland, FL), Medina Growin' Green Granular Organic 4N-0.9P-2.5K (hereafter referred to as MG) (Hondo, TX), and Nature Safe 8N-2.2P-4.2K (hereafter referred to as NS) (Griffin Industries, Coldspring, KY), and Control (no fertilizer).

Three water volumes (depths) were marked at 7.6 cm (3 in), 15.2 cm (6 in), or 22.9 cm (9 in) above substrate line. Tub s were filled with water ($\text{pH } 6.9$, $\text{EC} = 0.10 \text{ mS}\cdot\text{cm}^{-1}$) to volumes of approximately 20 L (5.3 gal), 40.5 L (10.7 gal), or 60.5 L (16 gal) to achieve those depths. Water volume was evaluated every other day, and water was added as needed to maintain volumes (depths).

Treatments were in a three (3) water depth x four (4) fertilizer type factorial arrangement for a total of twelve (12) treatments, with four single-tub replications per treatment. The experiment was repeated. Initial run (Run 1) of this experiment was conducted from 7 Dec. 2009 – 15 Mar. 2010. The second run (Run 2) of this experiment was conducted from 23 Mar. 2010 – 12 July 2010.

For Run 1, water pH and EC were measured by inserting the Hanna pH/EC/TDS meter (Hanna Instruments, Woonsocket, RI) into the center of each individual tub one week after fertilizer application and then weekly until Week 12. From Week 12 through Week 14, water pH, EC, and nitrate-nitrogen (NO_3), ammonia-nitrogen (NH_3) and ammonium-nitrogen (NH_4) were measured weekly by inserting the YSI Professional Plus meter (YSI Inc., Yellow Springs, OH) into the center of each individual tub. Additional

water samples were collected from each individual tub one week after fertilizer application (14 Dec. 2009) and at the end of Run 1 (15 Mar. 2010) and analyzed using ICAP for NO₃ and NH₄ determination. One (1) Nalgene[®] narrow mouth bottle (4 oz, 125 mL) was dipped into center of each individual tub to collect these water samples, capped, and sent directly to lab for analysis (Consolidated Plastics Company, Inc., Stow, OH). For Run 1, additional water samples were collected into one Nalgene[®] narrow mouth bottle (4 oz, 125 mL) from the leachate (drain) of each tub and sent directly to lab for analysis at the same collecting dates noted above.

Run 1 laboratory and meter data indicated there were no differences between water sampled from the center of the tubs and the leachate. Hence, for Run 2, water samples were only collected from the center of the tubs one week after fertilizer application (30 Mar. 2010) and at the end of Run 2 (12 July 2010) and analyzed using ICAP for NO₃ and NH₄ determination. For Run 2, water pH, EC, NO₃, NH₃, and NH₄ were measured one week after fertilizer application and continued weekly through duration of study by inserting the YSI Professional Plus meter into the center of each tub.

Composite substrate samples were collected for analysis for each run prior to fertilizer application (Run 1: 7 Dec. 2009; Run 2: 30 Mar. 2010), at the end of each run (Run 1: 15 Mar. 2010; Run 2: 12 July 2010) and analyzed using the same ICAP analyses. Four substrate samples were collected from each rep and treatment, then had those four samples blended to one sample for each rep and treatment for lab analysis. Twelve (12), empty, 11.4 L (3 gal) buckets and twelve (12), empty, Ziploc[®] bags were labeled to represent each rep and treatment. For each run and for each treatment, researchers used a 59 mL (2 oz) cup to collect substrate samples (2 oz) from the bottom

of each tub; for a total of four (4) samples per treatment (1 per replication). Those four (4) samples were placed into their respective treatment bucket, blended together, and then a 59 mL (2 oz) sample was taken from the blend and placed into an individual quart size Ziploc® bag. Excess water was drained prior to sealing bags and bags were sent directly to lab for analysis.

Data were analyzed as a completely randomized design using PROC MIXED by SAS 9.1 (SAS Institute, Inc., Cary, NC). Linear and quadratic analysis were conducted, and means were separated utilizing CONTRAST statements at $\alpha = 0.05$ (5%). Data were initially analyzed to detect significant differences between runs. If there were no significant differences between runs, data were pooled across runs and analyzed for interactions of water depth and fertilizer type. If data indicated significant differences in runs, data were not pooled, and runs were analyzed separately and then analyzed for interactions between water depth and fertilizer type within runs.

Results and Discussion

Data showed significant interactions between water depth and fertilizer type, and there were differences between runs so data were not pooled over run and were analyzed separately.

Run 1

EC increased after fertilizer application and continued to increase over the duration of the study with decreasing water depths (reduced water volume) (Table 4) (Fig. 3). Work by Tian (2008) included actively growing plants where EC increased after fertilizer application and decreased as nutrients were absorbed. Contrary to Tian (2008) findings, and since there were no plants in this study, EC displayed a positive linear trend

over time for each depth and fertilizer ($P < 0.0001$) (Fig. 3). EC was consistently higher at lower depths, lower at greater depths, and higher in MG fertilizer ($0.48 - 0.34 \text{ mS}\cdot\text{cm}^{-1}$) (Fig. 3C) compared to other fertilizer treatments (Table 4). Tian (2008) found that EC was lower in greater depths due to more water and thus a greater dilution factor. EC did not exceed Tian (2008) recommendation of 0.5 to $1.0 \text{ mS}\cdot\text{cm}^{-1}$. For all fertilizer treatments, EC decreased linearly across greater water depths (Fig. 3).

Lotus has the ability to tolerate a wide pH range (4.5 to 9.0) and is not affected by a pH range of 5.5 to 8.0 in water (Meyer, 1930). For all water depths and fertilizer treatments, pH displayed a positive linear trend over time ($P < 0.0001$) (Fig. 4). For all water depths, pH was higher in MG and NS than in Control and Conv treatments (Table 4). Among fertilizer treatments, pH fluctuation may be due to the presence of ammonia/ammonium within fertilizers (i.e. the higher the ammonia/ammonium level is, the lower the pH) (MG: $1799 \text{ mg}\cdot\text{L}^{-1} \text{ NH}_4$; NS $142 \text{ mg}\cdot\text{L}^{-1} \text{ NH}_4$; Conv $88000 \text{ mg}\cdot\text{L}^{-1} \text{ NH}_4$ released over 4 month period) (Table 3). In addition, algae growth was observed growing in MG and NS treatment tubs, and Tian (2008) stated that a pH increase may be caused by algae growth, nutrient changes in water-soil system, or metabolism of plants. Deas and Orlob (1999) found that when algae utilize and remove CO_2 , it results in an $[\text{OH}^-]$ increase and an associated increase in pH, and that it was not uncommon to observe an increase from 0.5 to 1.5 pH units. Chellemi and Lazaorvits (2002) found that pH increased with increased N rates of organic fertilizer, and that pH had a linear relationship over time with increased applications. In addition, Chellemi and Lazaorvits (2002) found pH differences were observed between organic fertilizers and conventional when applied at the same N rate, which supports our findings in Table 4. pH was

consistently higher in organic fertilizers (Table 4). However, pH changes over time were within acceptable pH ranges (5.5 to 8.0) for lotus growth (Meyer, 1930).

There was not a significant interaction between water depth and fertilizer type for NO_3 concentration. Data presented are for each fertilizer type, and there were no differences within water depths. Over time, NO_3 concentration increased linearly (Fig. 5) and was highest in MG ($6.9 \text{ mg}\cdot\text{L}^{-1}$) and lowest in NS ($4.4 \text{ mg}\cdot\text{L}^{-1}$) (Table 4). There were no differences over time, within water depths, or among fertilizer treatments and no interactions were present for NH_3 concentration (mean $0.18 \text{ mg}\cdot\text{L}^{-1}$), therefore data were not presented.

Over time, NH_4 concentration decreased linearly (Fig. 6). From lower to greater depths in Conv, MG, and NS, NH_4 concentration decreased linearly ($P < 0.0001$) (Fig. 6). NH_4 concentration was consistently higher in NS for all water depths (Table 4). NH_4 concentration was higher in lower depths and lower in greater depths with increased volume and dilution.

Water samples collected one week after fertilizer application had higher concentrations of macronutrients than those collected at the end of the Run (Table 5). Calcium (Ca), K, and Mg concentrations decreased over time within all water depths among all fertilizers (Table 5). Phosphorus (P) concentrations increased within the lower water depths among Control and Conv treatments (Table 5). Micronutrients Fe, Na, and Zn and trace element Al concentrations increased over time within all water depths and among all fertilizer types with exception of Zn decreasing by 50% within the middle water depth among MG treatment (Table 5). Nutrient availability is influenced by pH and when pH is below 5, Al becomes more available which appears to be the case in this

experiment (Table 4, 5, and 7) (Mengel et al., 2001). Over time, water pH was approximately 6 within lower depths among Control and Conv treatments (Table 4).

The differences in nutrient concentration between the initial (pre-fertilizer) substrate sample and substrate samples from the end of Run 1 are presented in Tables 6 – 7. Most macronutrient concentrations increased within all water depths and among all fertilizer types by termination of Run 1, except for the Control (Table 6). Over time, all macronutrient and micronutrient concentrations, with exception of P and Fe, increased among NS treatment within all water depths (Table 6). Within the nutrition analysis of fertilizers (Table 3), NS contained lower concentrations of P and Fe compared to the other treatments. Sodium (Na) concentration increased over time among all fertilizer treatments and within all water depths and was higher in MG (Table 6); MG contained the higher concentration of Na within its nutritional analysis (Table 3). Substrate EC exceeded Tian (2008) recommendations to not go above $0.5 - 1.0 \text{ mS}\cdot\text{cm}^{-1}$ (Table 7). For all fertilizer types, except for Control, and water depths, substrate EC was nearly $1.0 \text{ mS}\cdot\text{cm}^{-1}$ higher than initial (pre-fertilizer) sample (Table 7). Soluble salt (SS) concentration increased across all treatments, especially in NS (range: $625 - 1024 \text{ mg}\cdot\text{L}^{-1}$). NS contained a higher SS concentration within its nutritional analysis than MG by $1000 \text{ mg}\cdot\text{L}^{-1}$ (MG: $20832 \text{ mg}\cdot\text{L}^{-1}$; NS $21700 \text{ mg}\cdot\text{L}^{-1}$) (Table 3). There were no differences in pH from initial sample to final sample (data not shown).

Run 2

Similar to Run 1, EC increased linearly over time for each water depth and fertilizer type ($P < 0.0001$) (Fig. 7) with exception of 7.6 cm water depth among both MG and NS treatments (Fig. 7C and 7B). EC was consistently higher at lower depths, lower

at greater depths, and higher in MG fertilizer ($0.80 - 0.46 \text{ mS}\cdot\text{cm}^{-1}$) compared to other fertilizer treatments (Table 8). In this run, Control and Conv had a lower EC than other fertilizers (Table 8).

Over time, pH increased linearly for all water depths and fertilizer types, except for MG which displayed a quadratic trend ($P = 0.0043$) (Table 8) (Fig. 8). All pH ranges were within acceptable ranges (4.5 to 9.0) for lotus growth (Table 8).

King and Torbert (2007) stated that excessive levels of N can inhibit growth of submerged plants and that the concentration should not exceed 1 or 2 $\text{mg}\cdot\text{L}^{-1}$. In this Run, the NO_3 concentrations exceeded those recommendations (Table 8) yet were not as high as what was found in an unpublished outdoor research pond study where the plants flourished. Among MG and NS treatments, NO_3 concentration displayed linear trends ($P = 0.0007$ and $P = 0.0003$, respectively) (Fig. 9). In greater depths, Conv and MG treatments had similar concentration rates (7.23 and $7.65 \text{ mg}\cdot\text{L}^{-1}$, respectively) (Table 8) (Fig. 9B-9C).

NH_3 concentration in Control, MG, and NS increased linearly over time within all water depths (Fig. 10A, 10C, & 10D). NH_3 concentration was consistently higher among both MG ($0.14 - 0.21 \text{ mg}\cdot\text{L}^{-1}$) and NS ($0.15 - 0.22 \text{ mg}\cdot\text{L}^{-1}$) than in the other two fertilizer treatments, regardless of water depth (Table 8).

Typically, low temperatures and low pH result in higher rates of net ammonification rather than net nitrification (Britto and Kronzucker, 2002), which was not seen in this Run. Within all water depths, NH_4 concentration increased linearly over time in Conv ($1.6 - 2.3 \text{ mg}\cdot\text{L}^{-1}$) (Table 8) (Fig. 11B). In addition, over time, among MG and NS treatments, NH_4 concentration displayed linear trends (Fig. 11C-11D). NH_4

concentration in NS was consistently higher for all water depths (Table 10). Control had significantly lower NH_4 concentrations than other fertilizers (Table 8).

In contrast to Run 1 findings, water samples collected one week after fertilizer application showed lower concentrations of macronutrients than those samples collected at the end of Run 2 (Table 9). There were no differences (i.e. 0%) between the final concentration and the initial NO_3 and NH_4 concentrations within all rates among all fertilizers (data not shown). Calcium (Ca), Mg, and Na concentrations increased over time within all water depths and among all fertilizer treatments (Table 9). Zinc (Zn) concentrations decreased within all water depths among all fertilizers (Table 9). Run 2 took place during warmer months which contributed to an increased nutrient concentration in the water; there was an approximate six degree increase during this run (up from 21°C in Run 1 to 27°C in Run 2).

Over time, most substrate nutrient concentrations increased among NS treatment within all water depths, with exception of Conv where NO_3 and NH_4 concentrations increased within all water depths (Table 10). Substrate EC increased among MG and NS within decreasing water depths and exceeded Tian (2008) recommendations to not go above $0.5 - 1.0 \text{ mS}\cdot\text{cm}^{-1}$ (Table 11). Final substrate sample showed that EC was nearly $1.0 \text{ mS}\cdot\text{cm}^{-1}$ higher than initial (pre-fertilizer) sample for lower depths in MG ($0.90 \text{ mS}\cdot\text{cm}^{-1}$), lower and middle depths in NS ($0.80 - 0.70 \text{ mS}\cdot\text{cm}^{-1}$, respectively), and at greater depths in Conv ($0.10 \text{ mS}\cdot\text{cm}^{-1}$) (Table 11). Soluble salt (SS) concentration increased across all treatments, especially in MG ($342 - 482 \text{ mg}\cdot\text{L}^{-1}$). Within Conv treatment, SS decreased by $57 \text{ mg}\cdot\text{L}^{-1}$ by termination of Run 2. pH increased by

approximately one unit for all fertilizer types and water depths, with exception of greater depths in Control and middle depth in Conv (Table 11).

Xie and others (2009) found that deep water depths (10 cm and up) led to reduced nutrient availability which our study supports. Considering that all parameters measured decreased with greater depths (due to greater water volume and dilution factors), maintaining water depth around 15.2 cm (6 in) results in satisfactory EC levels for lotus growth. There would be no additional benefit to maintaining lower or greater depths according to the results of this study. Electrical conductivity (EC) is a strong factor influencing lotus growth and with greater temperatures and lower depths, EC could rise close to a toxic level as was seen in Run 2 among MG treatment. Ideal EC is around $0.5 \text{ mS}\cdot\text{cm}^{-1}$ according to Tian (2008) which our study supports with a 15.2 cm (6 in) water depth. Xie and others (2009) also found biomass accumulation was greater at lower water depths with high nutrient rates applied, whereas biomass was lower at greater depths with low nutrient rates; this would be useful to apply toward future studies. Although some of the measured parameters differed, our data suggest predictable changes in water and substrate chemistry based on water depth and fertilizer type. The results of this study will provide lotus growers with recommendations on acceptable water depth and organic fertilizers for production. Future research to learn more about nutrient changes over time and applying principles learned to container plants may be beneficial for plant growth and health.

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Table 1. Fertilizer types and contents for both runs.

Fertilizer type	Fertilizer treatments	Fertilizer contents
Conventional	Harrell's Polyon 16N-2.6P-9.9K	All Polymer Coated: Ammonium Nitrate Copper Sulfate EDTA Iron Chelate Magnesium Sulfate Manganese Sulfate Mono-Ammonium Phosphate Sodium Molybdate Sulfate of Potash Zinc Sulfate
Organic	Medina Granular 4N-0.9P-2.5K	Kelp meal, humate, pasteurized poultry manure, molasses, and greensand
	Nature Safe 8N-2.2P-4.2K	Meat meal, hydrolyzed feather meal, bone meal, blood meal & sulfate of potash

Table 2. Nutritional analysis of substrates.^z Units are mg·L⁻¹ with exception of pH and electrical conductivity.

	Sandy clay loam	Pine bark	1:1 (v:v) Sandy clay loam:pine bark
pH	5.6	4.3	5.1
Electrical conductivity (EC) (mS·cm ⁻¹)	0.15	0.57	0.21
Soluble salts (SS)	96	399	148
Phosphorus (P)	7	4	1
Potassium (K)	41	90	46
Magnesium (Mg)	241	90	29
Calcium (Ca)	341	23	13
Copper (Cu)	1.1	0.2	0.3
Iron (Fe)	169	4.7	18.3
Manganese (Mn)	11	2.3	1.0
Zinc (Zn)	0.5	0.3	0.1
Boron (B)	1.2	0.4	0.2
Aluminum (Al)	294	25	40
Sodium (Na)	115	8	14
Nitrate-Nitrogen (NO ₃)	2	3	2
Ammonium-Nitrogen (NH ₄)	<0.1	3	2

^z Analyzed using ICAP for determination.

Table 3. Nutritional analysis of fertilizers.^z Units are mg·L⁻¹ with exception of pH and electrical conductivity.

	Medina granular organic 4N-0.9P-2.5K (MG)	Nature safe 8N-2.2P-4.2K (NS)	Harrell's polyon 16N-2.6P-9.9K (Conv)
pH	6.4	6.2	-- ^y
Electrical conductivity (EC) (mS·cm ⁻¹)	30	31	--
Soluble salts (SS)	20832	21700	--
Phosphorus (P)	1031	489	6000
Potassium (K)	10616	12266	12000
Magnesium (Mg)	3049	1086	14000
Calcium (Ca)	765	486	--
Copper (Cu)	83	1.3	790
Iron (Fe)	22.7	<0.1	3300
Manganese (Mn)	12	0.6	1300
Zinc (Zn)	21	<0.1	790
Boron (B)	13	2.7	--
Aluminum (Al)	3.2	4.3	--
Sodium (Na)	6985	2991	--
Nitrate-Nitrogen (NO ₃)	301	66	72000
Ammonium-Nitrogen (NH ₄)	1799	142	88000

^z Analyzed using ICAP for determination.

^y – Indicates information not available according to label.

Table 4. Mean effect of water depth and fertilizer type on water EC, pH, NO₃-N, and NH₄-N pulled over weekly sampling dates (14 weeks: 7 Dec 2009 – 15 Mar. 2010) for Run 1 (n=56).^z

Water depth [cm (in)]	Fertilizer ^y	EC (mS·cm ⁻¹)	pH	NO ₃ -N (mg·L ⁻¹)	NH ₄ -N (mg·L ⁻¹)
7.6 (3)	Control	0.10d ^x	6.27c	4.8c ^w	1.11c
	Conv	0.14c	6.13d	5.1b	1.89c
	MG	0.48a	7.03b	6.9a	4.65b
	NS	0.34b	7.05a	4.4d	7.36a
15.2 (6)	Control	0.09d	6.20c		1.05c
	Conv	0.12c	6.10d		1.59c
	MG	0.39a	7.32a		3.19b
	NS	0.25b	7.04b		5.23a
22.9 (9)	Control	0.09d	6.42d		0.93c
	Conv	0.10c	6.49c		1.34b
	MG	0.34a	7.26a		3.25a
	NS	0.2b	7.01b		4.15a

^z Water EC, pH, NO₃-N, and NH₄-N were measured from each individual tub one week after fertilizer application (14 Dec. 2009) and then weekly until end of Run 1 (15 Mar. 2010).

^y Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K). Applied at 0.6 kg·m⁻³ N.

^x Lowercase letters denote mean separation among fertilizer treatments within water depths at p<0.05 (SAS Institute, 2004).

^w There were no statistical differences within water depths; means represent data pooled over water depths.

Table 5. Percent increase (positive values) or decrease (negative values) in nutrient concentrations in water samples from the initial sample (7 Dec. 2009) to the final sample (15 Mar. 2010) of Run 1. Formula utilized is: [(Final Concentration – Initial concentration)/(Initial concentration)*100] = % increase or decrease.

Water depth [cm (in)]	Fertilizer ^z	Macronutrients ^y (%)							Micronutrients ^x (%)					Trace element (%)
		Ca	K	Mg	P	Cu	Fe	Mn	Na	Zn	Al			
7.6 (3)	Control	-36	-22	-38	58	-33	757	-33	55	25	828			
	Conv	-53	-3	-52	51	20	100	0	70	20	71			
	MG	-35	-2	-28	-69	-30	90	-33	86	50	40			
	NS	-64	-12	-58	-83	-56	700	-73	40	0	350			
15.2 (6)	Control	-54	-23	-50	100	0	675	-50	50	25	805			
	Conv	-50	-4	-40	-64	-20	180	0	114	75	174			
22.9 (9)	MG	-73	-35	-54	-79	-55	47	-50	13	-50	63			
	NS	-83	-16	-76	-40	-17	1600	-56	27	0	1300			
	Control	-27	-3	-29	-30	0	1345	0	47	0	1427			
	Conv	-70	-7	-56	40	25	155	-43	36	50	279			
	MG	-60	-12	-51	-63	-56	18	-67	53	0	675			
	NS	-73	-32	-64	-45	25	1283	-55	3	0	650			

^z Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K). Applied at 0.6 kg·m⁻³ N.

