# NITRATE-NITROGEN SUFFICIENCY RANGES IN LEAF PETIOLE SAP OF PAC CHOI GROWN WITH ORGANIC AND CONVENTIONAL FERTILIZERS

By

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# Abstract

Petiole sap nitrate-nitrogen ($NO_3$-N) analysis with handheld meters is a valuable tool in applying in-season nitrogen (N) for many crops. Sufficiency levels have been determined for several leafy green crops, including lettuce (*Lactuca sativa* L.) and cabbage (*Brassica oleracea* L.), but not for pac choi (*Brassica rapa* L.). The response of pac choi to different fertilizer rates and sources [conventional and organic] has established optimal soluble N application rates and Cardy meter sufficiency ranges.

Greenhouse experiments were conducted during summer and fall of 2008 in Manhattan, KS. Conventional soluble fertilizer was formulated from inorganic salts with a 4 $NO_3$-N: 1 ammonium ratio. Phosphorus was held at 1.72mM and K at 0.83mM for all treatment levels. The organic soluble fertilizer, fish hydrolyzate (2N:1.72P:0.83K), was diluted to provide the same N levels as with conventional treatments. Both fertilizers were applied at rates of 0, 32, 75, 150, 225, 300, and 450 mg $\cdot$ $L^{-1}$. Seedlings were transplanted and fertilizer application began at 18 days. Plants were harvested at seven weeks (five weeks post transplanting) after receiving 15 fertilizer applications during production. Samples of the most recently matured leaves were harvested weekly and analyzed for petiole sap $NO_3$-N and leaf blade total N concentration. Leaf count, leaf length, and chlorophyll content were also measured weekly. Fresh and dry weights were determined on whole shoots and roots. Optimum yield was achieved at the 150 mg. $L^{-1}$ fertility rate with both conventional and organic fertilizers.

Field and high tunnel experiments were conducted during fall 2008 to validate the sufficiency ranges obtained from the greenhouse studies. Based on field and high tunnel results, sufficiency levels of $NO_3$-N for pac choi petiole sap during weeks 2 to 3 of production were 800-

1500 mg. $L^{-1}$, and then dropped to 600-1000 mg. $L^{-1}$ during weeks 4 through harvest for both conventional and organic fertilizers sources. These ranges could vary based on the variety of the crop, the fertility of soil, and certain environmental factors such as photoperiod, light intensity. However, we found that petiole sap nitrate always increased to the point associated with the maximum biomass, followed by a plateau where sap nitrate remained constant. This characteristic of the cardy meter can provide the growers with a practical methodology to generate their standard curves under specific conditions to guide in-season N applications.

Total N in leaf tissue showed fewer fertilizer rate effects than petiole sap $NO_3$-N. Chlorophyll content was not useful in evaluating pac choi N status.

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May

# Dedication

In the mist of high demanding part of our daily life, I see my children spirit encouraging me to beyond the ordinary to make the best of my capabilities.

To my children Ranim, Ibrahim, Jenna and Mustafa,
To my beloved husband Mohamad.
I love you.

# Chapter 1 - Introduction

One goal of most horticulture production systems is to achieve healthy growth and optimal yield of a crop within a particular climate and soil limitations. Soils can be augmented with various amendments to improve their physical characteristics and nutrients status. If excess nutrients are applied, however, these can be toxic to crops and result in off-site pollution, or simply be a waste of input dollars. To determine optimal nutrient levels for a particular yield goal, both soil and plant testing can be used.

Petiole sap analysis for diagnostic purposes has been practiced for more than a century and in recent years has gained considerable popularity among agriculturalists. A successful interpretation of tissue nutrient status is wholly dependent on the accuracy of normal or standard values with which analytical results are compared. This chapter presents a review about the vegetable crop (pac choi), system (fertilizer) used, and type of analysis done in these studies.

## Pac Choi (*Brassica rapa* Chinensis Group)

Chinese cabbage is a member of the Cruciferae family. The progenitor species in this family is *Brassica campestris*, and it is believed to have evolved in the Mediterranean area. It was introduced to China more than 2000 years ago and was not introduced to the United States until the late nineteenth century. It is grown throughout the year in California, Florida, and Hawaii, and in the spring and fall seasons in New Jersey. There are two main types of Chinese cabbages, the heading type (*Brassica rapa* L. subsp. *pekinensis*) and the non-heading type (*Brassica rapa* L. subsp. *chinensis*). Pac choi is the non-heading type and is characterized by dark green leaves and white petioles.

Two main varieties of Pac choi are a green, leafed white-stemmed pac choi, and a green-stemmed pac choi (Creasy, 2000). Most varieties are day-length sensitive, which means they will bolt as days lengthen. Susceptibility to bolting after transplanting is determined by photoperiod and temperature during the growing season (Tindall, 1983). Kalisz and Cebula (2006) noted that Chinese cabbage seedlings should be acclimated before they are transplanted so the plants can more successfully withstand adverse weather conditions of the spring season. Short-term exposure of plants to lower temperatures prior to transplanting is one of the hardening techniques used. From these sub-varieties, 'Mei Qing' is bolt resistant and tolerant to a wide range of temperatures characteristic of Kansas weather, so we chose to use it in our recent studies.