^y There were no mean differences (i.e. 0%) for macronutrients NO₃-N or NH₄-N; thus data were not presented within table.

^x There were no differences (i.e. 0%) for micronutrients or trace elements As, B, Cd, Cr, Ni, or Pb, respectively; thus data were not presented within table.

Table 6. Percent increase (positive values) or decrease (negative values) in nutrient concentrations in substrate samples from the initial sample (7 Dec. 2009) to the final sample (15 Mar. 2010) of Run 1. Formula utilized is: $[(\text{Final Concentration} - \text{Initial concentration})/(\text{Initial concentration}) * 100] = \% \text{ increase or decrease.}$

Water depth [cm (in)]	Fertilizer ^z	Macronutrients (%)							Micronutrients ^y (%)				Trace element (%)
		NH ₄ -N	NO ₃ -N	Ca	K	Mg	P	Fe	Mn	Na	Al		
7.6 (3)	Control	-68	-70	58	-24	109	68	-95	234	75	-94		
	Conv	519	1479	354	183	235	-17	-73	832	301	-95		
	MG	45	713	185	208	134	-86	-96	385	758	-96		
	NS	796	5395	556	318	232	31	-99	711	430	-97		
15.2 (6)	Control	-69	-74	94	-20	173	-21	-96	312	98	-94		
	Conv	366	2545	311	157	220	-86	-80	758	293	-95		
	MG	-6	172	158	146	132	190	-96	308	605	-96		
	NS	312	3722	484	220	214	18	-98	798	362	-97		
22.9 (9)	Control	-64	-62	118	-25	181	-86	-97	320	78	-95		
	Conv	312	1248	139	59	137	10	-90	306	212	-95		
	MG	35	129	145	94	91	114	-96	302	517	-95		
	NS	145	2697	312	181	155	-22	-97	742	284	-96		

^zFertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K). Applied at 0.6 kg·m⁻³ N.

^yThere were no differences (i.e. 0%) for micronutrients or trace elements As, B, Cd, Cr, Cu, Ni, Pb, or Zn, respectively; thus data were not presented within table.

Table 7. Final EC, SS, pH, and difference between initial substrate sample (7 Dec. 2009) and final substrate sample (15 Mar. 2010) EC and SS. Formula utilized: Final sample – Initial sample = difference.

Water depth [cm (in)]	Fertilizer ^z	Final analysis			Difference between initial and final samples	
		EC (mS·cm ⁻¹)	SS (mg·L ⁻¹)	pH	EC (mS·cm ⁻¹)	SS (mg·L ⁻¹)
7.6 (3)	Control	0.40	278	4.93	0	130
	Conv	1.24	868	4.68	1	720
	MG	1.09	764	5.10	1	616
	NS	1.67	1172	4.87	1	1024
15.2 (6)	Control	0.46	321	4.89	0	174
	Conv	1.17	816	4.68	1	668
	MG	0.94	660	5.20	1	512
	NS	1.30	911	4.81	1	764
22.9 (9)	Control	0.46	321	4.92	0	174
	Conv	0.76	529	4.91	1	382
	MG	0.82	573	5.08	1	425
	NS	1.10	773	4.69	1	625

^zFertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K). Applied at 0.6 kg·m⁻³ N.

Table 8. Mean effect of water depth and fertilizer type on water EC, pH, NO₃-N, and NH₄-N pulled over weekly sampling dates (14 weeks: 30 Mar. 2010 – 12 July 2010) for Run 2. (n=56).^z

Water depth [cm (in)]	Fertilizer ^y	EC (mS·cm ⁻¹)	pH	NO ₃ -N (mg·L ⁻¹)	NH ₃ -N (mg·L ⁻¹)	NH ₄ -N (mg·L ⁻¹)
7.6 (3)	Control	0.18d ^x	7.00b	5.56d	0.07b	1.0d
	Conv	0.19c	6.25d	7.09b	0.02b	2.3c
	MG	0.80a	7.03a	8.62a	0.21a	10.5b
	NS	0.49b	6.90c	6.33c	0.22a	12.3a
15.2 (6)	Control	0.16d	7.00a	5.35c	0.05b	0.8c
	Conv	0.19c	6.44d	7.53a	0.04b	2.2b
	MG	0.58a	6.93b	6.73b	0.18a	8.8a
	NS	0.37b	6.84c	6.67b	0.21a	9.1a
22.9 (9)	Control	0.15d	7.04a	5.45b	0.14a	0.7d
	Conv	0.16c	6.42d	7.23a	0.02b	1.6c
	MG	0.46a	7.00b	7.65a	0.14a	6.8b
	NS	0.31b	6.85c	5.31b	0.15a	8.3a

^z Water EC, pH, NO₃-N, and NH₄-N were measured from each individual tub one week after fertilizer application (30 Mar. 2010) and then weekly until end of Run 2 (12 July 2010).

^y Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K). Applied at 0.6 kg·m⁻³ N.

^x Lowercase letters denote mean separation among fertilizer treatments within water depths at p<0.05 (SAS Institute, 2004).

Table 9. Percent increase (positive values) or decrease (negative values) in nutrient concentrations in water samples from the initial sample (23 Mar. 2010) to the final sample (12 July 2010) of Run 2. Formula utilized is: [(Final Concentration – Initial concentration)/(Initial concentration)*100] = % increase or decrease.

Water depth [cm (in)]	Fertilizer ^z	Macronutrients ^y (%)						Micronutrients (%)						Trace elements ^x (%)	
		Ca	K	Mg	P	B	Cu	Fe	Mn	Na	Zn	Al	As		
7.6 (3)	Control	332	8	287	-42	0	125	-38	0	146	-20	-48	-33		
	Conv	267	-6	141	-84	-78	-8	106	-55	89	-50	321	-67		
	MG	379	1	118	95	25	86	-47	75	64	-33	-36	0		
	NS	713	-12	117	152	0	80	160	450	63	0	345	-20		
15.2 (6)	Control	438	-4	321	-32	0	125	95	0	107	-20	64	-33		
	Conv	447	20	227	600	0	100	-38	0	99	-43	-13	-43		
	MG	338	-8	87	95	25	40	61	100	49	0	-54	-20		
	NS	201	1	79	68	0	25	-43	25	89	0	-22	-20		
22.9 (9)	Control	340	-13	255	-14	-20	50	-80	0	111	0	-91	-20		
	Conv	511	13	193	-71	-33	60	63	0	122	-17	138	0		
	MG	159	-10	49	5	0	100	-48	0	55	0	-68	0		
	NS	197	0	77	-17	-33	80	-45	0	83	0	-57	-64		

^z Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-.2P-4.2K). Applied at 0.6 kg·m⁻³.

^y There were no mean differences (i.e. 0%) for macronutrients NH₄-N or NO₃-N; thus data were not presented within table.

^x There were no differences (i.e. 0%) for trace elements Cd, Cr, Ni, or Pb; thus data were not presented within table.

Table 10. Percent increase (positive values) or decrease (negative values) in nutrient concentrations in substrate samples from the initial sample (23 Mar. 2010) to the final sample (12 July 2010) of Run 2. Formula utilized is: [(Final Concentration – Initial concentration)/(Initial concentration)*100] = % increase or decrease.

Water depth [cm (in)]	Fertilizer ^z	Macronutrients (%)										Micronutrients ^y (%)				Trace element (%)
		NH ₄ -N	NO ₃ -N	Ca	K	Mg	P	Fe	Mn	Na	Al					
7.6 (3)	Control	-69	110	-52	-71	-67	-82	-99	-90	-61	-100					
	Conv	115	2226	-52	-71	-67	-82	-99	-78	29	-99					
	MG	58	-67	130	93	68	-8	-90	14	577	-92					
15.2 (6)	NS	-63	1367	391	176	216	-30	-97	194	556	-98					
	Control	-59	144	-86	-87	-84	-86	-99	-84	-18	-99					
	Conv	623	2620	-53	-70	-66	-75	-99	-70	35	-99					
22.9 (9)	MG	110	441	-28	-53	-45	-79	-97	-68	127	-97					
	NS	222	517	297	164	177	-14	-96	179	566	-97					
	Control	-31	671	-85	-73	-70	-86	-99	-78	-5	-99					
	Conv	1033	1368	-81	-84	-80	-88	-101	-90	-28	-100					
	MG	8	-12	-63	-54	-49	-80	-97	-80	105	-97					
	NS	68	546	287	117	172	-4	-94	161	483	-96					

^z Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2P-4.2K). Applied at 0.6 kg·m⁻³.

^y There were no differences (i.e. 0%) for micronutrients or trace elements As, B, Cd, Cr, Cu, Ni, Pb, or Zn, respectively; thus data were not presented within table.

Table 11. Final EC, SS, pH and difference between initial substrate sample (23 Mar. 2010) and final substrate sample (12 July 2010) of Run 2. Formula utilized is: Final sample – Initial sample = difference.

Water depth [cm (in)]	Final analysis				Difference between initial and final samples			
	Fertilizer ^z	EC (mS·cm ⁻¹)	SS (mg·L ⁻¹)	pH	EC (mS·cm ⁻¹)	SS (mg·L ⁻¹)	pH	
7.6 (3)	Control	0.6	451	5.7	0	304	1	
	Conv	0.1	91	6.0	0	-57	1	
	MG	0.9	630	5.8	1	482	1	
	NS	0.8	560	5.6	1	412	1	
15.2 (6)	Control	0.5	347	5.6	0	200	1	
	Conv	0.1	91	5.5	0	-57	0	
	MG	0.7	490	5.8	0	342	1	
	NS	0.7	518	5.7	1	370	1	
22.9 (9)	Control	0.6	442	5.6	0	295	0	
	Conv	0.1	89	5.7	1	-57	1	
	MG	0.7	490	5.8	0	342	1	
	NS	0.6	434	5.7	0	286	1	

^z Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-.2P-4.2K). Applied at 0.6 kg·m⁻³.

Fig. 1. Photo detailing drain components.



Fig. 2. Photo detailing attached drain to tub with Epoxy putty.



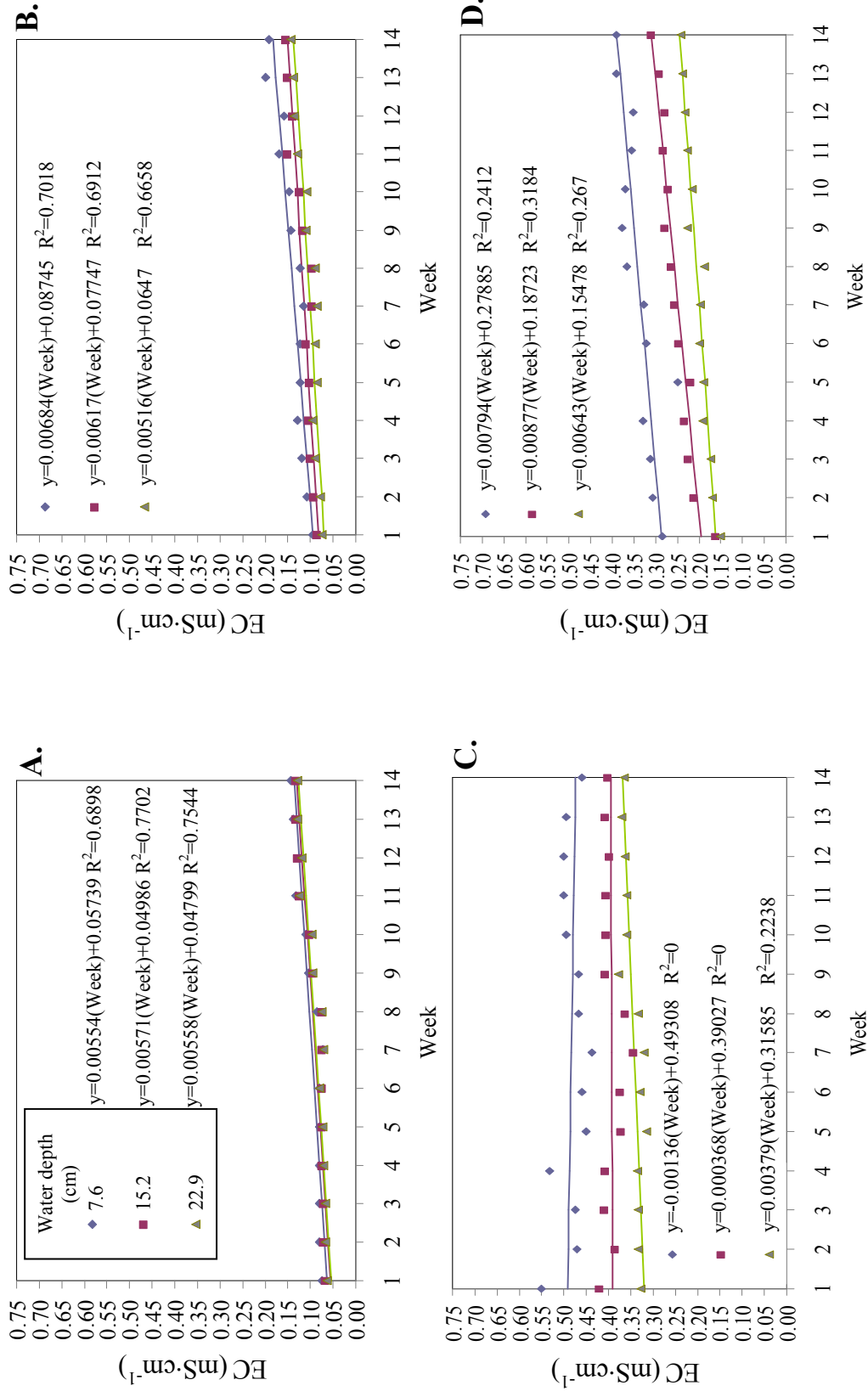


Fig. 3. Change in water EC for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Run 1 over time (14 weeks: 7 Dec 2009 – 15 Mar. 2010) for three water depths.

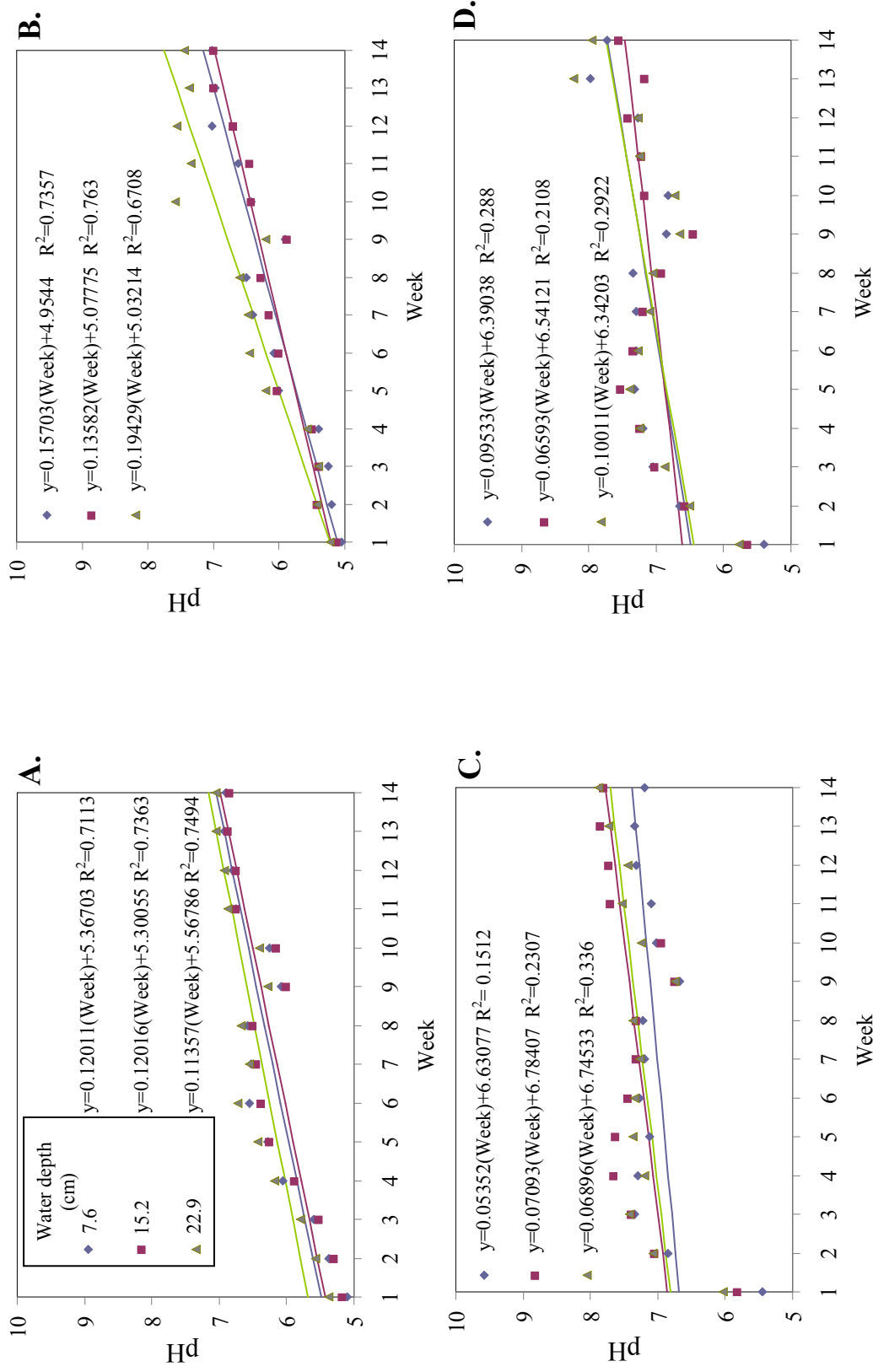


Fig. 4. Change in water pH for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Run 1 over time (14 weeks): 7 Dec 2009 – 15 Mar. 2010) for three water depths.

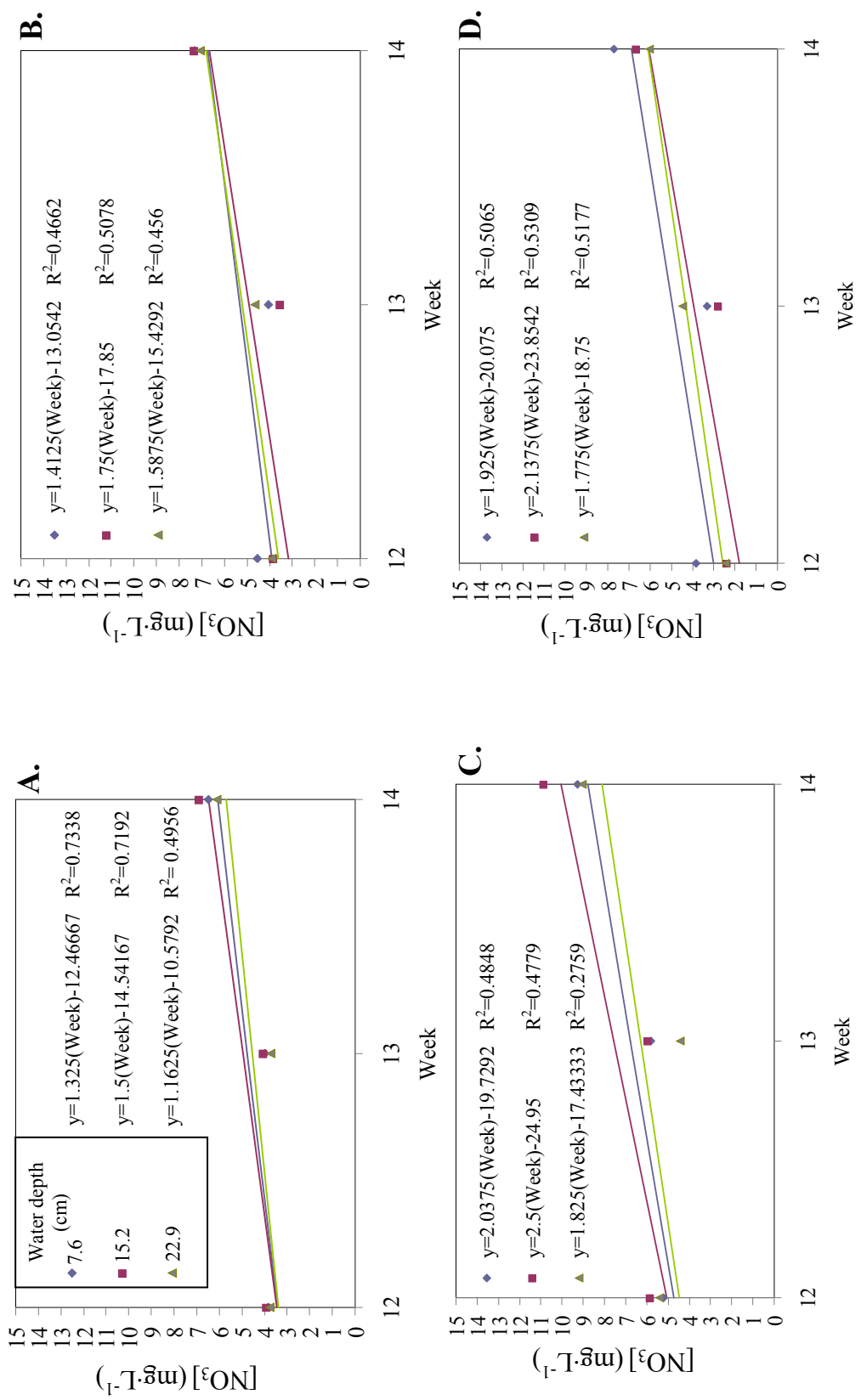


Fig. 5. Change in water NO₃ concentration for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Run 1 over time (3 weeks: 22 Feb. 2010 – 15 Mar. 2010) for three water depths.