Light intensity can also affect growth, quality, and nitrogen assimilation. In lettuce, 16 hours of supplementary lighting increased fresh weight, tip-burn ratings, head firmness, and reduced nitrate accumulation in the leaves compared to natural light in a greenhouse experiment (Chesworth, 2008). Also, in lettuce, total N and $NO_3$ concentration of the leaves were increased by the addition of N to the soil and by reduction of light intensity (Cantliffe, 1972a).

We found no studies available on the effect of light intensity on the pac choi plant. Pac choi requires adequate soil water and plant nutrients for optimum growth (Averbeke, Juma, & Tshikalange, 2007) and doesn't tolerate poorly drained conditions. The fresh leaf yield of pac choi ranges between 5 $t \cdot ha^{-1}$ and 30 $t \cdot ha^{-1}$ (Tindall, 1983), with date of planting being an important yield factor (Juma, et al., 2004-5).

# Organic Production

In 1995, the United States Department of Agriculture issued the following definition of organic production: Organic production is an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity. It is based on minimal use of off-farm inputs and management practices that restore, maintain, and enhance ecological harmony.

The National Organic Program (NOP, 2002) set standards which regulate the organic certification process, some of these requirements are as follows:

Soil fertility and crop nutrients are managed through tillage, crop rotations, cover crops, animal and crop waste, and some allowed mined non-refined materials.

Physical, biological, and mechanical means are used to control insects, weeds, and diseases. Organic seeds and transplants are required if commercially available.

Use of genetic engineering, ionizing radiation, and/or sewage sludge is prohibited.

Land must have no prohibited substances for at least three years before the harvest of an organic crop.

Organic fertilizers generally contain humus or plant materials that decompose gradually, releasing nutrients into the soil that are required for the plants. Specifically, organic fertilizers contain the three main nutrients that plants require: nitrogen, phosphorous, and potassium, plus other secondary nutrients and minerals. Two main types of organic fertilizers are plant-based and animal-based fertilizers. The plant-based generally has low to moderate N-P-K values, where nutrients become readily available in the soil for plants to use. Some provide an extra dose of trace minerals and micronutrients. The most common plant-based fertilizers include alfalfa, compost, corn-gluten meal, cottonseed meal, kelp seaweed, soybean meal, and cover crops. Animal-based fertilizers include poultry and livestock manures and composts, seabird guano, blood meal, bone meal, and fish products (fish emulsion, hydrolyzed fish powder, fish meal) (Chesworth, 2008).

Compost has long been used as a beneficial soil amendment in horticulture since it suppresses certain plant diseases, improves moisture retention and cation exchange capacity, provides micronutrients, and also works as a source of slow-release nitrogen and organic matter (Stoffella, 2001). Growers interested in resource conservation also appreciate that locally produced compost substitutes for peat and other nonrenewable products that provide supportive enterprises for recycling horticultural industry organic residuals while avoiding landfills, enhancing retail product market options. Many conventional producers rely on compost to maintain soil organic matter, particularly in intensive systems that do not support a substantive cover crop period which reduces populations of crop insects, pests, and phytopathogens that target their primary crops (Millner, 2009). Composts typically have low N content, ranging from 0.8% to 2.0% for mature compost that is stabilized and no longer phototoxic (Stoffella, 2001). Consequently, additional N may need to be supplied from other sources, which may include legume cover crops, blood and feather meal, and pelletized poultry litter.

## High Tunnels

High tunnels, also called hoop houses, are unheated passively ventilated, walk-in plastic-film- covered structures used to improve the crop environment (Lamont, 2005). They must have a source of water for irrigation and are used to produce a wide variety of crops directly in the soil, and less frequently, in artificial media. They are used by growers to provide season extension and to enhance crop quality and yield. High tunnels help growers produce crops outside of the normal season, thus meeting consumer demand on either end of the production curve when competition is lower and prices are less competitive. The modified climate inside the high tunnels creates the opportunity to produce crops that can't normally grow if unprotected, diversifying the farming system. By helping protect crops from weather-related damage such as sun scald, wind bruises and hail spots, and from pest damage, high tunnels can provide a higher percentage of top-grade fruits and vegetables at harvest. High tunnels provide a protected environment relative to the open field, making them more economical than glasshouses as protective covering due to their low construction and operating cost. As they have no energy cost, automated ventilation, or heating system (Lamont, 2002), it is common for producers to recover their investment costs within one or two years (Blomgren & Frisch, 2006).

## Nitrogen Testing

### *Soil testing*

The ability to estimate fertility of soil and improve its productivity and crop yields has intrigued humans for centuries. Soils are dynamic in nature and their fertility status is known to change across time as nutrients are removed by the harvest of crops for food, fiber, and energy production lost by leaching, runoff, or change in availability. Thus, accurate and timely assessments of a soil's ability to supply nutrients to plants is needed to facilitate profitable crop production, prevent accumulation or depletion of nutrients in soil, preserve environmental quality, conserve natural resources, and, ultimately, insure agricultural practices are sustainable.

Many scientists and observant stewards of the land have made significant contributions to our understanding of plant nutrition and soil fertility. One could classify Liebig (1840) as having been an early worker in the soil-testing field. However, to date, most significant advances in modern soil testing technology were realized in the 1900s, by scientists who pioneered early methods of soil-testing procedures and performed field research to observe and quantify the effects of fertilization rate on plant performance. In the early 1900s, many Land Grant institutions developed programs investigating the utility of soil testing. By the 1930s, and 1940s, soil-test laboratories were being developed throughout the United States and services were being used by local clientele.

Objectives of soil sampling are to accurately determine the available nutrient status of soils and to clearly indicate to the farmer the seriousness of any deficiency or excess that may exist in terms of various crops. Soil base analysis before planting is a very important indicator of the soil nutrient status which gives the chance to the grower to adjust the fertilization amendment s at a early stage of the season. From this basis, fertilizer needs are determined and results are expressed in such a way to permit an economic evaluation of the suggested fertilizer recommendations (Walsh, 1973).

Crops all over the world are probably more deficient in N than in any other element; up to 99% of N in soil is present in very complex organic compounds not available to the plants. This N slowly becomes available to plants through microbial decomposition of the organic matter and conversion to available inorganic forms of N. The rate at which microorganisms decompose soil organic matter is dependent on temperature, moisture, aeration, type of organic matter, pH, and other factors. The main form of available N, nitrate-nitrogen is subject to leaching, de-nitrification, and immobilization by microorganisms.

### *Plant testing*

Plant analysis plays an increasingly important role in the expanding technology of economic plant production. Many workers, and among the earliest Lagatu (1924) and Lundegardh (1954), have attempted to use tissue analysis to demonstrate the relationship between yield, fertilizer use, and plant composition, but there is renewed interest and greatly accelerated activity in the field in recent years. This is due to farmers demanding more accessible technology, which gives them a quick interpretation of crop nutrient status. This is important so that adjustments of fertility applications can be made during the current growing season, and not just to next year's crop.

Plant analyses could be classified as *a)* total chemical analysis, which measures both the elements that have already been incorporated into plant tissues and those that are still present as soluble constituents of the plant sap; or *b*) relative quantity (rapid tissue tests), which measures unassimilated, soluble contents of the plant sap.

Testing the sap of the plant during the season is preferred as it doesn't require much labor, and with modern technology, testing fresh sap can be done on-farm where results can be obtained quickly and provide insight on the nitrogen dynamics of the crop. In many cases, this rapid turnaround can be important in making fertilization decisions.

Once plant nutrition contents have been determined, the next question is naturally whether the values found are sufficient to prevent the plant from suffering from deficiency. Most research workers have therefore tried to define a critical/ sufficiency level as the point below which plant nutrient content can't fall without causing growth problems or reducing yield. Above this level, there is usually a band of optimal or adequate nutrient contents which are sufficient for satisfactory growth. The extent of this band varies according to species, variety, climate, and other factors. At even higher nutrient content, wasteful luxury consumption and perhaps toxicity can exist.

Results of plant tissue analysis along with results of soil analysis provide useful tools for the grower in managing the rate and timing of fertilizer applications for vegetables. However, each has limitations and they should not be used for purposes not intended. For example, tissue testing is not recommended if the crop has received a foliar spays containing nutrients, especially micronutrients. There is no way to completely remove residues from leaf surfaces which results in higher test than actually in the plant tissue.

A quick way to test petiole sap nitrate-nitrogen (PSNN) is the Cardy meter, a handheld digital device which measures nitrate levels with a specific-ion electrode meter. As researchers started using quick petiole sap test technology like the Cardy meter to establish sufficiency ranges for different crops, questions arose regarding procedures, techniques, and accuracy of the standards developed. Petiole sap tests may not have the desired accuracy for certain micronutrients compared to traditional laboratory analyses using whole leaves, but in some cases the grower needs to evaluate the need for speed versus accuracy for the nutrients to be determined.

Currently, petiole sap tests appear to have the most utility for the mobile nutrients such as N, P, and K. Special guidelines need to be considered while using the Cardy meter to get accurate results.

Nitrate conversions**:** **C**ardy meters can read out in nitrates or nitrate-nitrogen. It depends on the calibration. If the Cardy meters read out in nitrates, the reading must be divided by 4.43 to find the nitrate-nitrogen value, which can then be compared to chart values (most of the sufficiency ranges values are given in nitrate-nitrogen values).

Sap vs. dried petioles**:** There are some published literatures values for petiole nitrate-nitrogen, but these values are sometimes based on dried petioles and are not directly transformable to fresh sap nitrate-nitrogen concentrations. Only fresh petiole sap nutrient values can be used with a fresh petiole sap-testing procedure.

Time of the day: Temperature and time of day might influence plant sap nitrate content. Taking readings consistently between 9 a.m. and 11a.m. will yield the most consistent results. Reasonable standardization of temperature and weather conditions under which sampling is carried out will help provide for more consistent test results.

Leaf stage: A specific leaf part, at a well-defined stage of growth is important. In pac choi, the petioles of the most recently matured leaves (those leaves that have reached maximum size or essentially stopped expanding in size), should have changed from a juvenile light green color to a dark green color.

Leaf part: In most crops, the petiole is easily identified. In pac choi, the petiole is the midrib of the leaf after the leaf blade is removed.

Number of leaves: The number of leaves should be representative of the area or the plots being tested. The petioles should be chopped and mixed, and a sub-sample of the chopped petioles pieces used for the final sample to crush.

Equipment: A garlic press is used to squeeze the sap from the petiole pieces. If many samples are being tested, a hydraulic sap press is useful. Other utensils include a sampling knife, scissors, paper towels, chopping knife, board, and testing kits, insuring cleanness of all tools with distilled de-ionized water after each use.