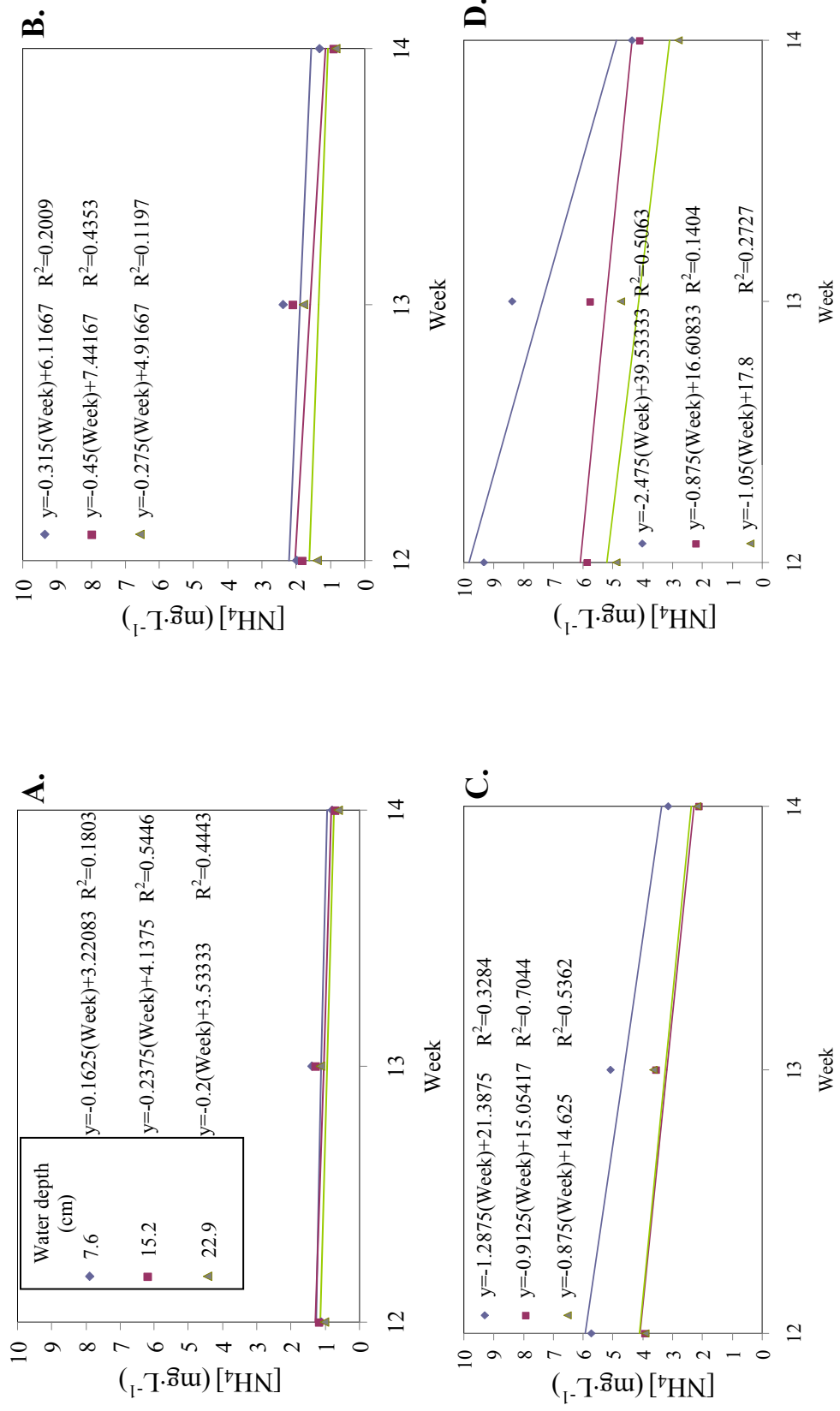


Fig. 6. Change in water NH_4 concentration for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS (Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Run 1 over time (3 weeks: 22 Feb. 2010 – 15 Mar. 2010) for three water depths.

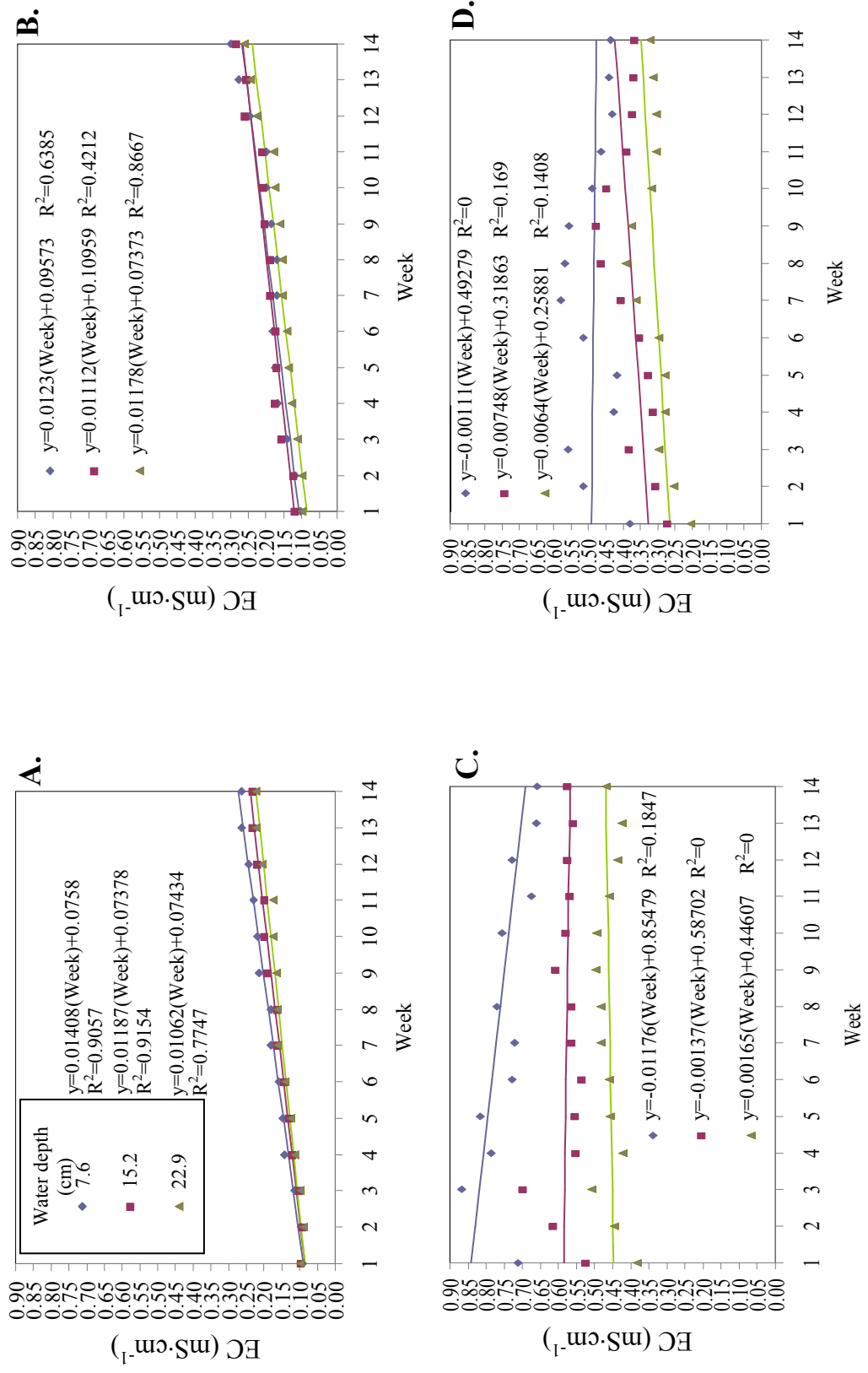


Fig. 7. Change in water EC for Control (zero fertilizer; n=4) (A), Conv (Harrell’s Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Run 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three water depths.

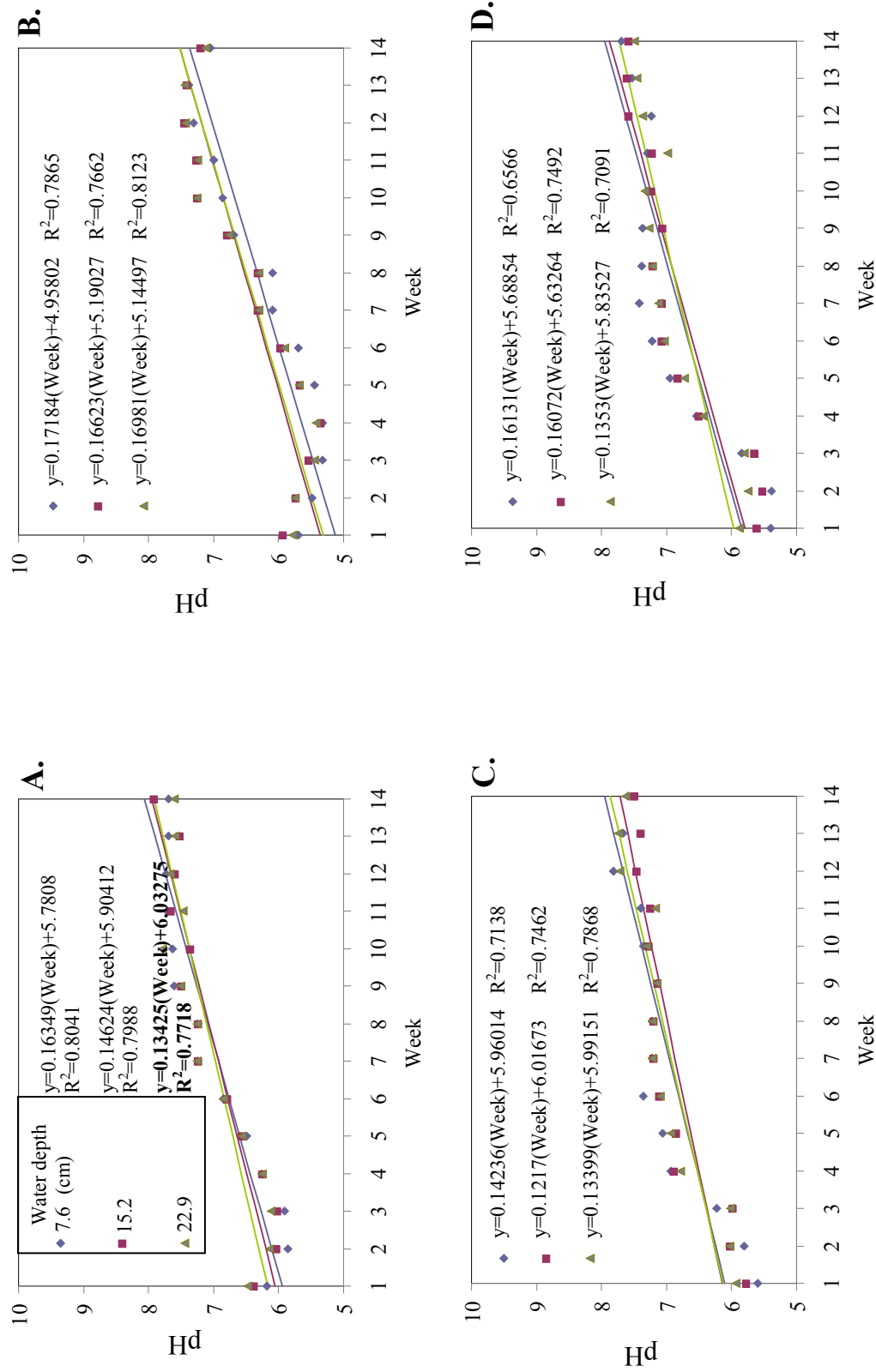


Fig. 8. Change in water pH for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Run 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three water depths.

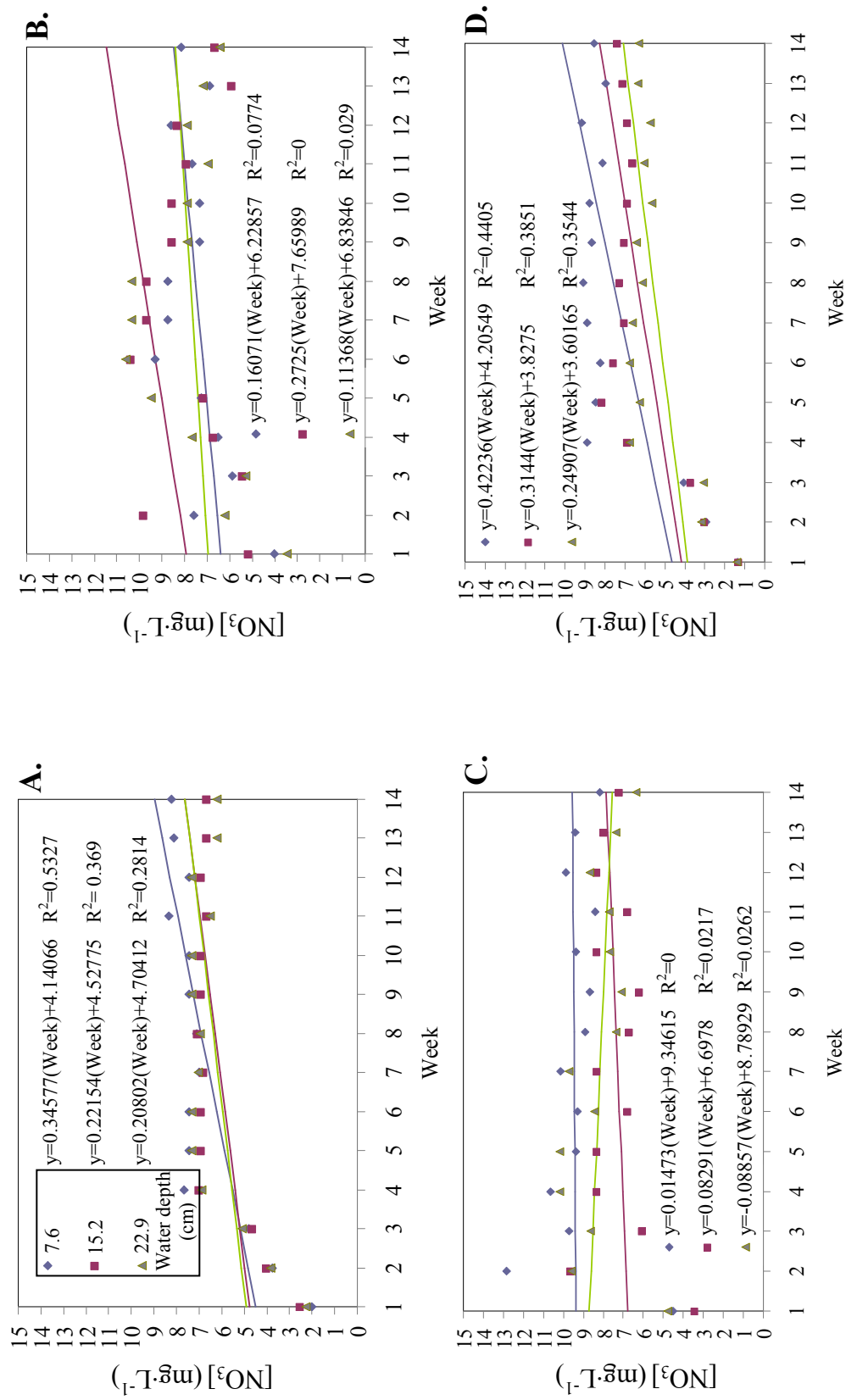


Fig. 9. Change in water NO_3^- concentration for Control (zero fertilizer; $n=4$) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; $n=4$) (B), MG (Medina Granular 4N-0.9P-2.5K; $n=4$) (C), and NS Nature Safe 8N-2.2P-4.2K; $n=4$) (D) treatments during Run 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three water depths.

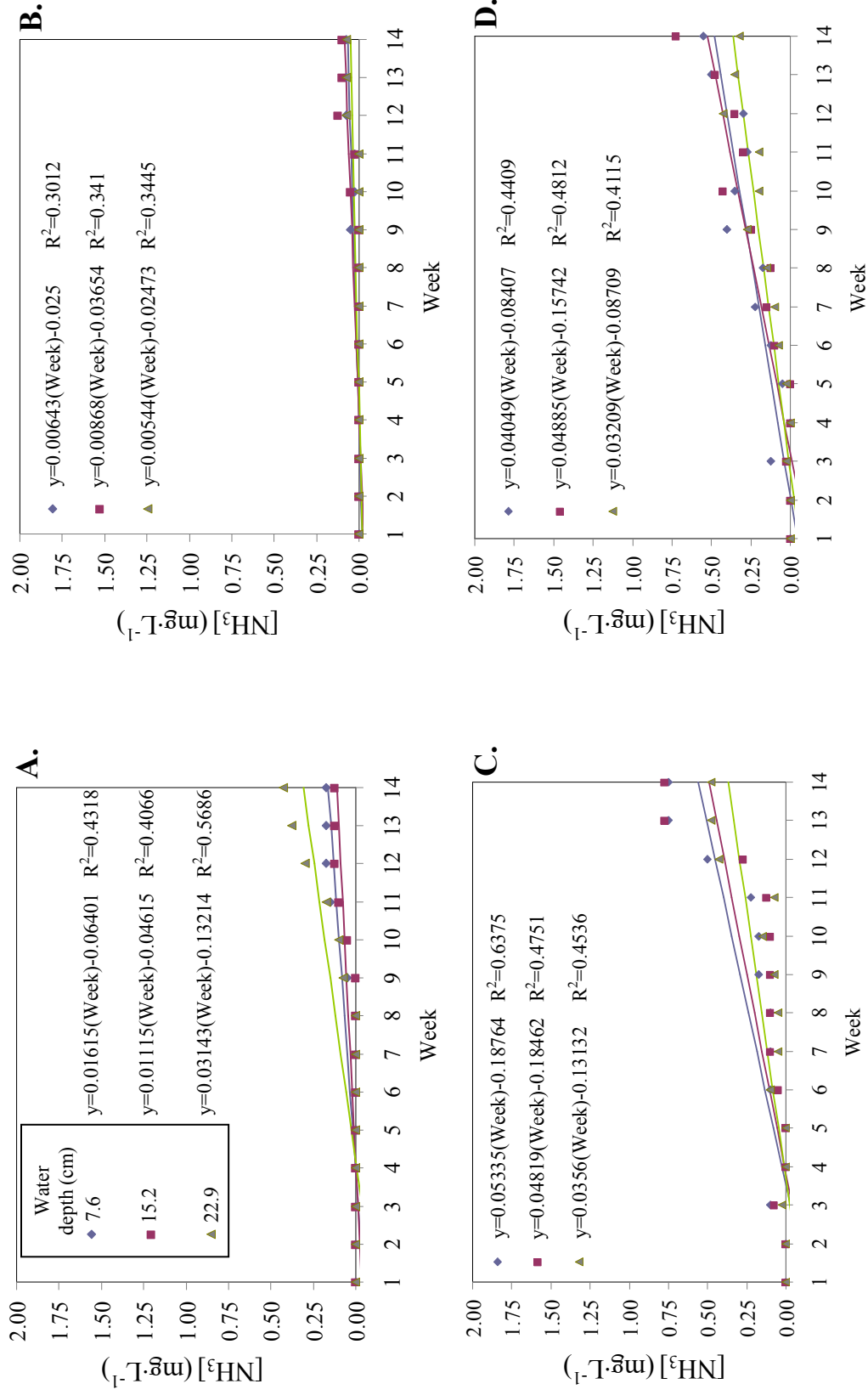


Fig. 10. Change in water NH_3 concentration for Control (zero fertilizer; $n=4$) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; $n=4$) (B), MG (Medina Granular 4N-0.9P-2.5K; $n=4$) (C), and NS Nature Safe 8N-2.2P-4.2K; $n=4$) (D) treatments during Run 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three water depths.