Storing petioles: Fresh, whole petioles can be stored on ice for up to eight hours or frozen overnight without appreciable changes in sap N concentrations. The leaf blades should be stripped from the petioles and the petioles placed in a plastic bag on ice in a cooler. Petioles may be stored at room temperature (70°F) in a plastic bag for up to two hours. If whole leaves or petioles are stored in open air, the petioles will wilt and nutrient readings will not be accurate. Only petioles should be stored not sap. Cold petioles must be warmed to room temperature before crushing, so that temperature differences between sap and meter do not affect results.

Reading time frame: Measurement of the pressed sap nutrient content must be made within one or two minutes of pressing, or as soon as the reading is stabilized. Otherwise, nitrate readings could change from the fresh petiole condition when the sap is exposed to air.

Calibration scale: Samples should always be read within the calibration (reading) scale of the Cardy meter. Readings outside of the calibrated range should be considered inaccurate. If sample sap nutrient concentrations are higher than the high end of the calibration scale, the sap must be diluted. Dilution can be done with distilled de-ionized water using about 20 to 50 parts water to one part sap. Expressed sap is placed on a sampling sheet (the sheet usually is cut into ½”strips) and the sheet placed on a sensor pad so that it covers both electrodes. The concentration of nitrates is read on the digital scale, which automatically switches among 1x, 10x, or 100x scales depending on the concentration of the nutrient in the sap. Meters should be used and stored in a cool, dry environment. Electrodes should be replaced after 500 measurements (Hochmuth, 1994).

Nitrate-nitrogen sufficiency ranges have been determined using the Cardy nitrate meter for some crops like lettuce, cabbage and broccoli (Schulbach, et al., 2007). Schulbach, Smith, Hartz, and Jackson were leader of a project that determined critical petiole nitrate-nitrogen levels for these crops by using Cardy meter based on field plots experiment at different development stages. Nitrogen amendments for this experiment were not specified.

Matthaus and Gysi (2001) developed sufficiency standards for wide ranges of vegetable crops including lettuce, Chinese cabbage, broccoli, and cauliflower. Their standards were based on field experiments using an Orion-Ion Analyser EA 904 to measure the nitrate concentration.

Coulombe (1999) developed nitrate-nitrogen fresh-sap sufficiency ranges for broccoli at field trials. In this experiment, all nutrients were held constant with the exception of nitrogen, which was broadcast prior to planting and side-dressed after planting. Three levels of N treatments were applied to give a total of 50 and 100 kg total N $ha^{-1}$, in addition to the control. To measure the fresh petiole sap nitrate, Merckoquant nitrate test strips were used.

No literature was found for fresh petiole sap nitrate sufficiency ranges for pac choi.

# Literature Cited

Averbeke, J., & Tshikalange. (2007). Yield response of African leafy vegetables to nitrogen, phosphorus, and potassium: The case of /i brassica rapa/ L. subsp. /i chinensis/ and /i solanum retroflexum/ dun. Water S. A., 33(3), 355.

Blomgren, T., & Frisch, T. (2006). High tunnels using low-cost technology to increase yields, improve quality, and extend the season. Produced by Cornell University Cooperative Extension. University of Vermont, Center for Sustainable Agriculture.

Cantliffe, D. J. (1972a). Nitrate accumulation in spinach grown under different light intensities. J. of American Society for Horticultural Science, 97, 152-154.

Chesworth, W. (2008). Encyclopedia of Soil Science. Dordrecht, Netherlands: Springer.

Coulombe, J. (1999). Evaluation of soil and petiole sap nitrate quick-tests for broccoli in quebec. Acta Horticulturae, (506), 147-152.

Creasy, R. (2000). The edible Asian garden / Rosalind creasy. Periplus.

Hochmuth, G. J. (1994). Plant petiole sap testing for vegetable crops. No. CIR 1144). Florida: University of Florida.

Juma, K. A., Netshithuthuni, N. C., Tshikalange, T. E., & and Van Averbeke, W. (2004-5). Improving production of indigenous vegetables. Pretoria, South Africa: Center for Organic and Smallholder Agriculture, Tshwane University of Technology,.

Kalisz, A., & Cebula, S. (2006). The effect of temperature on growth and chemical composition of chinese cabbage seedlings in spring period. Folia Hort., 18(1), 3-15.

Lamont, W. J. (2002). Penn State high-tunnel extension program. HortTechnology, 12(4), 732-735.

Lamont, W. J. (2005). Plastics: Modifying the microclimate for the production of vegetable crops. HortTechnology, 15(3), 477-481.

Matthaus, D., & Gysi, C. (2001). Plant-sap analysis in vegetables a tool to decide on nitrogen top dressing. Acta Horticulturae, (No.563), 93-102.

Millner, P. (2009). High-tunnel and organic horticulture: Compost, food safety, and crop quality. HortScience, 44(2), 242-245.

Schulbach, K., Smith, R., Hartz, T., & Jackson, L. (2007). Guide to nitrogee quick-tests for vegetables with 'Cardy' nitrate meter No. 95-0582. California: CDFA.

Stoffella, P. (2001). Compost utilization in horticultural cropping systems. Hoboken: CRC Press.

Tindall, H. D. (1983). Vegetables in the tropics (pp. 416-418). London: Macmillen Press.

Walsh, L. M. (1973). Soil testing and plant analysis. Edited by leo M. Walsh and James D. Beaton. Managing editor: Richard C. Dinauer. Assistant editors: Patricia McMahan and Sheryl S. Schoenfeld. Soil Science Society of America.