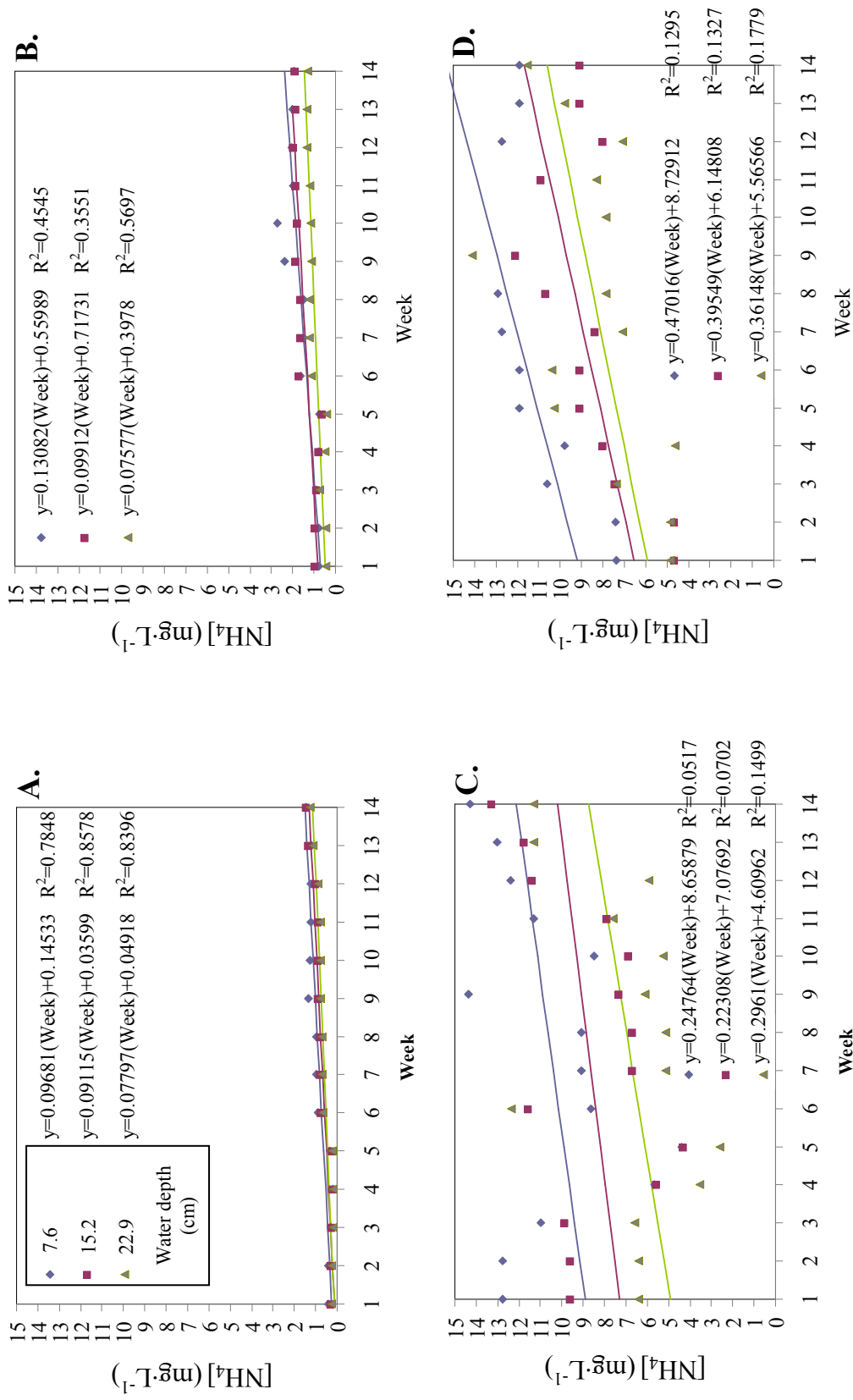


Fig. 11. Change in water NH_4 concentration for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS (Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Run 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three water depths.

CHAPTER III
EFFECT OF FERTILIZER TYPE AND RATE ON
WATER AND SUBSTRATE EC, pH,
NITRATE-NITROGEN (NO₃) AND AMMONIUM-NITROGEN (NH₄)
IN A SIMULATED POND SYSTEM

Abstract

Two small-scale simulation pond experiments were conducted to investigate the effects of fertilizer type (conventional, organic, or no fertilizer) and fertilizer rate on substrate and water EC, pH, nitrate-nitrogen (NO₃), ammonia-nitrogen (NH₃), and ammonium-nitrogen (NH₄) concentration over a three-month period to determine potential fertilizer rates and fertilizer types conducive for lotus (*Nelumbo* sp.) growth. Both experiments were conducted with intention of finding an acceptable fertilizer rate to apply for lotus pond production. EC is a strong factor influencing lotus growth and with greater temperatures and higher fertilizer rates, EC could rise close to a toxic level (1.0 mS·cm⁻¹) as was seen in Expt. 2 among Medina Growin' Green (organic) treatments. Substrate and water EC, pH, NO₃, NH₃, and NH₄ were higher among organic fertilizer treatments and with higher rates. Variables tested were within recommended levels for lotus growth, with exception of 1.3 kg·m⁻³ N (2.25 lb·yd⁻³ N) rate in Expt. 2. All

measured parameters among all fertilizer treatments and within all fertilizer rates increased linearly over time, with exception of pH.

Introduction

Sacred Lotus (*Nelumbo nucifera* Gaertn.) is an aquatic emergent angiosperm that belongs to the Nelumbaceae family and the genus *Nelumbo* Adans. Lotus has rhizomes that elongate with two types of emerging leaves: Floating and upright (standing) leaves (Nohara and Kimura, 1997). There are two species within the genus *Nelumbo*: *N. nucifera* Gaertn. and *N. lutea* (Willd.) Pers. North American native *N. lutea* (American Lotus) is distributed from eastern North America, extending south to Columbia. *N. nucifera* (Asian or Sacred Lotus) is distributed throughout Asia and Oceania, from Russia to Australia. Both species of lotus prefer shallow (approximately one meter), still water with a mud bottom where its rhizomes will spread rapidly into a water depth of two and a half (2.5) meters (Cook et al., 1974; Main et al., 2006).

Lotus has been cultivated for over three thousand years in the Far East where it continues to be used for medicine, food, and for cultural and religious activities (La-Ongsri, 2004; Shen-Miller, 2002). Each part of lotus is consumed throughout Asia for food or used for medicinal purposes, including rhizome, nodes, runners, seed, young shoot, leaf, stalk, petal, stamen, and pericarp (or fruit receptacle, seedpod) (Nguyen, 2001; Qichao and Xingyan, 2005; Wang and Zhang, 2004). Lotus' diverse nature and international popularity as a vegetable, ornamental, and/or medicinal would suggest that there may be opportunities for growing and marketing this plant locally. Its status as a living fossil symbolizes its worldly significance and stature and as an ancient prayer still

heard in Tibet today: Om mani padme hum, which translates “Oh, the jewel [is] in the heart of the lotus” (Slocum and Robinson, 1996).

Lotus can be grown in ponds, containers, greenhouses, and raised beds. For container and pond production, several substrates and depths have been suggested but the information is not well documented. Natural fertilizers (i.e. bone meal, blood meal, seaweed extracts) supply nutrients over a longer period than most synthetic ones and are less likely to burn plants (Gillman, 2008). The continued use of synthetic, mineral nitrogen fertilizers tends to lower pH values and deplete soil organic matter content (Wen, 1982). In contrast, once organic fertilizers are applied, gardeners would rarely need to apply them more than once a year because of their slow release and at times, one application can last several years (Coleman, 1989; Gillman, 2008). Nielsen and Thorup-Kristensen (2004) suggested that an ideal organic substrate blend should supply most of the nutrients needed for plant growth and limit the need for additional soluble nutrients. Nonetheless, this increased utilization has taken place mainly in dry, upland crop fields where crops other than aquatic plants are grown (Hesse, 1982). The improvement of physical properties of soils may have more relevance to dry soils rather than flooded soils; yet there is limited information available. In aquatic plant production (more notably *Nelumbo* sp.), there is no evidence of research conducted on the effects of organic fertilizers and/or amendments within anaerobic environments.

Organic fertilizer is a major choice in China, although there is not any scientific information available on its effects. Although animal manure has been recommended as an incorporated fertilizer, Shen-Miller and others (2002) disagree. In their studies, manure proved fatal to young seedlings and they reported clay as an essential component

for nutrient retention and as a minor nutrient source for lotus production. Slocum and Robinson (1996) suggested that one-part of well-rotted and composted cow manure be mixed with two- to three-parts topsoil. This discrepancy illustrates a need for more research on organic lotus production that serves as a foundation for this dissertation. The objective of this study is to investigate how selected rates of organic fertilizers influence water EC, pH, and nutrient concentration in an aquatic environment. Findings will provide useful guidelines for the aquatic plant industry.

Materials and Methods

A small-scale simulation pond experiment was conducted to compare the effect of fertilizer type (conventional, organic, or no fertilizer) and fertilizer rate on water EC, pH, nitrate-nitrogen (NO_3), and ammonium-nitrogen (NH_4) concentration over a three month period. All research was conducted in a glass greenhouse with computer controlled evaporative cooling pads and fans with XLS 15 Firebreak shade cloth (Svensson, Kinna, Sweden) in Auburn, AL at lat. 32.6°N. Temperature set-points were 29°C day and 27°C night with ambient light.

Experiment utilized a 1:1 (v:v) Marvyn sandy clay loam: pine bark substrate. Physical properties of the soil and nutritional analysis of individual and combined components were determined by the Soil Testing Laboratory at Auburn University (Table 1 – 3).

Forty, [68.1 L (18 gal), 59.4 cm (L) x 46.7 cm (W) x 39.1 cm (D) (23.4 in x 18.4 in x 15.4 in)] storage tubs (Rubbermaid®, Atlanta, GA) were filled to ¼ container depth (10.2 cm, 26.5 L) with substrate. Tubs were modified using basic schedule 40 PVC plumbing parts to include a drain. Prior to addition of substrate, a 5.1 cm (2 in) hole was

centered 7.6 cm (3 in) from tub bottom on one short side of each tub and cut with a 5.1 cm (2 in) arbored hole saw drill bit. The slip socket end of a 3.8 cm x 2.5 cm (1.5 in x 1 in) bushing and a 1.9 cm x 2.5 cm (0.75 in x 1 in) threaded male adapter were cemented together using PVC primer and cement with a 3.2 cm (1.25 in) piece of aquarium filter sleeve (Spectrum Brands, Madison, WI) glued in between the PVC pieces (Fig. 1). Filter was inserted to prevent substrate loss through the drain when sampling leachate. Epoxy putty was used to seal around the drains both inside and outside of each tub (Fig. 2). Each drain was fitted with nylon water shut off valves (Gilmour®, Somerset, PA). Leaks were minimal, but those that occurred were patched with epoxy putty; silicone did not work.

After tubs were filled with substrate, fertilizer treatments were applied and hand blended at their respective treatment rates. Four fertilizer types were used: Conventional controlled-release fertilizer, Harrell's Polyon 16N-2.6P-9.9K (16N-6P₂O₅-12K₂O) (hereafter referred to as Conv) (Harrell's LLC, Lakeland, FL), Medina Growin' Green Granular Organic 4N-0.9P-2.5K (4N-2P₂O₅-3K₂O) (hereafter referred to as MG) (Hondo, TX), and Nature Safe 8N-2.2P-4.2K (8N-5P₂O₅-5K₂O) (hereafter referred to as NS) (Griffin Industries, Coldspring, KY), and Control (no fertilizer).

For Experiment 1, each fertilizer type, with exception of Control, was applied at three treatment rates: 0.44 kg·m⁻³ N (0.75 lb·yd⁻³ N) (high), 0.3 kg·m⁻³ N (0.5 lb·yd⁻³ N) (med), and 0.15 kg·m⁻³ N (0.25 lb·yd⁻³ N) (low). In Experiment 2, the treatment rates were higher: 1.33 kg·m⁻³ N (2.25 lb·yd⁻³ N) (high), 0.89 kg·m⁻³ N (1.5 lb·yd⁻³ N) (med), and 0.44 kg·m⁻³ N (0.75 lb·yd⁻³ N) (low).

Water depth was marked at 22.9 cm (9 in) above substrate line to maintain designated water volume. Tubs were filled to approximately 80.7 L (17.7 gal) with water (pH 6.9, EC = 0.10 mS·cm⁻¹) to respective volume. Water volume was evaluated every other day, and water was added as needed to maintain volume.

Treatments were in a three (3) fertilizer rate x four (4) fertilizer type factorial arrangement for a total of ten (10) treatments; with four single-tub replications per treatment. There were two experiments. The first experiment (Expt. 1) was conducted from 7 Dec. 2009 – 15 Mar. 2010. The second experiment (Expt. 2) was conducted from 23 Mar. 2010 – 12 July 2010.

For Expt. 1, water pH and EC were measured by inserting the Hanna pH/EC/TDS meter (Hanna Instruments, Woonsocket, RI) into the center of each individual tub one week after fertilizer application and then weekly until Week 12. From Week 12 through Week 14, water pH, EC, and nitrate-nitrogen (NO₃) and ammonium-nitrogen (NH₄) were measured weekly by inserting the YSI Professional Plus meter (YSI Inc., Yellow Springs, OH) into the center of each individual tub. Additional water samples were collected from each individual tub one week after fertilizer application (14 Dec. 2009) and at the end of Expt. 1 (15 Mar. 2010) and analyzed using ICAP for NO₃ and NH₄ determination. One (1) Nalgene® narrow mouth bottle (4 oz, 125 mL) was dipped into center of each individual tub to collect these water samples, capped, and sent directly to lab for analysis (Consolidated Plastics Company, Inc., Stow, OH). For Expt.1, additional water samples were collected into one Nalgene® narrow mouth bottle (4 oz, 125 mL) from the leachate of each tub and sent directly to lab for analysis at the same collecting dates noted above.

Expt. 1 laboratory and meter data indicated there were no differences between water sampled from the center of the tubs and the leachate. Hence, for Expt. 2, water samples were only collected from the center of the tubs one week after fertilizer application (30 Mar. 2010) and at the end of Expt. 2 (12 July 2010) and analyzed using ICAP for NO₃ and NH₄ determination. For Expt. 2, water pH, EC, NO₃, and NH₄ were measured by inserting the YSI Professional Plus meter into the center of each individual tub one week after fertilizer application and continued weekly through duration of study.

Composite substrate samples were collected for analysis for each run prior to fertilizer application (Expt. 1: 7 Dec. 2009; Expt. 2: 30 Mar. 2010), at the end of each experiment (Expt. 1: 15 Mar. 2010; Expt. 2: 12 July 2010) and analyzed using the same ICAP analyses. Twelve (12), empty, 11.4 L (3 gal) buckets and twelve (12), empty, Ziploc® bags were labeled to represent each treatment. For each experiment and for each treatment, researchers used a 59 mL cup (2 oz) to collect substrate samples (2 oz) from the bottom of each tub; for a total of four (4) samples per treatment (1/rep). Those four (4) samples were placed into their respective treatment bucket, mixed together, and then a 2 oz sample was taken from the mix and placed into an individual quart size Ziploc® bag. Excess water was drained prior to sealing bags and bags were sent directly to lab for analysis.

Data were analyzed as a completely randomized design using PROC MIXED by SAS 9.1 (SAS Institute, Inc., Cary, NC). Linear and quadratic analysis were conducted and means were separated utilizing CONTRAST statements at $\alpha = 0.05$ (5%). Unless otherwise stated, all data were analyzed at the 5% level.

Results and Discussion

Expt. 1 – Low Rate

Tian (2008) found water EC increased after fertilizer application and decreased as nutrients were absorbed with actively growing plants. Since there were no plants in this study, EC increased linearly after fertilizer application and continued to increase over the duration of the study with increasing rates in Conv, MG, and NS ($P < 0.0001$) (Fig. 3B, 3C, and 3D). EC was consistently lower at low rates, higher at high rates, and was highest in MG fertilizer across all rates ($0.16 - 0.34 \text{ mS}\cdot\text{cm}^{-1}$) (Fig. 3C) compared to other fertilizer treatments (Table 4). Within all rates, water EC was lower in Control and Conv treatments (Table 4). EC did not exceed Tian (2008) recommendation of 0.5 to 1.0 $\text{mS}\cdot\text{cm}^{-1}$, which led to utilizing higher rates in Expt. 2 to verify data from Expt. 1 in order to define the limits of fertilizer supplements.

Lotus is not affected by a pH range of 5.5 to 8.0 in water and has the ability to tolerate a wide pH range (4.5 to 9.0) (Meyer, 1930). For Control and Conv treatments, pH displayed a positive linear trend over time for each rate ($P < 0.0001$) (Fig. 4A-4B). Over time, for each rate, pH displayed quadratic trends for MG ($P=0.0402$) and a positive linear trend for NS ($P=0.0013$) (Fig. 4C-4D). Within all fertilizer rates, pH was higher in MG and NS than in Control and Conv treatments (Table 4). Chellemi and Lazaorvits (2002) found that pH increased with increased N rates of organic fertilizer and that it had a linear relationship over time with increased applications, which supports our findings in Table 4.

There was no significant interaction between fertilizer rate and type for NO_3 concentration. Over time, NO_3 concentration displayed positive linear trends for each

rate and fertilizer type ($P < 0.0001$) (Fig. 5) and there were no differences within rates among fertilizer type (Table 4). NO_3 was highest in MG ($5.9 \text{ mg}\cdot\text{L}^{-1}$) (Fig. 5C). There were no differences found within fertilizer rates among Control, NS, and Conv treatments ($4.2, 4.9, \text{ and } 4.7 \text{ mg}\cdot\text{L}^{-1}$ respectively) (Table 4). There were no differences over time, within fertilizer rates, or among fertilizer types and no interactions were present for NH_3 concentration (mean $0.15 \text{ mg}\cdot\text{L}^{-1}$), therefore data were not presented.

Over time and within rates, NH_4 concentration decreased linearly among fertilizer types ($P < 0.0001$) (Fig. 6). NH_4 concentration was consistently higher in MG within all rates (Table 4). NH_4 concentration was higher within high rates among MG and NS (3.68 and $3.3 \text{ mg}\cdot\text{L}^{-1}$, respectively) (Table 4),

Water samples collected one week after fertilizer application had higher levels of macronutrients than those collected at the end of Expt. 1 (Table 5). Calcium (Ca), K, and Mg concentrations decreased across all fertilizer types and within all rates, with exception of Conv (high rate) for K which increased by 14% (Table 5). Micronutrients B, Mn, and Na increased across all fertilizer types and rates (Table 5).

The differences in nutrient concentration between the initial (pre-fertilizer) substrate sample and substrate samples from the end of Expt. 1 are presented in Table 6. Over time, all macronutrient and micronutrient concentrations, with exception of Al and Fe, increased among NS treatment within all fertilizer rates (Table 6). Sodium (Na) concentration increased over time among MG and NS treatments within all rates (Table 6). Substrate EC exceeded Tian (2008) recommendations to not go above $0.5 - 1.0 \text{ mS}\cdot\text{cm}^{-1}$ (Table 7). Within 0.3 and $0.44 \text{ kg}\cdot\text{m}^{-3}$ rates among all fertilizer types, except for Control, substrate EC was nearly $1.0 \text{ mS}\cdot\text{cm}^{-1}$ higher than initial (pre-fertilizer) sample

(Table 7). Soluble salt (SS) concentration increased across all treatments, especially NS (range: 265 – 807 mg·L⁻¹). There were no differences in pH from initial sample to final sample (data not shown).

Expt. 2 – High Rate

Similar to Expt. 1, water EC increased linearly over time for each rate and fertilizer type ($P < 0.0001$) (Fig. 7). Water EC was consistently higher in MG across all rates and lower in Control (Table 8). A toxic EC level above 1.0 mS·cm⁻¹ was reached among MG (1.2 mS·cm⁻¹) within the high rate (Table 8) (Fig. 7C), which goes above Tian (2008) recommendations for lotus growth.

Over time, within all rates and among all fertilizer treatments, except MG, water pH displayed quadratic trends ($P < 0.0001$; Control $P = 0.0154$) (Fig. 8). Both MG and NS displayed higher pH ranges within all rates (range MG: 7.06 – 7.19; NS: 6.72 – 7.34) and Conv displayed a lower pH (5.52 – 6.44) (Table 8). Contrary to Chellemi and Lazaorvits (2002) findings, pH was higher within the low rate whereas it was lower within the high rate (Table 8). All pH ranges were within acceptable ranges (4.5 to 9.0) for lotus growth (Table 8).

Over time, NO₃ concentration displayed quadratic trends among Conv and NS treatments ($P < 0.0001$, $P = 0.0097$, respectively) (Fig. 9B and 9D), and positive linear trends among MG ($P = 0.0007$) (Fig. 9C). MG had higher NO₃ concentrations across all rates (range 14.3 – 16.3 mg·L⁻¹) while NS (range 9.8 – 13.36 mg·L⁻¹) and Conv (range 9.55 – 12.1 mg·L⁻¹) were lower (Table 8).

Contrary to Chellemi and Lazaorvits (2002) and Expt. 1 findings, NH₃ concentration displayed positive linear trends over time for each rate and fertilizer type

($P < 0.0001$) (Fig. 10A-10C), except NS which displayed a quadratic trend ($P < 0.0001$) (Fig. 10D).

Over time, NH_4 concentration displayed positive linear trends among all fertilizers, except Control ($P < 0.0001$) (Fig. 11). Although, in the nutritional analysis of treatments (Table 3), NS contained a lower concentration of NH_4 and Conv contained a higher NH_4 concentration, NH_4 concentration was consistently higher in NS across all fertilizer rates (range 7.3 to 23.7 $\text{mg}\cdot\text{L}^{-1}$) and Control was lower (range 1.3 to 4.4 $\text{mg}\cdot\text{L}^{-1}$) (Table 8). Nonetheless, considering Conv was a controlled-release fertilizer, NH_4 concentration release was far slower, explaining its lower water concentration in this experiment.

Contrary to Expt. 1 data, water samples collected one week after fertilizer application had lower nutrient concentrations than those collected at end of Expt. 2 (Table 9). Over time, macronutrients Ca, K, Mg, and micronutrient Na concentrations increased across all fertilizer rates and types (except for Control) (Table 9). Expt. 2 took place during warmer months which may have contributed to increased nutrient concentrations; there was an approximate six degree difference (up from 21 to 27°C).

The differences in nutrient concentration between the initial (pre-fertilizer) substrate sample and substrate samples from the end of Expt. 2 are presented in Tables 10 - 11. Over time, NS had the higher nutrient concentration increase across all nutrients, with exception of NH_4 where Conv increased within all rates and NO_3 where Conv increased within both 0.44 and 0.89 $\text{kg}\cdot\text{m}^{-3}$ N rates (Table 10). Once again, EC exceeded Tian (2008) recommendations of 1.0 $\text{mS}\cdot\text{cm}^{-1}$ and SS concentration increased across all fertilizer types and rates (Table 11). pH increased by approximately one unit across all

fertilizer types and rates, with exception of Control, and the low and high rates for Conv (Table 11).

For Expt. 1, a toxic EC of $1.0 \text{ mS}\cdot\text{cm}^{-1}$ was not reached which led to the utilization of higher rates for Expt. 2. The toxic EC level was surpassed readily with increased rates of 0.89 to $1.33 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ in both Conv and MG treatments. Both experiments were conducted with intention of finding an acceptable fertilizer rate to apply for a field study (Chapter 4). It was through utilizing $0.89 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ in the E.V. Smith study that led to conduct fertilizer rate experiments to determine a rate suitable for lotus growth within Tian's (2008) EC recommendations. Using $0.89 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ in the E.V. Smith study led to the demise of most of the lotus ponds. Since $0.89 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ proved to be too much, researchers determined a $0.6 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ ($1 \text{ lb}\cdot\text{yd}^{-3} \text{ N}$) rate would be low enough to grow lotus outdoors in a pond, based on these greenhouse studies (Chapter 4). Unfortunately, for reasons yet discovered, the $0.6 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ rate proved to be too high in the outdoor study (Chapter 4). This led to the removal of substrate and fertilizer in order to begin anew; this time with $0.44 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ ($0.75 \text{ lb}\cdot\text{yd}^{-3} \text{ N}$) (Chapter 4). Considering that these experiments were in a glass greenhouse with a sunscreen shade, researchers question if sunlight and heat had anything to do with potentially increasing water EC levels. More research needs to be conducted to determine the cause and effect as to why the water EC rose outside in the ponds. Future research to learn more about nutrient changes over time and application of principles learned may be beneficial to optimize plant growth and health.

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Table 1. Fertilizer types and contents for both experiments.

Fertilizer type	Fertilizer treatments	Fertilizer contents
Conventional	Harrell's Polyon 16N-2.6P-9.9K	All Polymer Coated: Ammonium Nitrate Copper Sulfate EDTA Iron Chelate Magnesium Sulfate Manganese Sulfate Mono-Ammonium Phosphate Sodium Molybdate Sulfate of Potash Zinc Sulfate
Organic	Medina Granular 4N-0.9P-2.5K	Kelp meal, humate, pasteurized poultry manure, molasses, and greensand
	Nature Safe 8N-2.2P-4.2K	Meat meal, hydrolyzed feather meal, bone meal, blood meal & sulfate of potash

Table 2. Nutritional analysis of substrates.^z Units are mg·L⁻¹ with exception of pH and electrical conductivity.

	Sandy clay loam	Pine bark	1:1 (v:v) Sandy clay loam:pine bark
pH	5.6	4.3	5.1
Electrical conductivity (EC) (mS·cm ⁻¹)	0.15	0.57	0.21
Soluble salts (SS)	96	399	148
Phosphorus (P)	7	4	1
Potassium (K)	41	90	46
Magnesium (Mg)	241	90	29
Calcium (Ca)	341	23	13
Copper (Cu)	1.1	0.2	0.3
Iron (Fe)	169	4.7	18.3
Manganese (Mn)	11	2.3	1.0
Zinc (Zn)	0.5	0.3	0.1
Boron (B)	1.2	0.4	0.2
Aluminum (Al)	294	25	40
Sodium (Na)	115	8	14
Nitrate-Nitrogen (NO ₃)	2	3	2
Ammonium-Nitrogen (NH ₄)	<0.1	3	2

^z Analyzed using ICAP for determination.

Table 3. Nutritional analysis of fertilizers.^z Units are mg·L⁻¹ with exception of pH and electrical conductivity.

	Medina granular organic 4N-0.9P-2.5K (MG)	Nature safe 8N-2.2P-4.2K (NS)	Harrell's polyon 16N-2.6P-9.9K (Conv)
pH	6.4	6.2	-- ^y
Electrical conductivity (EC) (mS·cm ⁻¹)	30	31	--
Soluble salts (SS)	20832	21700	--
Phosphorus (P)	1031	489	6000
Potassium (K)	10616	12266	12000
Magnesium (Mg)	3049	1086	14000
Calcium (Ca)	765	486	--
Copper (Cu)	83	1.3	790
Iron (Fe)	22.7	<0.1	3300
Manganese (Mn)	12	0.6	1300
Zinc (Zn)	21	<0.1	790
Boron (B)	13	2.7	--
Aluminum (Al)	3.2	4.3	--
Sodium (Na)	6985	2991	--
Nitrate-Nitrogen (NO ₃)	301	66	72000
Ammonium-Nitrogen (NH ₄)	1799	142	88000

^z Analyzed using ICAP determination.

^y – Indicates information not available according to label.

Table 4. Mean effect of fertilizer rate and type on water EC, pH, NO₃-N, and NH₄-N pulled over weekly sampling dates (14 weeks: 7 Dec 2009 – 15 Mar. 2010) for Expt. 1 (n=56).^z

Fertilizer rate [kg·m ⁻³ N (lb·yd ⁻³ N)]	Fertilizer ^y	EC (mS·cm ⁻¹)	pH	NO ₃ -N (mg·L ⁻¹)	NH ₄ -N (mg·L ⁻¹)
0.15 (0.25)	Control	0.1bc ^x	6.14c	4.2b ^w	1.03bc
	Conv	0.09c	6.49b	4.7b	1.04c
	MG	0.16a	7.13a	5.9a	1.79a
0.3 (0.5)	NS	0.11b	7.15a	4.9b	1.61ab
	Conv	0.09c	6.41b		0.98b
	MG	0.25a	7.23a		2.58a
0.44 (0.75)	NS	0.15b	7.19a		2.42a
	Conv	0.1c	6.43c		1.12c
	MG	0.34a	7.23a		3.68a
	NS	0.2b	6.87b		3.3b

^z Water EC, pH, NO₃-N, and NH₄-N were measured from each individual tub one week after fertilizer application (14 Dec. 2009) and then weekly until end of Expt. 1 (15 Mar. 2010).

^y Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K).

^x Lowercase letters denote mean separation among fertilizer treatments within fertilizer rates at p<0.05 (SAS Institute, 2004).

^w There were no statistical differences within fertilizer rates; means represent data pooled over fertilizer rates.

Table 5. Percent increase (positive values) or decrease (negative values) in nutrient concentrations in water samples from the initial sample (7 Dec. 2009) to the final sample (15 Mar. 2010) of Expt. 1. Formula utilized is: [(Final Concentration – Initial concentration)/(Initial concentration)*100] = % increase or decrease.

Fertilizer rate [kg·m ⁻³ N (lb·yd ⁻³ N)]	Fertilizer ^z	Macronutrients ^y (%)						Micronutrients ^x (%)					
		Ca	K	Mg	P	Al	B	Cu	Fe	Mn	Na		
0.15 (0.25)	Control	-58	-5	-34	200	1594	0	0	1467	0	40		
	Conv	-51	-1	-36	-33	444	0	0	400	0	45		
	MG	-48	-22	-35	-63	750	-25	-34	68	-14	35		
	NS	-70	-15	-54	-61	400	50	0	400	0	41		
0.3 (0.5)	Conv	-60	-3	-52	100	409	0	0	343	0	27		
	MG	-66	-35	-53	-88	200	-33	-38	-32	-20	18		
	NS	-68	-10	-58	13	550	0	0	500	0	38		
	Conv	-66	14	-37	133	270	0	0	164	0	59		
0.44 (0.75)	MG	-45	-26	-42	-77	300	-20	-33	26	-33	25		
	NS	-81	-7	-53	-36	100	0	0	120	-43	44		

^z Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K).

^y There were no mean differences (i.e. 0%) for macronutrients NH₄-N or NO₃-N; thus data were not presented within table.

^x There were no mean differences (i.e. 0%) for micronutrients As, Cd, Cr, Ni, Pb, or Zn; thus data were not presented within table.

Table 6. Percent increase (positive values) or decrease (negative values) in nutrient concentrations in substrate samples from the initial sample (7 Dec. 2009) to the final sample (15 Mar. 2010) of Expt. 1. Formula utilized is: $[(\text{Final Concentration} - \text{Initial Concentration})/(\text{Initial Concentration}) * 100] = \% \text{ increase or decrease.}$

Fertilizer rate [kg·m ⁻³ N (lb·yd ⁻³ N)]	Fertilizer ^z	Macronutrients (%)							Micronutrients ^y (%)				
		NH ₄ -N	NO ₃ -N	Ca	K	Mg	P	Al	Fe	Mn	Na		
0.15 (0.25)	Control	-72	-88	101	-30	161	3	-94	-95	351	111		
	Conv	-41	175	166	-2	90	-26	-95	-93	418	198		
	MG	-48	20	142	25	100	79	-96	-97	306	311		
0.3 (0.5)	NS	223	3864	552	197	243	31	-96	-98	805	388		
	Conv	-6	789	190	13	119	-11	-95	-86	485	195		
	MG	-35	400	184	84	131	51	-96	-96	313	504		
0.44 (0.75)	NS	112	4098	514	150	228	11	-96	-98	945	331		
	Conv	44	1444	142	11	173	159	-96	-97	350	177		
	MG	16	294	60	46	96	46	-92	-93	106	263		
	NS	101	630	289	63	196	9	-95	-87	704	244		

^zFertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K).

^y There were no mean differences (i.e. 0%) for micronutrients As, B, Cd, Cr, Cu, Ni, Pb, or Zn; thus data were not presented within table.

Table 7. Final EC, SS, pH, and difference between initial substrate sample (7 Dec. 2009) and final substrate sample's (15 Mar. 2010) EC and SS in Expt. 1.
Formula utilized: Final sample – Initial sample = difference.

Fertilizer rate [kg·m ⁻³ N (lb·yd ⁻³ N)]	Fertilizer ^z	Final analysis			Difference between initial and final samples	
		EC (mS·cm ⁻¹)	SS (mg·L ⁻¹)	pH ^y	EC (mS·cm ⁻¹)	SS (mg·L ⁻¹)
0.15 (0.25)	Control	0.45	331	4.84	0	185
	Conv	0.61	430	4.85	0	282
	MG	0.67	469	5.01	0	321
0.3 (0.5)	NS	0.59	412	4.91	1	807
	Conv	0.76	529	4.79	1	382
	MG	0.88	616	5.05	1	469
0.44 (0.75)	NS	1.24	868	4.71	1	720
	Conv	0.95	668	4.81	1	521
	MG	0.56	391	5.30	0	243
	NS	1.36	955	4.77	0	265

^zFertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K).

^y There were no mean differences between initial and final pH, thus data were not presented in final column.

Table 8. Mean effect of fertilizer rate and type on water EC, pH, NO₃-N, and NH₄-N pulled over weekly sampling dates (14 weeks: 30 Mar. 2010 – 12 July 2010) for Expt. 2. (n=56).^z

Fertilizer rate [kg·m ⁻³ N (lb·yd ⁻³ N)]	Fertilizer ^y	EC (mS·cm ⁻¹)	pH	NO ₃ -N (mg·L ⁻¹)	NH ₃ -N (mg·L ⁻¹)	NH ₄ -N (mg·L ⁻¹)
	Control	0.13c ^x	6.38cd	5.91b	0.04b	0.65b
0.44 (0.75)	Conv	0.16c	6.44c	9.55b	0.009b	1.3b
	MG	0.46a	7.12a	14.33a	0.33ab	6.1a
	NS	0.35b	6.72b	9.8b	0.28ab	7.3a
0.89 (1.5)	Conv	0.2c	6.28c	11.65b	0.007b	2.6b
	MG	0.83a	7.06b	16.41a	0.15b	11.7a
	NS	0.5b	7.34a	12.33b	0.88a	14.2a
1.33 (2.25)	Conv	0.86b	5.52d	12.1b	0.06b	4.4c
	MG	1.2a	7.19b	16.3a	0.69a	18.1b
	NS	0.68c	7.22a	13.36b	0.58a	23.7a

^z Water EC, pH, NO₃-N, NH₃-N and NH₄-N were measured from each individual tub one week after fertilizer application (30 Mar. 2010) and then weekly until end of Expt. 2 (12 July 2010).

^y Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-2.2P-4.2K).

^x Lowercase letters denote mean separation among fertilizer treatments within fertilizer rates at p<0.05 (SAS Institute, 2004).

Table 9. Percent increase (positive values) or decrease (negative values) in nutrient concentrations in water samples from the initial sample (23 Mar. 2010) to the final sample (12 July 2010) of Expt. 2. Formula utilized is: [(Final Concentration – Initial concentration)/(Initial concentration)*100] = % increase or decrease.

Fertilizer rate [kg·m ⁻³ N (lb·yd ⁻³ N)]	Fertilizer ^z	Macronutrients ^y (%)										Macronutrients ^x (%)												
		Ca	K	Mg	P	Al	B	Cu	Fe	Mn	Na	Zn	Ca	K	Mg	P	Al	B	Cu	Fe	Mn	Na	Zn	
0.44 (0.75)	Control	448	-3	20	-1	-6	0	0	-4	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0
	Conv	354	35	132	25	53	-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	33	
	MG	494	82	178	474	-88	0	-71	0	0	0	0	0	0	0	0	0	0	0	0	0	-20	-50	
	NS	1659	124	363	29	-84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	15	
0.89 (1.5)	Conv	465	89	176	71	22	-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	125	29	
	MG	963	119	349	1489	-76	-20	25	0	0	0	0	0	0	0	0	0	0	0	0	0	80	39	
	NS	988	114	248	62	-85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150	25	
	Conv	1357	482	479	1000	-52	-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	175	71	
1.33 (2.25)	MG	359	138	173	613	-69	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	33	48	
	NS	2013	152	316	81	-50	-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	175	55	

^z Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-.2P-4.2K).

^y There were no mean differences (i.e. 0%) for macronutrients NH₄-N or NO₃-N; thus data were not presented within table.

^x There were no mean differences (i.e. 0%) for micronutrients As, Cd, Cr, Ni, Pb, or Zn; thus data were not presented within table.

Table 10. Percent increase (positive values) or decrease (negative values) in nutrient concentrations in substrate samples from the initial sample (23 Mar. 2010) to the final sample (12 July 2010) of Expt. 2. Formula utilized is: [(Final Concentration – Initial concentration)/(Initial concentration)*100] = % increase or decrease.

Fertilizer rate [kg·m ⁻³ N (lb·yd ⁻³ N)]	Fertilizer ^z	Macronutrients (%)							Micronutrients ^y (%)				
		NH ₄ -N	NO ₃ -N	Ca	K	Mg	P	Al	Fe	Mn	Na		
0.44 (0.75)	Control	-71	-100	-82	-87	-82	-84	-99	-98	-87	-61		
	Conv	843	1204	-99	-84	-82	-86	-100	-99	-87	-51		
	MG	451	-71	-73	-72	-68	-86	-98	-98	-85	25		
0.89 (1.5)	NS	128	52	153	85	94	33	-82	-76	135	428		
	Conv	2706	4909	-54	-65	-60	-86	-100	-99	-76	-55		
	MG	554	-73	-71	-76	-72	-78	-98	-98	-84	7		
1.33 (2.25)	NS	634	761	235	167	65	80	-89	-77	172	624		
	Conv	2285	1084	-82	-87	-84	-86	-100	-99	-90	-60		
	MG	674	-69	-61	-66	-62	-81	-99	-99	-80	52		
	NS	1806	1572	601	221	206	29	-95	-92	220	790		

^z Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.9P-2.5K); NS (Nature Safe 8N-.2P-4.2K).

^y There were no mean differences (i.e. 0%) for trace elements As, B, Cd, Cr, Cu, Ni, Pb, or Zn; thus data were not presented within table.

Table 11. Final EC, SS, pH, and difference between initial substrate sample (23 Mar. 2010) and final substrate sample's (12 July 2010) EC and SS in Expt. 2. Formula utilized: Final sample – Initial sample = difference.

Fertilizer rate [kg·m ⁻³ N (lb·yd ⁻³ N)]	Fertilizer ^z	Final analysis			Difference between initial and final samples		
		EC (mS·cm ⁻¹)	SS (mg·L ⁻¹)	pH	EC (mS·cm ⁻¹)	SS (mg·L ⁻¹)	pH
0.44 (0.75)	Control	0.4	278	5.53	0	135	0
	Conv	1.4	955	5.59	1	807	0
	MG	0.62	434	5.82	0	286	1
	NS	0.45	315	5.70	0	167	1
0.89 (1.5)	Conv	2.5	1736	5.68	2	1588	1
	MG	0.98	686	5.77	1	538	1
	NS	0.65	455	5.98	0	307	1
1.33 (2.25)	Conv	2.4	1649	5.59	2	1502	0
	MG	0.98	686	6.03	1	538	1
	NS	1.50	1050	5.80	1	902	1

^z Fertilizer treatments: Control (no fertilizer); Conv (Harrell's Polyon 16N-2.6P-9.9K); MG (Medina Granular Organic 4N-0.P-2.5K); NS (Nature Safe 8N-.2P-4.2K).

Fig. 1. Photo detailing drain components.



Fig. 2. Photo detailing attached drain to tub with Epoxy putty.



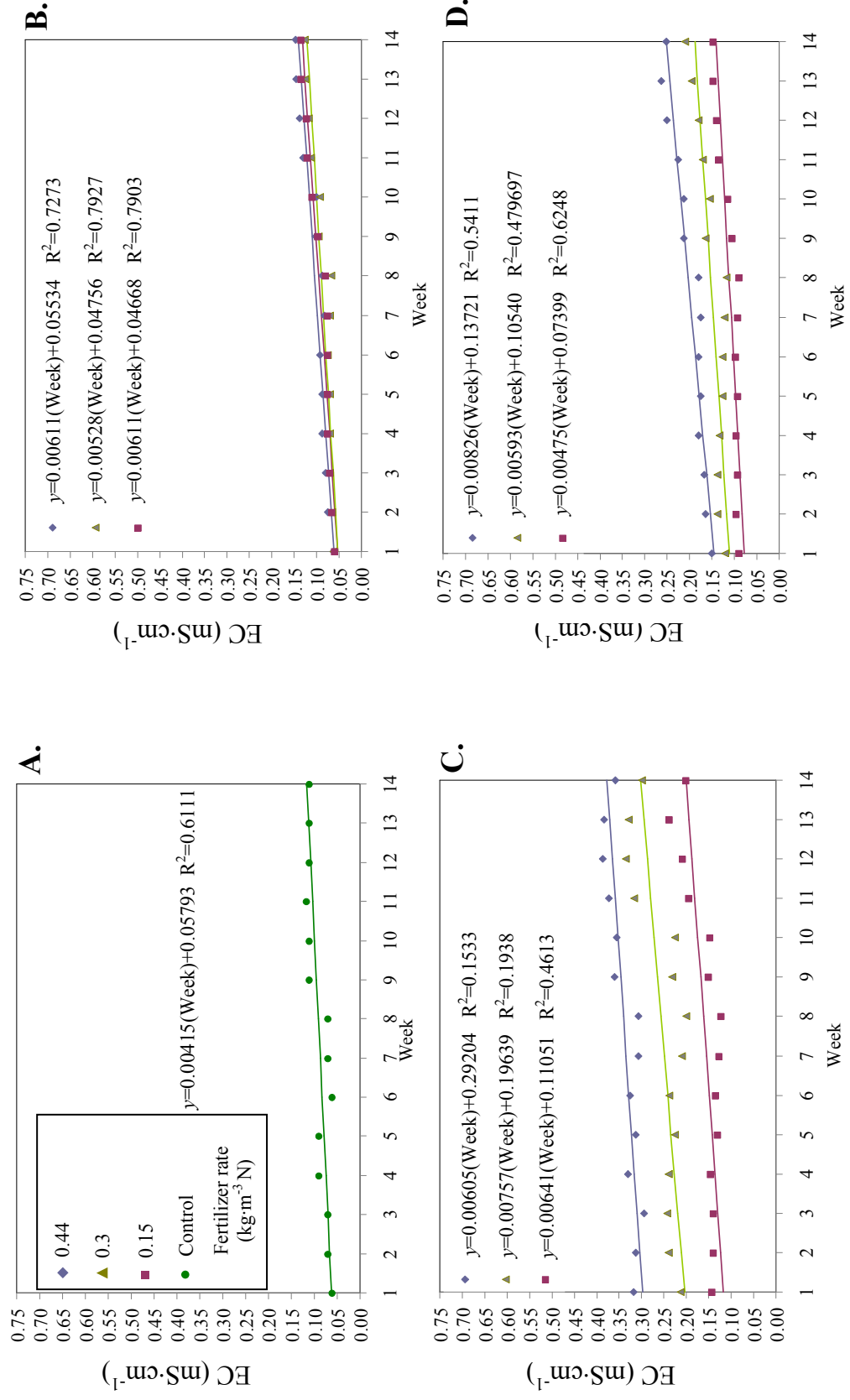


Fig. 3. Change in water EC for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 1 over time (14 weeks: 7 Dec 2009 – 15 Mar. 2010) for three fertilizer rates.