# Chapter 2 - Nitrate-Nitrogen Sufficiency Ranges in Leaf Petiole Sap of Pac Choi Produced with Organic versus Inorganic Fertilizers

## Introduction

Research has shown that plant analysis can determine the nitrogen (N) needs of many crops (Gardner, 1989a; Gardner and Pew, 1974). Regular tissue analysis should enable farmers to recognize and correct an incipient nutrient deficiency, but this is impractical for vegetables like pac choi (*Brassica rapa* L. Chinensis group) with a short growth period. Petiole sap nitrate is a highly sensitive indicator of plant nutrient status and is simple, practical, and fast (Amor, 2006; Drews and Fischer, 1992; Hochmuth, Maynard, Vavrina, and Hanlon, 1991). Need for a reliable method of testing N status has encouraged research on the nitrate test for petiole sap in vegetables. Petiole sap nitrate-nitrogen (PSNN) levels associated with maximum growth rate or yield have been determined for vegetable crops like cabbage (*Brassica oleracea* L., Capitata group); (Gardner, 1989a; Schulbach, Smith, Hartz, and Jackson, 2007) broccoli (*Brassica oleracea* L. Italica group) (Gardner, 1989b; Hochmuth, 1994), lettuce, (*Lactuca sativa* L.) (Schulbach et al., 2007) and collards (*Brassica oleracea* L. Acephala group) (Hochmuth, 1994), but not for pac choi. The objectives of this study were to 1) establish $NO_3$-N petiole sap sufficiency ranges for pac choi 2) determine if standards differ by fertilizer source (organic and inorganic), and 3) validate standards determined in greenhouse experiments with field results.

# Materials and Methods

## Greenhouse Study 1- Experiment

A greenhouse experiment with pac choi (*Brassica rapa* L. Chinensis group 'Mei Qing'; Johnny's Selected Seed, Winslow, Maine) was conducted during the summer of 2008 (22 June – 5 Aug.) in the glass greenhouse range of the Kansas State University Throckmorton Plant Sciences Center, Manhattan, KS.

Treatments were arranged in a randomized complete-block factorial design with seven (N rates) x two fertilizer sources (conventional and organic). The number of blocks (replicates) differed between parameters, with all being measured in six blocks except for total leaf N and PSNN, which were measured as the average of two blocks, resulting in three replications.

Pac choi seeds were sown on 22 June in plugs using a Farfard super-fine germinating mix (Hummert International, Topeka, KS.). Plugs were placed under mist for 18 days and seedlings were transplanted on 10 July into 14-cm pots (2000 $cm^3$). The plants were grown in a root media containing 70% Fafard peatmoss (Hummert, Topeka, KS), 30% Therm-O-Rock coarse perlite (Hummert, Topeka, KS) with dolomotic lime (19 $g/m^3$), gypsum (2.83 $g/m^3$), and micromix (2.83 $g/m^3$). A surfactant (Suffusion Granular; OHP Inc., Mainland, PA) was top-dressed on each pot at a rate of 4.7 g/pot to allow the peat-based media to re-wet.

Nutrient treatments included seven concentrations of N (0, 32, 75, 150, 225, 300, and 450 $mg^{\cdot} L^{-1}$) from two sources of fertilizer (conventional and organic). The conventional soluble fertilizer was formulated from inorganic salts to result in a 4 $NO_3$-N: 1 $NH_4$-N ratio. Constant levels of other nutrients were supplied (Table 1). The organic soluble fertilizer was fish hydrolysate (Neptune's Harvest, Gloucester, Mass.) with 2N: 1.72P: 0.83K diluted at each application to get the exact N concentration for different rates. This resulted in ranges of 27 to 387 mg. $L^{-1}$ P and 13 to 123 mg. $L^{-1}$ K (Table 2). Temperature was monitored using HOBO Environmental Monitors (Onset Computer Corp., Bourne, MA). Air temperature averaged 22.6ºC during the night and 27.7 ºC during the day. The water source used for the experiment was municipal water, which had a very low alkalinity (39 mg· $L^{-1}$). Plants were fertigated when gravimetric weight of sentinel pots decreased by 30% from container capacity due to water loss. A measured volume of fertilizer solution was applied at each watering to result in a controlled leaching fraction of 0.15. Total number of applications of fertilizer by the end of the growing season was 15.

By mid-July, organic plants did not look like they were growing normally. By 19 July, we had lost 15 organic plants and the rest were not doing well. At this point, the experiment was modified to continue with planned treatments on the conventional plants, as organic plants were not making a good comparison.

Saturated media extract (SME) method (Berghage, 1987) was used on root medium samples collected from dead organic pots. Distilled water was added to a beaker with the root medium until the sample was saturated. After samples were mixed and allowed to equilibrate for 1h, solution was extracted by squeezing the saturated sample through cheese cloth, and samples were measured for EC, pH using an Accumet Excel XL 20 pH/ conductivity meter (Fisher Scientific LLC, Denver, CO.).

Ammonia-N was measured using an Alpkem RFA Autoanalyzer methodology number A303-S021 (Alpkem Corporation, Clackamas, OR.). Nitrate-N was also measured, using Alpkem RFA Autoanalyzer methodology number A303-S170 (Alpekum Corporation, Clackamas, OR.). Ortho Phosphorus (ammonium molybdate blue colorometric procedure) was measured, using an Alpkem RFA Autoanalyzer methodology number A303-S200-13 (Alpkem Corporation, Clackamas, OR.). Potassium was measured with Flame Atomic Absorption Spectrophotometer 3110 (Perkin Elmer Corp., Norwalk, CT.).

Diagnostics were performed on the organic plants and potting mix to determine why the organic plants were not thriving so that modifications could be made in future experiments.

For the conventional treatments only, the pour-through method (Cavins, Whipker, and Fonteno, 2008) was used to monitor pH and EC. Pots were fertilized and allowed to equilibrate for 1h. Pots were then placed over a plastic saucer. Distilled water (75 mL) was added and the leachate was collected. Electrical conductivity (EC) and pH were measured on each sample using an Accumet Excel XL 20 pH/ conductivity meter (Fisher Scientific LLC, Denver, CO.).

The saturated medium extract (SME) was used on root medium samples collected at final harvest 5 Aug. EC, pH, and N-P-K were measured according to the Berghage method mentioned before.

Leaf samples were taken weekly once plants were two weeks old. The youngest, fully expanded leaf was collected from each pot, and measured for PSNN and total leaf N. The blades were separated from the petioles. Blades were dried in a forced-air oven at 70ºC for 72 hours and then ground in a stainless steel Wiley mill (Scientific Apparatus, Philadelphia, PA.) to pass a 20-mesh screen for total N analysis using a dry combustion procedure (TruSpec CN, LECO Corp, St. Joseph, MO.) at the Kansas State University Soil Testing Laboratory, Manhattan, KS, (Wright, 2001) potassium (k) levels were measured at harvest using a sulfuric acid/hydrogen peroxide-digested plant material, Flame Atomic Absorption Spectrophotometer 3110 (Perkin Elmer Instruments, Norwalk, CT.).

Petioles were chopped and pressed with a garlic press to extract plant sap. Fresh sap was analyzed immediately for $NO_3$-N with a handheld ion-specific electrode (Cardy nitrate $NO_3^{-}$ meter, Horiba, Ltd., Kyoto, Japan) (Hochmuth, 1994). The meter was calibrated before analysis and after every 10 measurements with a standard of 2,000 $mg \cdot L^{-1}$ $NO_3$, and slope was adjusted with a 150 $mg \cdot L^{-1}$ $NO_3$ solution. A few drops of the petiole sap were placed on a sampling sheet; the reading was recorded after the value had stabilized. Meter readings were in units of $mg \cdot L^{-1}$ $NO_3$ and were converted to $NO_3$-N (Hartz, 2007). To obtain a sufficient petiole sap, samples were combined between reps in each block.

Plant chlorophyll content was recorded weekly using a chlorophyll meter, SPAD-502, (Spectrum Technologies, Inc., Plainfield, IL) and by taking the average reading of three leaves located on the second whorl per pot. Leaf length was also measured weekly. Shoot fresh and dry weights (dried for a minimum of 48 h at 70ºC in a forced-air oven) were recorded at the end of the experiment. This study was completed with only the conventional pac choi plants.

Data were analyzed using the MIXED procedure (SAS Version 9.1.3, Cary, NC.). Statistics included F-tests and LS-means (least-square means) for fixed effects and contrasts, testing the equality of means for pairs of adjacent N rates with a 14 degrees of freedom error term (i.e., N rates 0 versus 32, 32 versus 75, etc.). Optimum yield was determined to be the point at which there were no significant differences between that point and the next higher nutrient level. Nutrient sufficiency ranges were calculated at the 95% confidence interval of PSNN at the optimum nutrient level. Regression analysis between the PSNN and total leaf nitrogen were performed, and regression equations were calculated for individual sampling dates.

## Greenhouse Study 1- Diagnostics

To find out why the organic pac choi plants died, the pour-through method (Cavins et al., 2008) was used to collect leachate from root media of the organic and conventional pots. EC and pH were measured on the leachate of each sample and compared between organic and conventional plants of the pac choi root media using an Accumet Excel XL 20 pH/ conductivity meter (Fisher Scientific LLC, Denver, CO.). Results showed no significant differences in EC and pH in the root media of organic or conventional plants (results not shown), which eliminated the salt toxicity possibility.

Another possibility investigated was micronutrient toxicity in the root media. A side experiment was conducted in fall 2008 (3 Sept. - 29 Sept.) in the glass greenhouse range of the Kansas State University Throckmorton Plant Sciences Center, Manhattan, KS.

Treatments were arranged in RCBD factorial design with two treatments (fallow pots - just root media with no plants) and one fertilizer source (organic), using eight replicates.

The first root media was the same source used in greenhouse study 1 and was prepared using 70% Fafard peatmoss (Hummert, Topeka, KS), 30% Therm-O-Rock coarse perlite (Hummert, Topeka, KS) with dolomotic lime (672 $g/ft^3$), gypsum (100.5 $g/ft^3$), and micromix (100.5 $g/ft^3$). A surfactant (Suffusion Granular; OHP Inc., Mainland, PA) was top dressed on each pot at a rate of 4.7 g/pot to allow the peat-based media to re-wet.