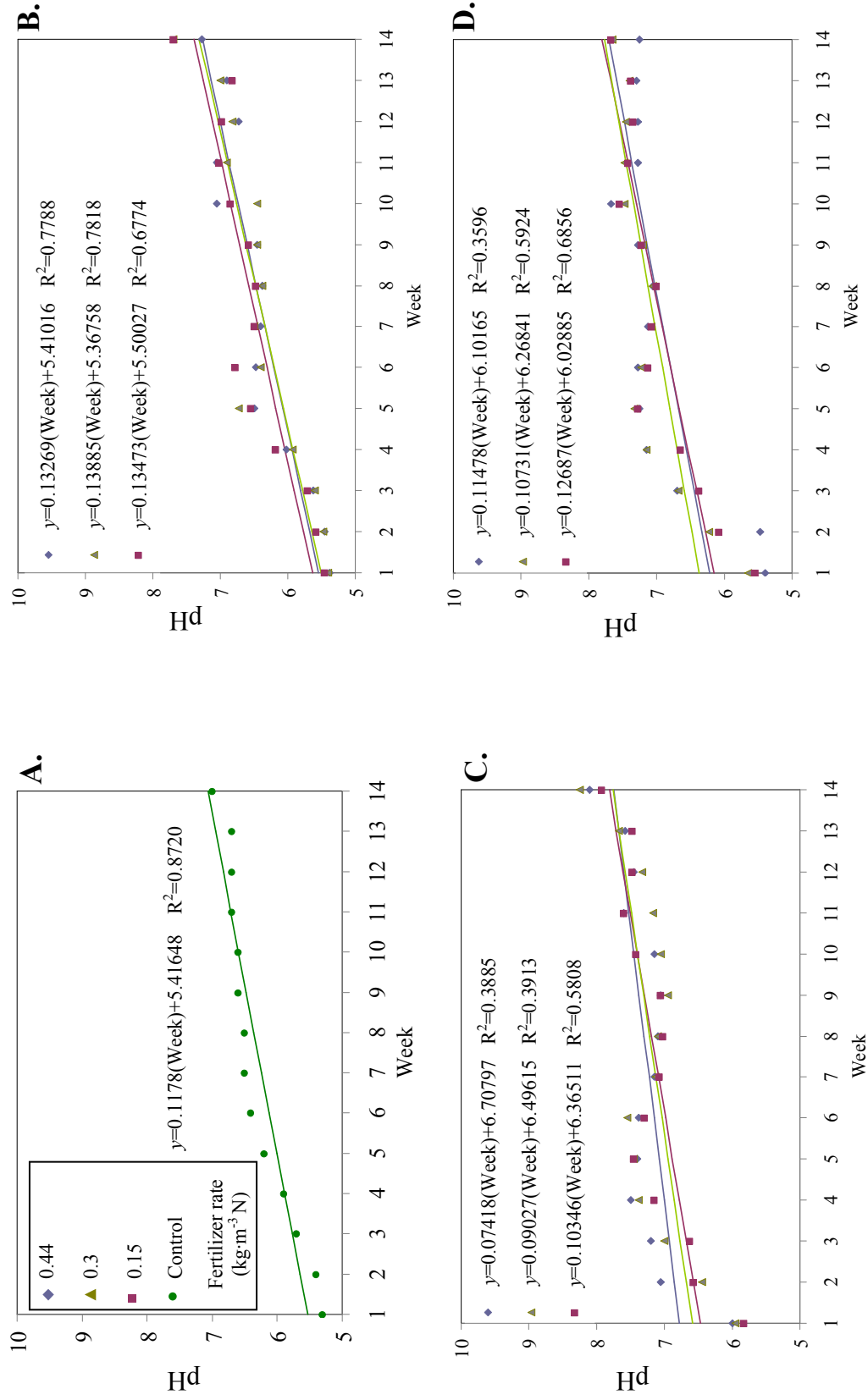


Fig. 4. Change in water pH for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 1 over time (14 weeks: 7 Dec 2009 – 15 Mar. 2010) for three fertilizer rates.

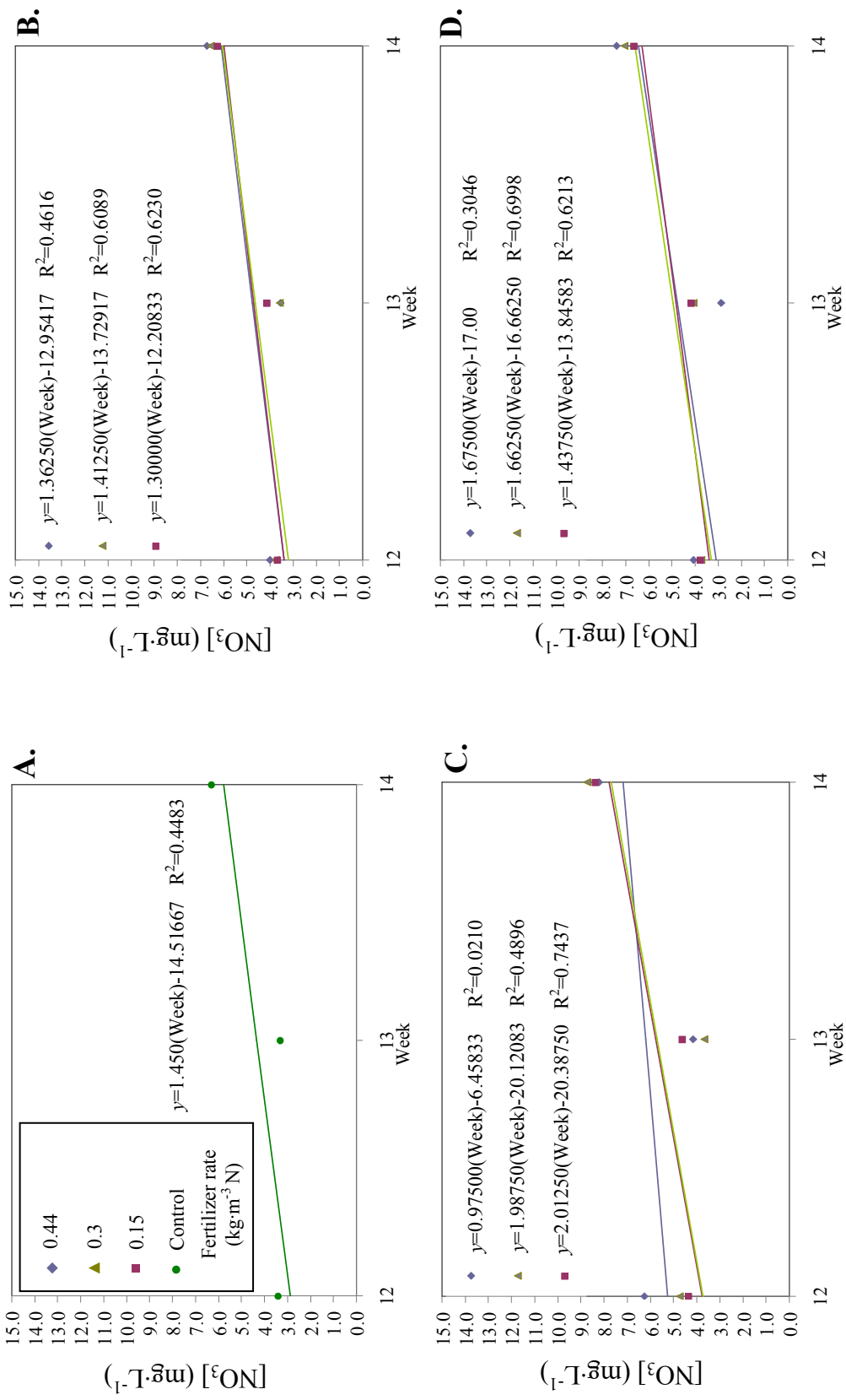


Fig. 5. Change in water NO₃ concentration for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 1 over time (3 weeks: 22 Feb. 2010 – 15 Mar. 2010) for three fertilizer rates.

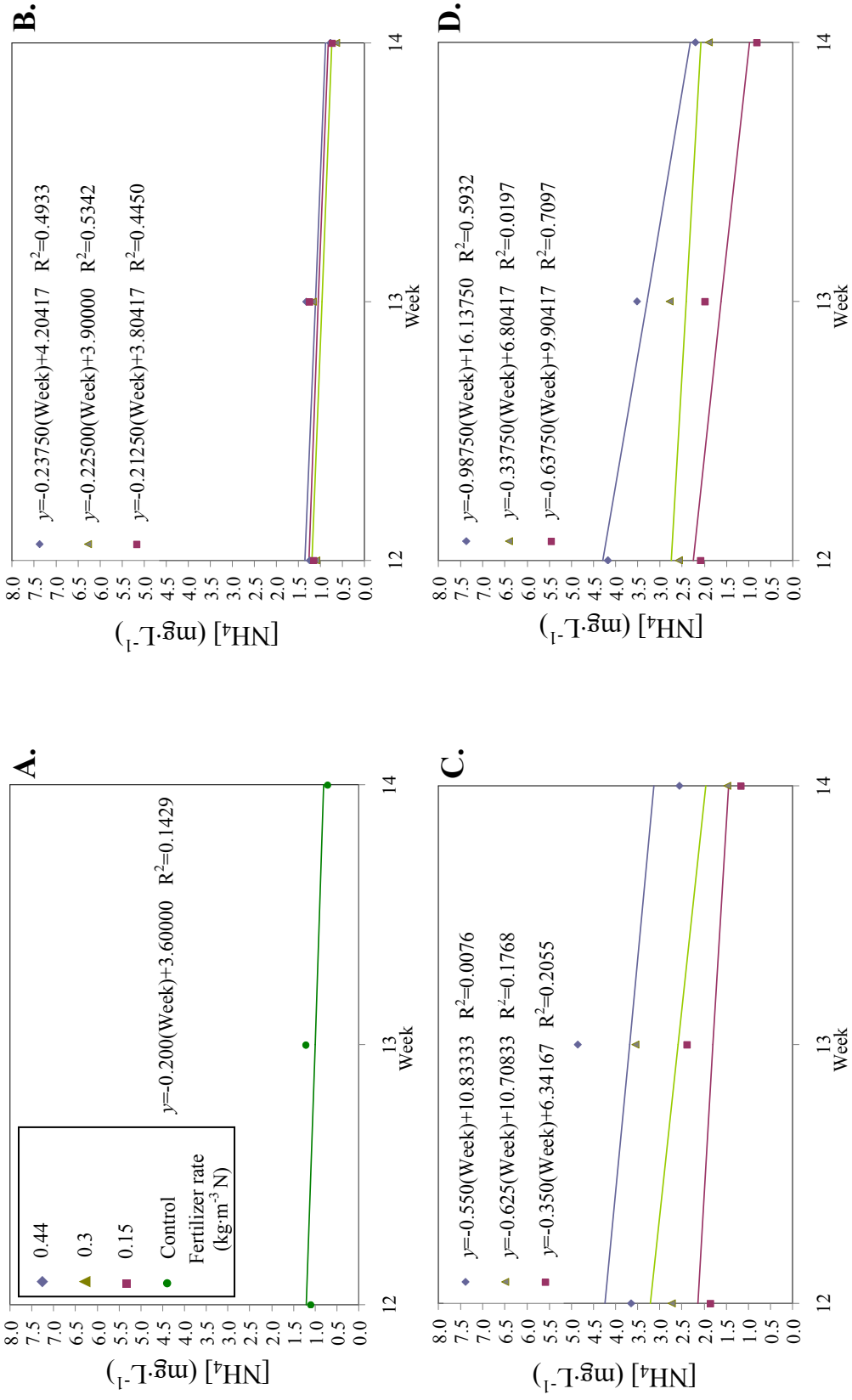


Fig. 6. Change in water NH₄ concentration for Control (zero fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 1 over time (3 weeks: 22 Feb. 2010 – 15 Mar. 2010) for three fertilizer rates.

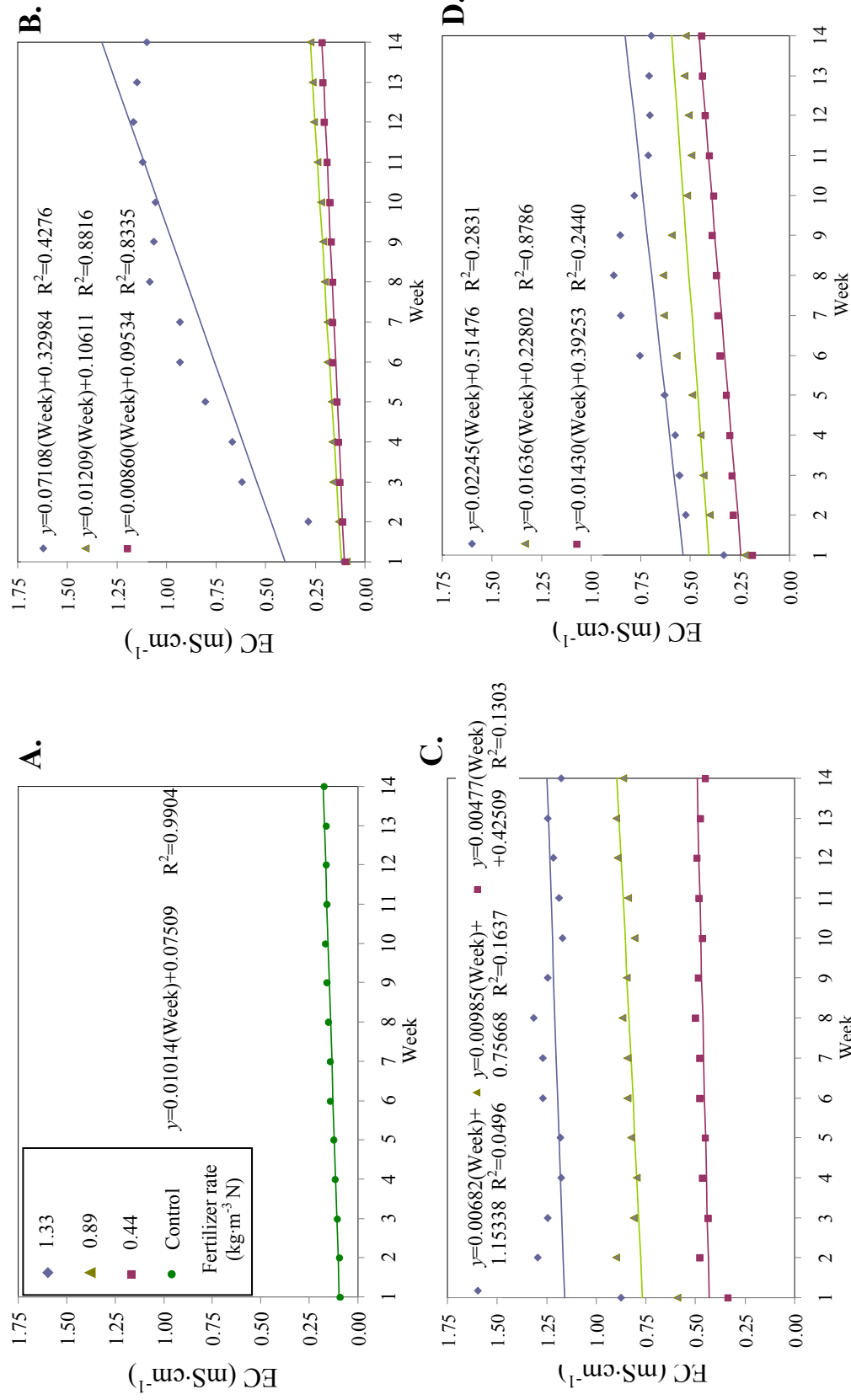


Fig. 7. Change of water EC for Control (no fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three fertilizer rates.

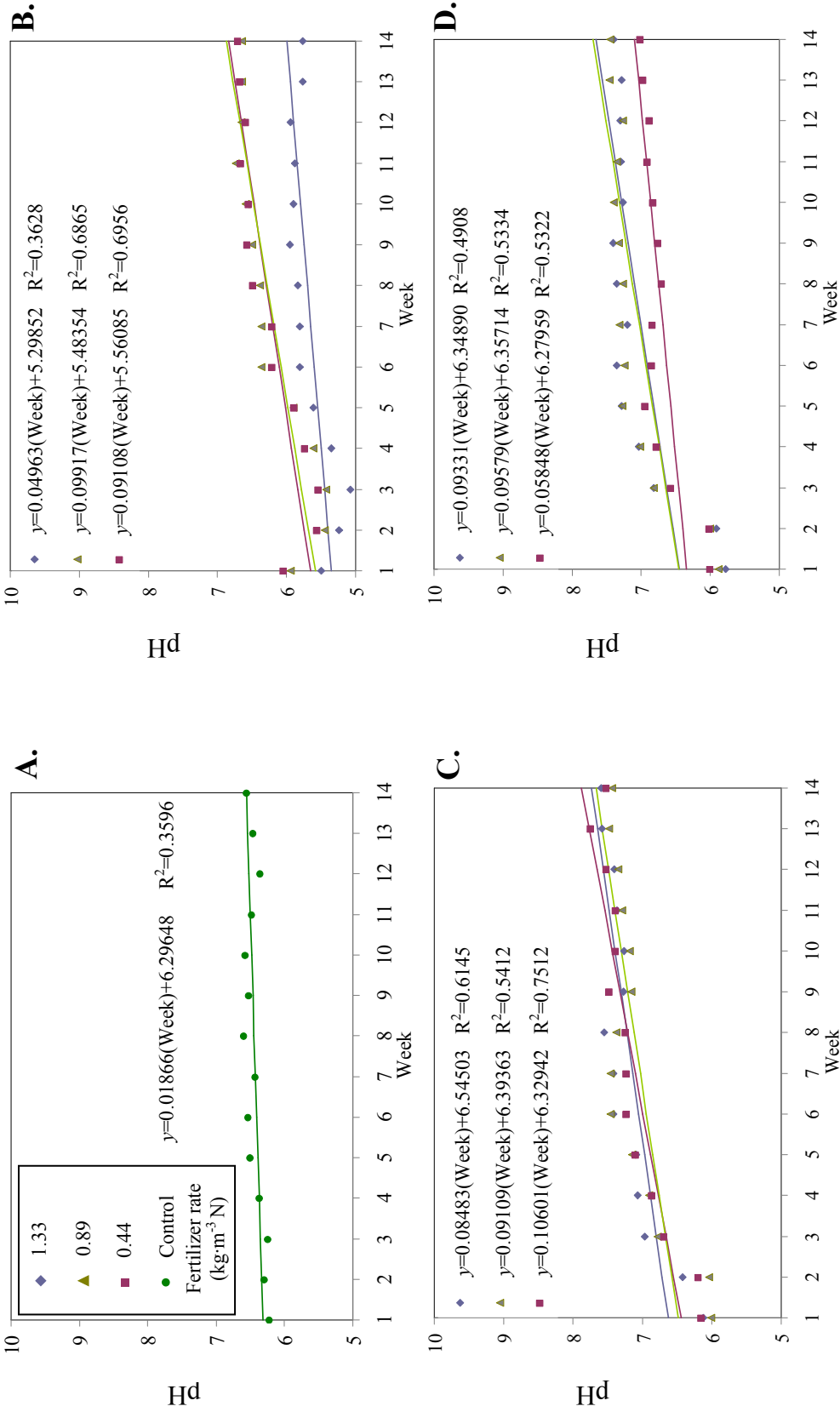


Fig. 8. Change of water pH for Control (no fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three fertilizer rates.