The other root media was a pre-mixed bark-based Sunshine Mix Special Blend E6340, (SunGro Horticulture, Bellevue, WA) containing organic starter nutrients of 0.61 $NH_4$-N, 1.4

$NO_3$-N, and 0.3 P mg.$L^{-1}$. The organic soluble fertilizer was fish hydrolyzate (Neptune's Harvest, Gloucester, MA) at 150 mg. $L^{-1}$. Fallow pots were fertigated every three days for the period of 26 days.

The pour-through method (Cavins et al., 2008) was used to collect leachate from the pots on 03 Sept., 10 Sept., 17 Sept., and 29 Sept. Micronutrients (Cu, Mn, Zn, Al) were measured using an Inductively coupled plasma (ICP) spectrometer, 720-ES ICP Optical Emission Spectrometer (Varian Austrailia Pty Ltd, Mulgrave, Vic Australia), and ammonium ($NH_4$-N) using Indophenol Colorimetric Reaction A303-S021 ammonia nitrogen, (Alpkem Corporation Clackamas, OR), at the Kansas State University Soil Testing Laboratory, Manhattan, KS.

Results showed no significant differences of Cu, Mn, Zn, or Al over the period of the experiment. Statistical analysis showed a significant accumulation of $NH_4$-N in the Fafard peatmoss media starting at seven days and increasing throughout the testing period, but not in the pre-mixed bark-based root media (Table 3). This suggests some kind of ammonium toxicity in the organic fertilizer using the Fafard peatmoss mixed-root media.

Further studies need to be done to assess the ammonium toxicity in this root media.

## Greenhouse study 2

A second nutrient rate was conducted during the fall 2008 (1 Nov. - 23 Dec.) in the glass greenhouse range of the Kansas State University Throckmorton Plant Sciences Center, Manhattan, KS. Materials and methods were similar to greenhouse study 1 except for the following; *a)* As a result of the diagnostic experiment done on different root media, pac choi plants were transplanted on 18 Nov. into a bark-based root medium Sunshine Mix Special

Blend E6340, (SunGro Horticulture, Bellevue, WA) containing organic starter nutrients of (0.61 $NH_4$-N, 1.4 $NO_3$-N, and 0.3 P) mg. $L^{-1}$. *b)* Since this study was conducted during late fall and winter, supplemental light from 400-watt, HPS lamps (P.L. light Systems, Beamsville, Ontario, CA.) was provided daily for 12 h from11 Dec. to 23 Dec. *c)* Temperature was monitored using HOBO Environmental Monitors (Onset Computer Corp., Bourne, MA). Air temperature averaged 18.2ºC during the night and 22.3 ºC during the day *d)* In addition to all other factors, leaf count was measured on a weekly basis e) Statistical analysis was performed using a MIXED procedure (SAS Version 9.1.3, Cary, NC.) to compare organic versus conventional fertilizers at different N fertility rates.

## Outdoor Study

A field experiment with pac choi (*Brassica rapa* L.Chinensis group 'Mei Qing'; Johnny's Selected Seed, Winslow, ME) was conducted during fall (05 Sept. - 18 Oct.) 2008. Research plots were located at the Kansas State University Horticulture Research Center (Olathe, KS.). The high-tunnels at the Olathe research center were established in 2002. Half or the high-tunnels and field plots have been managed with organic amendments and half with conventional amendments (Knewtson, J.S. 2008). Organic plots were managed in compliance with USDA National Organic Program standards, and were inspected and certified in 2003, 2006, 2007 and 2008.

For this study, beginning in 2007, each high tunnel or field plot was subdivided into three 3.2x6.1 m plots to which one of three fertilizer levels were assigned (control, low, and high) following a latin square design to avoid bias due to position effects in the high-tunnels.

Fertilizers rates were determined based on soil analysis at the beginning of the study in 2007, and recommendations for vegetable crops in Kansas (Marr et al., 1988).

Trials were performed on a Kennebec silt loam in 3x3.2m open field plots and adjacent 3x3.2m high-tunnel plots with 1.5 m sidewalls (Stuppy, North Kansas City, MO.). Tunnels were covered with single-layer 6-mil (0.153mm) K-polyethylene (Klerk's Plastic Product Manufacturing, Inc., Richburg, SC). Plants were arranged as 72 split plots with 12 treatment combinations in a Latin square design, associated with open fields and high-tunnels, conventional and organic, and fertilization at three N rates (control, low, and high) (Figure 2-1).

All plots were planted to a buckwheat cover crop (*Fagopyrum sagittatum* Gilib.) in the summer. Control plots received no supplemental fertilizer. The low treatments received pre-plant fertilizer amendment applied one time per year (in the spring). Compost application rates were based on the assumption that 50% of the nitrogen from compost would be available to plants during the growing season, while 100% would be available from conventional fertilizers (Warman and Havard, 1997). Jack's Peat-Lite 20N:4.4P:16.6K J. R. Peters, Inc., (Allentown, MO) at a rate of 87 lb N/hectare was applied for conventional plots and a mixed-source compost (Microleverage 0.6N: 0.4P: 4.4K, Hughesville, MO.) at a rate of 176 lb N/hectare was applied for organic plots. Starting two weeks after planting, high fertility treatments plots received additional soluble fertilizer at a rate of 7.24 kg/ha applied three times (09 Sept., 18 Sept., and 28 Sept.) during the growing season. Organic plots received fish hydrolyzate 2.23 N- 4.35P2O5- 0.3K2O (Neptune's Harvest, Gloucester, MA.), the conventional plots received 21.6 g/20 $ft^2$plot $KNO_3$ and 6.24g/20 $ft^2$ plot $Ca(NO_3)_2$, this rate calculated to apply an amount of calcium equivalent to that present in the fish hydrolyzate (Talavera-Bianchi, M., 2009).