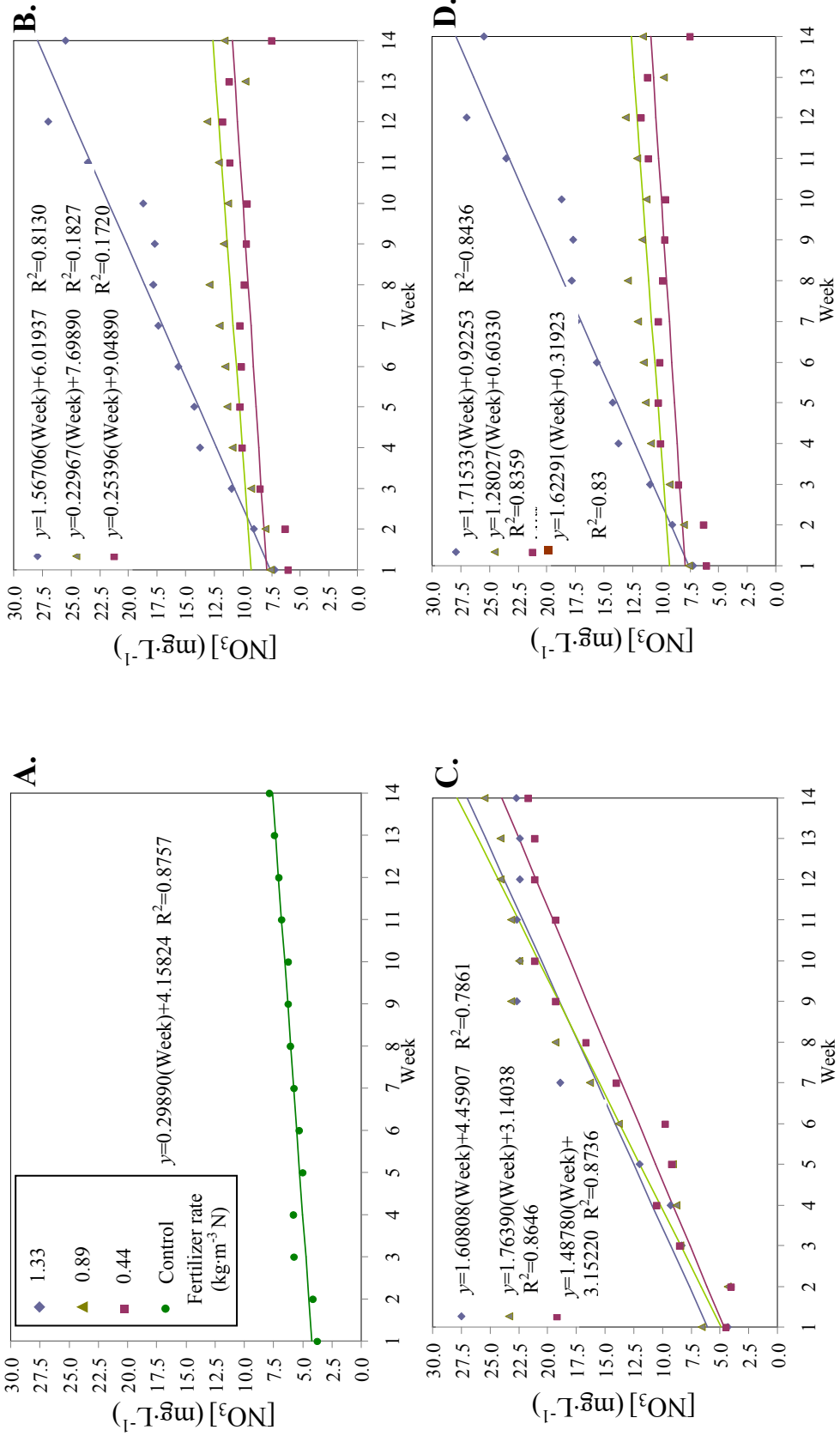


Fig. 9. Change of water NO₃ for Control (no fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three fertilizer rates.

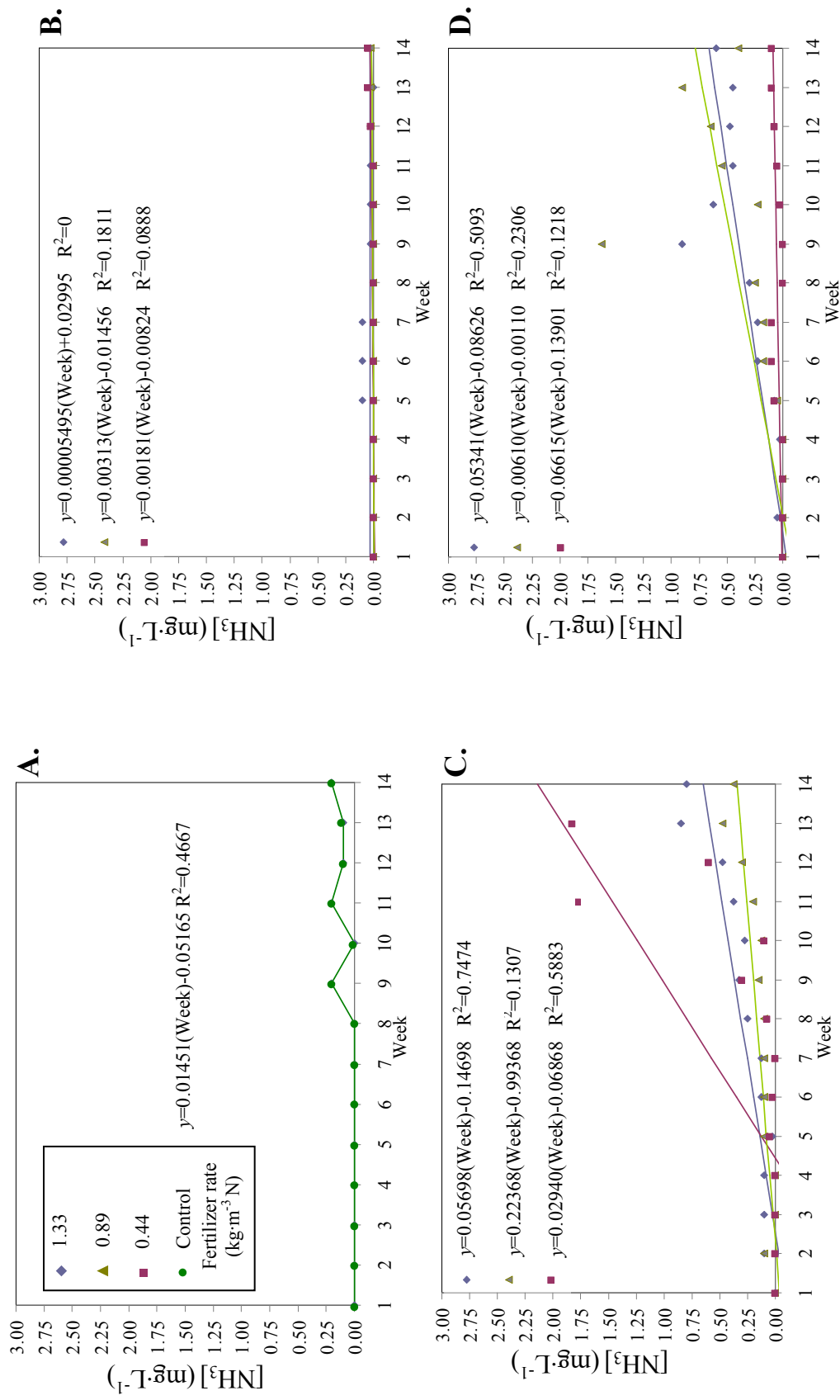


Fig. 10. Change of water NH_3 for Control (no fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three fertilizer rates.

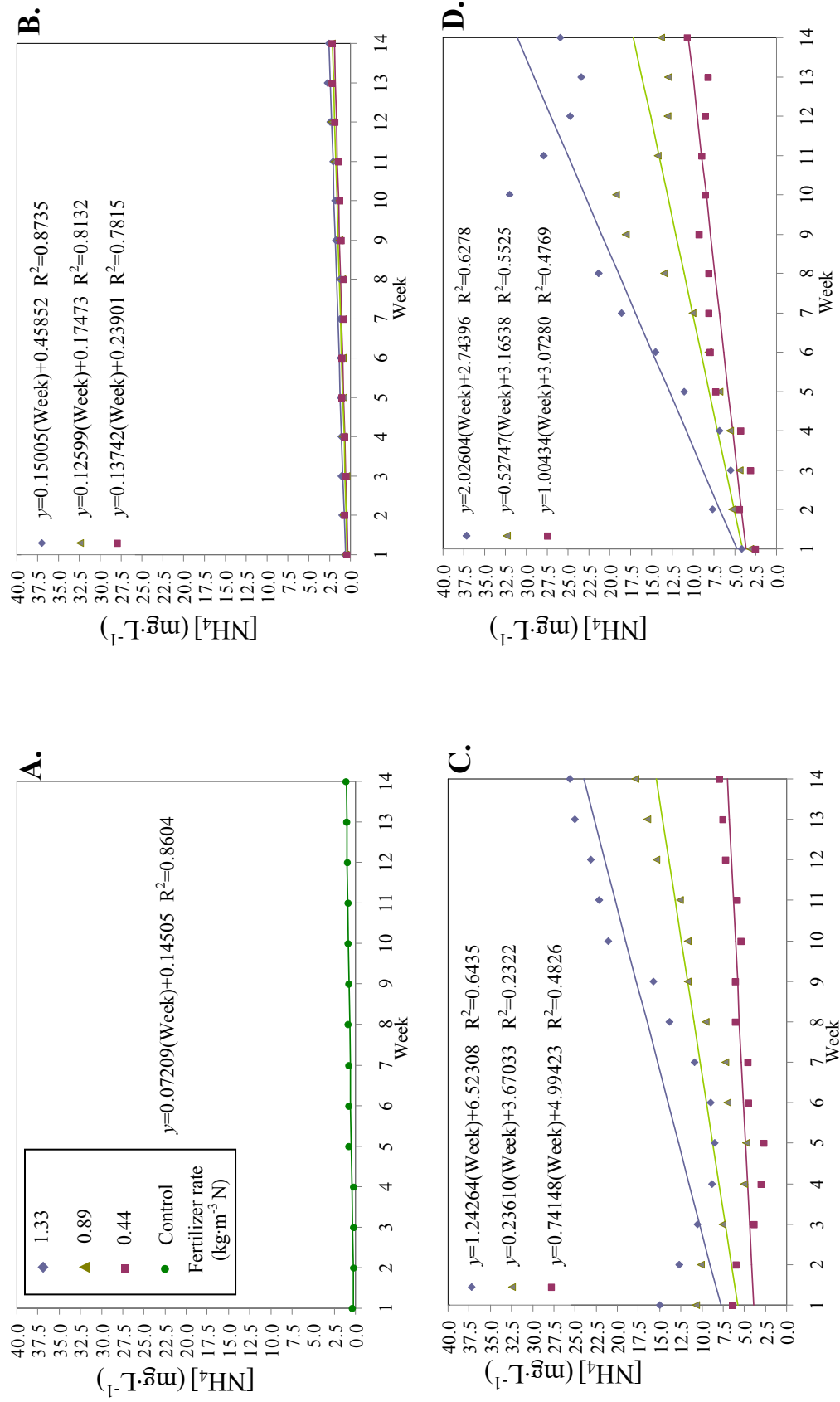


Fig. 11. Change of water NH_4 for Control (no fertilizer; n=4) (A), Conv (Harrell's Polyon 16N-2.6P-9.9K; n=4) (B), MG (Medina Granular 4N-0.9P-2.5K; n=4) (C), and NS Nature Safe 8N-2.2P-4.2K; n=4) (D) treatments during Expt. 2 over time (14 weeks: 30 Mar. 2010 – 12 July 2010) for three fertilizer rates.

CHAPTER IV

POND EXPERIMENT: FERTILIZER TYPE AFFECTS LOTUS GROWTH

Abstract

Two cultivars of lotus (*Nelumbo nucifera* ‘Hubei #5’ and ‘Space 36’) were used in this study to investigate their growth response to organic and conventional fertilizer types in 9 m³ in-ground ponds with a substrate volume of 1.5 m³ (2 yd³); consisting of a 1:1 (v:v) clay loam: pine bark substrate blend. Fertilizer treatments were applied at their general recommended rates of N per cubic yard for container production, which was 0.89 kg·m⁻³ N (1.5 lb·yd⁻³ N). Planting date was not delayed after fertilizer application in pond experiment and there were no overflow pipes or berms present. Heavy rain caused all the ponds to overflow into each other. Experimental treatments were compromised or lost; thus data were not shown. The second experiment adjusted for first experiment deficiencies by using above-ground ponds and reduced fertilizer rate of 0.6 kg·m⁻³ N (1 lb·yd⁻³ N). However, once again, toxic levels of soluble salts resulted in the required removal of substrate, fertilizer, and plants. Resetting the experiment and applying 0.4 kg·m⁻³ N (0.75 lb·yd⁻³ N) did not result in toxicity. Organic and conventional fertilizers both affected water EC, pH, and nutrient concentrations. Electrical conductivity (EC) was different among fertilizers & supported findings that EC should not exceed 0.5 – 1.0 mS·cm⁻¹.

E.V. Smith Alabama Agricultural Experiment Station

Materials and Methods

A pond experiment was conducted to compare the effect of fertilizer type (conventional or organic) on growth response of *N. nucifera* ‘Space 36’ and ‘Hubei #5’ cultivars in addition to fertilizer types effect on water EC, pH, nitrate-nitrogen (NO₃), and ammonium-nitrogen (NH₄) concentrations over one growing season. All research was conducted outside in an open field under full sun in Shorter, AL at lat. 32°N.

Experiment utilized a 1:1 (v:v) clay loam: pine bark blend. Physical properties of the field soil and nutritional analysis of individual and combined components were determined by the Soil Testing Laboratory at Auburn University (Tables 1 – 3).

Twelve (12), [9 m³ (11.7 yd³), 1.5 m (L) x 6 m (W) x 1 m (D) (5 ft x 20 ft x 3 ft)] ponds were dug in an open field during Fall 2008. Ponds were lined with landscape fabric (Cassco, Montgomery, AL) and secured with anchor pins prior to the addition of the 1 mm (45 mil) EPDM Firestone Pond Liner (AZPonds and Supplies, Reading, PA) (Fig. 1). Pond liner was secured using anchor pins. Plywood dividers 1.2 m (W) x 4.2 m (L) (4 ft x 8 ft) were installed in the middle of each pond to separate the cultivars within each pond. Ponds were filled with approximately 1.5 m³ (2 yd³) of pond substrate to an approximate depth of 0.5 m (1.5 ft) and a volume of 4250 L (1122 gal). Ponds were filled with water to be 0.5 m (1.5 ft) above the substrate, hence making their respective volume to approximately 8500 L (2244 gal). After ponds were filled with substrate and water, fertilizer treatments were applied at the general recommended rate of N for container production, 0.89 kg·m⁻³ N (1.5 lb·yd⁻³ N) and blended into substrate by hand on 5 May 2009. Three fertilizer treatments were used: Conventional controlled-release

fertilizer, Harrell's Polyon 16N-2.6P-9.9K (hereafter referred to as Conv) (Harrell's LLC, Lakeland, FL), Medina Growin' Green Granular Organic 4N-0.9P-2.5K (hereafter referred to as MG) (Hondo, TX), and Nature Safe 8N-2.2P-4.2K (hereafter referred to as NS) (Griffin Industries, Coldspring, KY).

The experimental design was a split-plot design with two *N. nucifera* cultivars grown in one pond (i.e. one pond = one whole plot). There were four blocks (four replicates) with three whole plots (ponds) each, for a total of twelve (12) ponds. Each of the three fertilizer treatments were randomly assigned to one whole plot (pond) within a block. Each whole plot (pond) was divided into two subplots (i.e. pond half = subplot for each cultivar). Each cultivar was randomly assigned to one subplot (pond half) within each whole plot (i.e. each cultivar was placed on either side of the divider within one pond).

Two to three node lotus rhizome-propagules of 'Space 36' and 'Hubei #5' were divided from stock plants with young leaves, and planted into ponds on 6 May 2009. Three lotus rhizomes per cultivar were planted per pond half (pond half = ~1.5 m x 3 m x 1 m). Experiment was conducted from 6 May 2009 – 13 Nov. 2009.

Water and substrate samples were collected from each individual pond one week prior to fertilizer application (29 Apr. 2009), one week after fertilizer application (13 May 2009) and at the end of experiment (13 Nov. 2009) and analyzed using ICAP for NO₃ and NH₄ determination. One (1) Nalgene[®] narrow mouth bottle (4 oz, 125 mL) was dipped into center of each pond to collect water samples, capped, and sent directly to lab for analysis (Consolidated Plastics Company, Inc., Stow, OH). Researchers used a 0.2 L

cup (8 oz) and collected substrate samples from each pond half, placing them into plastic bags to be sent to lab for same ICAP analyses.

Results and Discussion

On 7 May 2009, the day after planting and two days after fertilizer application, Shorter, AL received 15 cm – 23 cm (6 – 9 in) of rain, which caused ponds and fertilizer to overflow into each other, due to the lack of berms or overflow stand pipes. Six out of twelve ponds went out of production. Chellemi and Lazaorvits (2002) stated that phytotoxic and negative growth effects could be avoided by delaying planting date, which was applied in subsequent experiments.

Soil analysis revealed much variation due to the fertilizer treatments overflowing into each other during the heavy rain (Table 6a). Whereas soil analyses showed great variation, water analyses illustrated that most elements were within acceptable limits for production (Table 6b) with acceptable nutrient ranges noted in Table 6c.

Although dividers were installed, ‘Space 36’ proved to be very aggressive and overtook all actively growing ponds by growing over, under, and around the dividers; hence integrity of cultivar treatments was compromised.

On 13 Nov. 2009, only five out of the twelve ponds were harvestable. All four reps of Conv treatments and one MG treatment were harvested. None of the other ponds contained harvestable rhizomes.

Rhizomes and runners were harvested from the substrate by hand and rinsed with irrigation water. All leaves, rhizomes, and runners were bagged separately. Rhizome and runner fresh weight were recorded separately by cultivar. Dry weight of vegetation was recorded.

Lack of berms around the ponds or overflow stand pipes resulted in fertilizer overflowing banks and mingling into adjacent ponds. Total rhizome harvest weight from Conv ponds was 136 kg (300 lbs) which covered 0.004 ha (0.01 acres = 500 ft²); overall yield was 292 kg·ha⁻¹.

Above-Ground Pond Experiment in Auburn, AL

Materials and Methods

An above-ground pond experiment was conducted to compare the effect of fertilizer type (conventional or organic) on growth response of *N. nucifera* ‘Space 36’ and ‘E 2’ cultivars in addition to fertilizer types effect on water EC, pH, nitrate-nitrogen (NO₃), and ammonium-nitrogen (NH₄) concentrations over one growing season. All research was conducted outside, in an open field under full sun in Auburn, AL at lat. 32°N.

Experiment utilized a 1:1 (v:v) clay loam: pine bark blend. Physical properties of the field soil and nutritional analysis of individual and combined components were determined by the Soil Testing Laboratory at Auburn University (Tables 1 – 3).

On 5 Apr. 2010, twelve (12), round blue poly stock ponds [3785 L (1000 gal), 2.7 m (diameter) x 0.7 m (depth) (9 ft x 2.3 ft)] were placed on top of landscape fabric (Cassco, Montgomery, AL) in an open field with 1 m (3 ft) spacing between each pond. Landscape fabric was secured with anchor pins (Cassco, Montgomery, AL). Ponds were modified using basic schedule 40 PVC plumbing parts to include an overflow pipe (Fig. 2). Ponds came with a 3.8 cm (1.5 in) hole already molded for drainage 7.6 cm (3 in) from tank bottom. Prior to addition of substrate, the slip socket end of a 3.8 cm x 1.27 cm (1.5 in x 0.5 in) bushing was cemented into the 3.8 cm (1.5 in) hole using PVC primer

and cement (Oatey, OH). A 15.2 cm (6 in) section of 1.27 cm (0.5 in) PVC pipe was primed and cemented to bushing on the inside of tank. One end of 1.27 cm (0.5 in) elbow was cemented to the end of the 15.2 cm (6 in) long piece of 1.27 cm (0.5 in) pipe inside the tank. A 30.5 cm (12 in) section of 1.27 cm (0.5 in) pipe was cemented to other end of elbow as shown in Fig. 2. In addition, plywood dividers were installed to each tank half to separate cultivars.

Ponds were filled with 0.76 m³ (~1 yd³) of pond substrate to an approximate depth of 15.2 cm (6 in) and a volume of 901 L (238 gal) on 13 Apr. 2010. After ponds were filled with substrate, fertilizer treatments were applied a week later (20 Apr. 2010) at the rate of 0.6 kg·m⁻³ N (1 lb·yd⁻³ N) which was based on greenhouse evaluations of fertilizer rates (Chapter 3) and blended in by hand. Three fertilizer treatments were used: Conventional controlled-release fertilizer, Harrell's Polyon 16N-2.6P-9.9K (hereafter referred to as Conv) (Harrell's LLC, Lakeland, FL), Medina Growin' Green Granular Organic 4N-0.9P-2.5K (hereafter referred to as MG) (Hondo, TX), and Nature Safe 8N-2.2P-4.2K (hereafter referred to as NS) (Griffin Industries, Coldspring, KY).

Water depth was marked at 15.2 cm (6 in) above substrate line to maintain water depth. Installation of overflow pipes helped maintain maximum water depth. Ponds were filled with water (pH 6.88, EC = 0.10 mS·cm⁻¹) to an approximate volume of 1802 L (476 gal). Water volume was evaluated every other day, and water was added as needed to maintain depths.

The experimental design was a split-plot design with two *N. nucifera* cultivars grown in one pond (i.e. one pond = one whole plot). There were four blocks (four replicates) with three whole plots (ponds) each, for a total of twelve (12) ponds. Each of

the three fertilizer treatments were randomly assigned to one whole plot (pond) within a block. Each whole plot (pond) was divided into two subplots (i.e. pond half = subplot for each cultivar). Each cultivar was randomly assigned to one subplot (pond half) within each whole plot (i.e. each cultivar was placed on either side of the divider within one pond).

Two to three node lotus rhizome-propagules of 'Space 36' and 'E 2' were divided from stock plants with young leaves, and planted into ponds on 27 Apr. 2010. Two lotus rhizomes per cultivar were planted per pond half.

Although planting date was delayed one week after fertilizer application, plants displayed soluble salt (SS) toxicity symptoms with yellowing and stunted new leaves with lack of vigor, digressing to total decline and death. Five rhizomes in MG and NS and three in Conv died and were removed and replaced on 20 May 2010. These replacement plants failed to thrive, had low vigor and died as well. Water EC indicated higher levels than results from previous greenhouse studies predicted (Chapter 3). Water EC levels were higher among MG and NS ($2.1 \text{ mS}\cdot\text{cm}^{-1}$ and $1.2 \text{ mS}\cdot\text{cm}^{-1}$, respectively) which exceeds Tian (2008) recommendations of $0.5 \text{ mS}\cdot\text{cm}^{-1}$.

On 4 June 2010, plant debris, substrate, and plywood dividers were removed from ponds. On 7 June 2010, new pond substrate was added to each pond as described above with a $0.44 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ ($0.75 \text{ lb}\cdot\text{yd}^{-3} \text{ N}$) rate of respective fertilizer treatments to each pond since the $0.6 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ ($1 \text{ lb}\cdot\text{yd}^{-3} \text{ N}$) rate resulted in toxic levels of soluble salts. On 11 June 2010, two to three node lotus rhizome-propagules of 'E 2' were divided from stock plants with young leaves, and planted into ponds. One lotus rhizome was planted per pond. The experimental design was a completely randomized design with four replicates.

Each replicate contained one of three fertilizer treatments, for a total of twelve ponds. Experiment was conducted from 5 Apr. – 8 Oct. 2010.

On 7 Oct. 2010, all ponds were drained by cutting the overflow pipes at the substrate line and removed. Leaves and petioles were also removed at the substrate line. Lotus rhizomes and runners were harvested on 8 Oct. 2010. Rhizomes and runners were rinsed with water, separated and weighed immediately for fresh weights.

Water pH, EC, nitrate-nitrogen (NO_3), and ammonium-nitrogen (NH_4) were measured one week after fertilizer application and then weekly for duration of both experiments by inserting the YSI Professional Plus meter (YSI Inc., Yellow Springs, OH) into the center of each pond. Additional water samples were collected from each individual pond one week after fertilizer application (11 June 2010) and at termination (8 Oct. 2010) and analyzed using ICAP for NO_3 and NH_4 determination. One (1) Nalgene[®] narrow mouth bottle (4 oz, 125 mL) was dipped into center of each individual tub to collect these water samples, capped, and sent directly to lab for analysis (Consolidated Plastics Company, Inc., Stow, OH). Samples were analyzed using ICAP for NO_3 and NH_4 determination. Researchers used a 0.2 L cup (8 oz) and collected substrate samples from each pond, placing them into plastic bags to be sent to lab for same ICAP analyses.

Data were analyzed as a complete randomized design using PROC MIXED by SAS 9.2 (SAS Institute, Inc., Cary, NC). Means were separated utilizing CONTRAST statements at $\alpha = 0.05$ (5%). Unless otherwise stated, all data were analyzed at the 5% level.

Results and Discussion

As Tian (2008) found, EC increased after fertilizer application and decreased as plants grew and nutrients were absorbed by actively growing plants. Water EC decreased linearly over time among all fertilizer treatments. Water samples revealed that the initial fertilization levels yielded EC readings of $1.2 \text{ mS}\cdot\text{cm}^{-1}$ (Week 7). This high level was identified as the cause of soluble salt (SS) toxicity and then demise; which contradicts Nguyen (2001) findings that EC of 2.8 to $3.1 \text{ mS}\cdot\text{cm}^{-1}$ was optimal for lotus growth. This experiment supported Tian (2008) findings that an EC around $0.5 \text{ mS}\cdot\text{cm}^{-1}$ is ideal and should not exceed $1.0 \text{ mS}\cdot\text{cm}^{-1}$, even for large plants (Table 5a).

Water pH increased linearly after fertilization and as nutrients were absorbed by actively growing plants, pH decreased by termination of study. Tian (2008) stated that a pH increase may be caused by algae growth, nutrient changes in water-soil system, or metabolism of plants. Lotus has the ability to tolerate a wide pH range (4.5 to 9.0), and is not affected by a range of 5.5 to 8.0 in water (Meyer, 1930). All water pH ranges were within ideal ranges for lotus growth (Table 5a).

Water temperature increased linearly from 24°C (75.2°F) in Week 7 to 32°C (89.6°F) by Week 10 and decreased to 19°C (66°F) by harvest in Week 24. Temperatures were well within range for lotus growth and consistent with Yang and others (2006) recommendations of 22 to 32°C (72 to 90°F) (Table 5a).

Water NO_3 concentration increased with lower temperatures until midway through study and decreased with higher temperatures. King and others (2007) stated that excessive levels of N can inhibit growth of submerged plants and that the concentration should not exceed 1 or $2 \text{ mg}\cdot\text{L}^{-1}$; treatment means well exceeded those

recommendations and still had sustained growth until harvest (mean average: MG 8.7 mg·L⁻¹; NS 5.8 mg·L⁻¹; Conv 9.5 mg·L⁻¹) (Table 5a). There were no differences among MG and Conv yet differences were found between the other treatments.

Consistent with Chellemi and Lazaorvits (2002), all fertilizer treatments displayed a quadratic relationship over time between NH₃ and fertilizer type. Although NS contained the lowest amount of NH₃ within the nutritional analysis of fertilizer treatments (Table 3), NS displayed a higher NH₃ concentration (5.35 mg·L⁻¹) (Table 5a). According to the fertilizer analysis, Conv had the highest NH₃ concentration (Table 3), yet as a controlled release fertilizer, the release was far slower than NS which explained its overall mean of 0.93 mg·L⁻¹ (Table 5a). MG was similar to Conv and there were differences between NS and the other two treatments for NH₃ (Table 5a).

Water NH₄ concentration increased slightly in the middle of the experiment perhaps due to the conversion of organic-N to NH₄ via ammonification process as described by Tiquia and Tam (2000). Typically, low temperatures and pH result in higher rates of net ammonification rather than net nitrification (Britto and Kronzucker, 2002), yet that was not seen in this experiment. Although there were higher NH₄ concentrations found in water, temperature and pH means were uniform and not low. Conv was significantly lower (4.48 mg·L⁻¹) (Table 5a).

According to ICAP water analysis, there were no differences from one week after fertilizer application, mid-way through study, or termination nor among fertilizer types for P, B, Cu, Mn, Fe, Zn, and trace elements Al, As, Cd, Cr, Pb, and Ni (data not shown). There were differences over time and no difference among fertilizer types for Ca, Mg, NH₄, and NO₃ (Table 6b and 6c). Those same macronutrients along with K, Na, EC, and

SS had higher levels mid-way through study than at termination; suggesting that the nutrients were absorbed into plant tissue (Table 8). There were differences among all fertilizer treatments for K, Na, EC, and SS for water analyzed mid-way through study (Table 6b). EC decreased from $0.82 \text{ mS}\cdot\text{cm}^{-1}$ mid-way through study in MG to $0.34 \text{ mS}\cdot\text{cm}^{-1}$ by termination; EC decreased as plants grew and nutrients were absorbed, which supports Tian (2008) findings.

Substrate analysis indicated that there were no differences from the initial (pre-fertilizer) sample to termination nor among fertilizer types for Ca (range 39.3 – 76.8 ppm), K (20 - 30 ppm), and P (range 0.58 - 5.6 ppm) (Table 7a), nor for Cu (range 0.13 - 0.38 ppm) or Zn (range 0.13 - 0.18 ppm) (Table 7a). EC increased from initial sample 0.21 to $0.7 \text{ mS}\cdot\text{cm}^{-1}$ by termination (Table 7b and 7c). Magnesium (Mg), Na, SS, and pH increased from pre-fertilizer sample to termination whereas N, Al, and Fe decreased.

Plant tissue analysis revealed high levels of micronutrients B, Fe, Mn, Na, Zn and trace elements Cr, Cu, and Ni compared to water and substrate analysis (Table 8). Although lotus was generally susceptible to high salt levels, Na absorption was substantial within plant tissue: Range 676 (Conv); 721 (NS); 733 (MG) (Table 8). Generally, Ni, Cr, Zn, and Mn increase P concentration within plant tissue, yet high Al can decrease the uptake of P; which may explain why the percentage of P is not high. Chromium (Cr), Cu, and Ni can be associated with reduced N concentrations within plant tissue (N range 3.6 -3.9%). Chromium (Cr), Cu, Mn, Ni, and Zn excess can induce Fe deficiencies yet Fe levels were quite high within plant tissue in comparison to water and substrate analysis (Tables 6 – 7b). The findings are suggestive of trace element toxicity, however, the visual appearance of plants grown were healthy as shown in Fig. 3. An

unpublished study found that lotus is able to hyper-accumulate many nutrients well over that of most plants (W. Orozco, unpublished data).

There were no differences across fertilizers for rhizome and runner fresh weight (rhizome average: 14.1 kg; runner average: 7.1 kg) (Table 5b). These findings support Chellemi and Lazaorvits (2002) whom observed vegetable plant health to be similar between organic and conventional fertilizers. Adegbidi and others (2003) found that utilization of organic materials increased biomass production and contributed to the sustainability of production systems.

It was through utilizing $0.89 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ in the E.V. Smith study that led to conduct fertilizer rate experiments to determine a $\text{kg}\cdot\text{m}^{-3} \text{ N}$ rate suitable for lotus growth within Tian's (2008) EC recommendations. Using $0.89 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ in the E.V. Smith study led to the demise of most of the lotus ponds. Since $0.89 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ proved to be too much, researchers determined a $0.6 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ ($1 \text{ lb}\cdot\text{yd}^{-3} \text{ N}$) rate would be low enough to grow lotus outdoors in a pond. Unfortunately, for reasons yet discovered, the $0.6 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ rate proved to be too high. This led to the removal of substrate and fertilizer in order to begin anew; this time with $0.44 \text{ kg}\cdot\text{m}^{-3} \text{ N}$ ($0.75 \text{ lb}\cdot\text{yd}^{-3} \text{ N}$). Considering that previous experiments were in a glass greenhouse with a sunscreen shade (Chapter 2 and 3), researchers question if sunlight and subsequent increased water temperatures and controlled higher night temperatures had anything to do with the increased water EC levels. More research needs to be conducted to determine fertilizer release rates and environmental variable interaction and levels on cause and effect of water EC level variability in outdoor ponds. This information is important to provide target best

management practices fertilizer recommendations to maximize growth and production of lotus while minimizing environmental impacts.

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Fig. 1. Photo illustration of pond layout at E.V. Smith for in-ground pond experiment.



Fig. 2. Photo depicting tank fitted with overflow pipe for above-ground pond experiment.



Fig. 3. Photo illustration from Aug. 2010 of 'E 2' lotus plants for above-ground pond experiment.



Table 1. Fertilizer types and contents for both experiments.

Fertilizer type	Fertilizer treatments	Fertilizer contents
Conventional	Harrell's Polyon 16N-2.6P-9.9K	All Polymer Coated: Ammonium Nitrate Copper Sulfate EDTA Iron Chelate Magnesium Sulfate Manganese Sulfate Mono-Ammonium Phosphate Sodium Molybdate Sulfate of Potash Zinc Sulfate
Organic	Medina Granular 4N-0.9P-2.5K	Kelp meal, humate, pasteurized poultry manure, molasses, and greensand
	Nature Safe 8N-2.2P-4.2K	Meat meal, hydrolyzed feather meal, bone meal, blood meal & sulfate of potash

Table 2. Nutritional analysis of substrates.^z Units are mg·L⁻¹ with exception of pH and electrical conductivity.

	Clay loam	Pine bark
pH	5.6	4.3
Electrical conductivity (EC) (mS·cm ⁻¹)	0.15	0.57
Phosphorus (P)	2	3
Potassium (K)	17	41
Magnesium (Mg)	34	77
Calcium (Ca)	89.7	215
Copper (Cu)	0	0
Iron (Fe)	45	41
Manganese (Mn)	12	14
Zinc (Zn)	0	1
Boron (B)	1	1
Aluminum (Al)	38	41
Sodium (Na)	15	17
Nitrate-Nitrogen (NO ₃)	2	3
Ammonium-Nitrogen (NH ₄)	<0.1	3

^z Analyzed using ICAP for determination.

Table 3. Nutritional analysis of fertilizers.^z Units are mg·L⁻¹ with exception of pH and electrical conductivity.

	Medina granular organic 4N-0.9P-2.5K (MG)	Nature safe 8N-2.2P-4.2K (NS)	Harrell's polyon 16N-2.6P-9.9K (Conv)
pH	6.4	6.2	-- ^y
Electrical conductivity (EC) (mS·cm ⁻¹)	30	31	--
Soluble salts (SS)	20832	21700	--
Phosphorus (P)	1031	489	6000
Potassium (K)	10616	12266	12000
Magnesium (Mg)	3049	1086	14000
Calcium (Ca)	765	486	--
Copper (Cu)	83	1.3	790
Iron (Fe)	22.7	<0.1	3300
Manganese (Mn)	12	0.6	1300
Zinc (Zn)	21	<0.1	790
Boron (B)	13	2.7	--
Aluminum (Al)	3.2	4.3	--
Sodium (Na)	6985	2991	--
Nitrate-Nitrogen (NO ₃)	301	66	72000
Ammonium-Nitrogen (NH ₄)	1799	142	88000

^z Analyzed using ICAP for determination.

^y – Indicates information not available according to label.

Table 4a. Range fluctuation in substrate EC, pH, and nutrient concentrations in substrate samples from E.V. Smith study which utilized 0.89 kg·m⁻³ N (1.5 lb·yd⁻³ N). Macronutrient units are in mg·L⁻¹.

Treatment	EC mS·cm ⁻¹	pH	NH ₄	P	K	Mg	Ca
Conv	1.12 – 1.86	5.5 – 6.8	1.8 – 30	3.5 – 139	262.5 – 621.5	135.5 – 414	491 – 1755
MG	0.5 – 1	5.3 – 6.1	0.6 – 5	2 – 177.5	207 – 423.5	130.5 – 196	455 – 886
NS	0.5 – 1	5.1 – 7.3	8.1 – 31	11 – 138	137.5 – 423.5	100 – 279	400 – 1845.5

Table 4b. Range fluctuation in water EC, pH, and nutrient concentrations in water samples from E.V. Smith study which utilized 0.89 kg·m⁻³ N (1.5 lb·yd⁻³ N). Macronutrient units are in mg·L⁻¹.

Treatment	EC mS·cm ⁻¹	pH	NH ₄	P	K	Mg	Ca
Conv	0.4 – 0.5	7.4 – 8.1	0.4 – 5.9	0.3 – 0.7	43 – 79	28 – 58	26 – 51
MG	0.6 – 0.8	7.4 – 7.7	5.7 – 26.3	0.1 – 22.5	41 – 78	30 – 79	26 – 177
NS	0.5 – 0.6	7.5 – 8	14.4 – 34.6	0.1 – 0.6	53 – 77	27 – 53	25 – 49

Table 4c. Acceptable water pH, EC, and nutrient concentration range for lotus production.^z Units are in mg·L⁻¹.

EC mS·cm ⁻¹	pH	P	K	Mg	Ca
0.5 – 1	4.5 – 9	10 – 15	30 – 50	15 – 20	20 – 40

^z (Tian, 2008; Yeager et al., 2007)

Table 5a. Effect of fertilizer rate 0.44 kg·m⁻³ N (0.75 lb·yd⁻³ N) and type on water EC, pH, temperature, NO₃, NH₃, NH₄ over time.

Treatment	EC mS·cm ⁻¹	pH	Temp°C	NO ₃ mg·L ⁻¹	NH ₃ mg·L ⁻¹	NH ₄ mg·L ⁻¹
MG	0.71a ^z	7.55b	27.1ab	8.77a	1.93b	9.04a
NS	0.49b	7.75a	27b	5.82b	5.35a	9.61a
Conv	0.38c	7.67ab	27.3a	9.51a	0.93b	4.48b

^z Lowercase letters denote mean separation at p<0.05 (Sas Institute, 2004).

Table 5b. Mean lotus rhizome and runner fresh weights. Units are in kg.

Treatment	Rhizome	Runner
MG	15.2 ^z	9.5
NS	12.6	7.1
Conv	14.5	4.5

^z No differences in means found at p<0.05 (Sas Institute, 2004).

Table 6a. Effect of fertilizer type and rate 0.44 kg·m⁻³ N (0.75 lb·yd⁻³ N) on water nutrient concentration from first water analysis (11 June 2010). Units are in mg·L⁻¹.

Treatment	Macronutrients						Micronutrients						Trace elements					
	NH ₄	NO ₃	Ca	K	Mg	P	B	Cu	Fe	Mn	Na	Zn	Al	As	Cd	Cr	Ni	Pb
MG	16	0.8	34	62	37	2	0.1	0.2	0.7	0.3	70	0.1	0.1	0.1	0.1	0.1	0.1	0.1
NS	4	0.4	38	55	31	0	0.1	0.1	0.4	42	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
Conv	3.5	0.9	16	24	21	0	0.1	1.1	0.1	33	0.1	0.1	1.6	0.1	0.1	0.2	0.1	0.1

Table 6b. Effect of fertilizer type and rate 0.44 kg·m⁻³ N (0.75 lb·yd⁻³ N) on water nutrient concentration from mid-way through study (11 Aug. 2010). Units are in mg·L⁻¹ with exception of EC and pH.

Treatment	Macronutrients						Micronutrient			EC		
	NH ₄	NO ₃	Ca	K	Mg		Na		(mS·cm ⁻¹)	SS	pH	
MG	16.1	0.5	34.8	93.3a ^z	63.3		60a		0.82a	570.8a	7.9	
NS	3.5	0.4	29.8	61ab	49.3		20.3b		0.46b	321b	8.0	
Conv	3.6	0.9	30.5	29.3b	59.5		13b		0.37b	257b	7.8	

^z Lowercase letters denote mean separation at p<0.05 (Sas Institute, 2004); if no differences then letters are omitted.

Table 6c. Effect of fertilizer type and rate 0.44 kg·m⁻³ N (0.75 lb·yd⁻³ N) on water nutrient concentration from sample collected at termination (8 Oct. 2010). Units are in mg·L⁻¹ with exception of EC and pH.

Treatment	Macronutrients						Micronutrient			EC		
	NH ₄	NO ₃	Ca	K	Mg		Na		(mS·cm ⁻¹)	SS	pH	
MG	0.8	0	21	14.3	23.5		31.5a ^z		0.34	239.3	7.9	
NS	0	0	23.5	14	21		20b		0.31	219.3	7.9	
Conv	0	0	21	21	21		20.3b		0.35	243.5	8	

^z Lowercase letters denote mean separation at p<0.05 (Sas Institute, 2004); if no differences then letters are omitted.

Table 7a. Effect of fertilizer type and rate 0.44 kg·m⁻³ N (0.75 lb·yd⁻³ N) on substrate nutrient concentration between initial and final sample. There were no differences over time (pre-fert versus termination) for means presented. Units are in mg·L⁻¹.

Treatment	Macronutrients				Micronutrients			
	Ca	K	P	Zn	Cu	Zn		
MG	39.3	20	0.58	0.13	0.25			
NS	76.8	24.8	5.6	0.18	0.38			
Conv	61.8	30	0.6	0.13	0.13			

Table 7b. Nutritional analysis of initial (pre-fert) substrate sample. Units are in mg·L⁻¹ with exception of EC and pH.

Treatment	Macronutrients				Micronutrients				EC		
	NH ₄	NO ₃	Mg	Fe	Mn	Na	Al	AI			
Pre-fert sample	2	2	29	18.3	1	14	40	40	0.21	148	5.1

Table 7c. Effect of fertilizer type and rate 0.44 kg·m⁻³ N (0.75 lb·yd⁻³ N) on substrate nutrient concentration from sample collected at termination (8 Oct. 2010). Units are in mg·L⁻¹ with exception of EC and pH.

Treatment	Macronutrients				Micronutrients				EC		
	NH ₄	NO ₃	Mg	Fe	Mn	Na	Al	AI			
MG	0.08	0.18	68.8	1.9	0.1	44.5a ^z	3.6	3.6	0.7	486	6.6
NS	0.3	0.13	72.5	0.8	0.2	32.8b	1.6	1.6	0.7	495	6.7
Conv	0.08	0.1	82.8	0.4	0.1	27.3b	0.8	0.8	0.6	465	6.6

^z Lowercase letters denote mean separation at p<0.05 (Sas Institute, 2004); if no differences then letters are omitted.

Table 8. Effect of fertilizers on nutrition in newly expanded leaves of 'E 2' harvested August 2010. Units are in mg·L⁻¹ except where otherwise noted.

Fertilizer	Macronutrients				Micronutrients				Trace elements								
	N (%)	Ca (%)	K (%)	Mg (%)	P (%)	B	Cu	Fe	Mn	Zn	Al	As	Cd	Cr	Na	Ni	Pb
MG	3.9	0.3	3.7a ^z	0.6	0.5a	52	42	92	137	52.8	24	0.1	0.1	10	734	83	0.1
NS	3.8	0.5	3.4b	0.6	0.4b	52	25	87	113	39.7	30	0.1	0.1	8	721	62	0.1
Conv	3.6	0.4	3.5ab	0.6	0.4ab	47	19	71	129	49.5	21	0.1	0.1	5	676	43	0.1

^z Lowercase letters denote mean separation at p<0.05 (Sas Institute, 2004); if no differences then letters are omitted.