Pac choi seeds were started in a greenhouse (08 Aug.) in 13x26 flats using commercial media, Sunshine Mix Special blend E6340 (SunGro Horticulture, Bellevue, WA) supplemented with MicroLeverage compost until transplanted.

Pac choi plants transplanted on 05 Sept. to high-tunnel or field plots (3 x 3.2m), each fertility plot had 20 plants/ row, two plants across the row, for the inner two rows and 20 plants per row, one plant across the row for the outer boarder one. Plants sampled were from the center of the two inner rows, avoiding the plants in between fertility rates. Sampled plants were chosen based on random number generator (RNG).

Leaf samples were taken weekly once plants were two weeks old. Three plants from each plot from each fertility level were sampled on 20 Sept., 27 Sept., and 06 Oct. and 11 Oct. The youngest fully expanded leaf was collected from each plant. PSNN, total leaf N, and K levels were measured at harvest (same procedure as the greenhouse study). Shoot fresh weight and dry weight were recorded at the end of the experiment. Data were analyzed using a MIXED procedure, SAS Version 9.3.1 (Cary, NC.), analysis of variance (ANOVA) was used to detect significant differences between treatments.

## Results and discussion

### Greenhouse Studies

Since greenhouse experiment 2 included both organic and conventional treatments, we will discuss its results first and then compare it to greenhouse experiment 1.

### *Plant measurements and final yield*

In greenhouse experiment 2, conventional treatment yielded greater fresh and dry weights at 75, 150, and 450 mg·L$^{-1}$ N rates compared to organic treatments (Figure 2-2, a, b). The maximum fresh yield for both organic (31.0 g/plant) and conventional (40.0 g/plant) was obtained at the N rate of 150 mg· L$^{-1}$. Fresh weight, dry weight, number of leaves, and leaf-length data consistently indicated the maximum yield was obtained at 150 mg.L$^{-1}$ N rate application for both conventional and organic fertilizers.

This agrees with greenhouse experiment 1, as the highest yield for conventional pac choi plants (87.5 g/plant) was also obtained at 150 mg·L$^{-1}$ (Figure 2-3,a,b) .

In experiment 2, root dry weight showed no differences between N rates (Figure 2-4), but it varied more than aboveground dry matter. N rate and fertilizer source interacted significantly at 225 mg· L$^{-1}$, where the weight of the root system at 225 mg.L$^{-1}$N rate was significantly higher for organic than conventional treatment. Leaf count and leaf-length measurements at harvest were consistent with the yield data (fresh and dry weight) for both conventional and organic treatments (Figure 2- 5, a, b), with a significantly higher number of leaves at the 450 mg. L$^{-1}$ N rate in conventional versus organic. Although conventional leaf-length at 75 and 150 mg· L$^{-1}$ did not increase significantly, leaf count and final yield were higher at that rate. The same results was generated from greenhouse experiment 1 as the leaf-length measurements for conventional pac choi plants at harvest the highest at 150 mg·L$^{-1}$ (Figure 2-6).

Despite the fact that the SPAD meter chlorophyll content has been used effectively in detecting N deficiency in some crops like cabbage and carrots (Westerveld, McKeown, Scott-Dupree, and McDonald, 2004; Westerveld et al., 2004), it was not a good diagnostic tool for evaluating pac choi N status. There were no significant differences among N rates throughout the growing period for this parameter in greenhouse experiment 2 (Figure 2-7), as well as greenhouse experiment 1 (Figure 2-8).

The significant decline in pac choi fresh and dry weight in the conventional treatment beyond 150 mg.L$^{-1}$ N and in the organic treatment beyond 300 mg. L$^{-1}$ N could be explained by the significant increase in EC (2.9 mS/cm) in the root media, as more concentrated fertilizer was applied, up to 450 mg.L$^{-1}$ in both organic and conventional treatments (Figure 2-9). Results agree with those obtained from greenhouse study 1 (Figure 2-10).

Literature for similar crops; as no reference found for pac choi; showed that lettuce had a 10% yield reduction with a 1.4 mS/cm EC level in the root media, (Evans, 2006). Scuderi, Giuffrida, and Noto (2009) showed use of nutrient solutions with an EC up to 3.6 mS cm$^{-1}$ resulted in a 25% yield reduction in lettuce as compared with the lowest EC tested (0.8 mS cm$^{-1}$ ). Root media pH for greenhouse study 2 stayed within the acceptable range (5.5 - 7.0) (Albright, 2010) (Figure 2-11).

### ***Sufficiency petiole sap $NO_3$-N ranges***

In experiment 2, PSNN concentrations, as determined by the Cardy meter, resulted in similar trends for both conventional and organic (Figure 2-12). The nitrate concentration in sap derived from the petiole of pac choi leaves increased significantly with increasing N rate up to 225 mg·L$^{-1}$ in week 2 and up to either 150 mg·L$^{-1}$ or 225 mg. L$^{-1}$ in weeks 3, 4, and 5 (Figure 2-12). At the maximum yield rate of 150 mg·L$^{-1}$ N rate, conventional petiole sap nitrogen was higher than organic only in week 5. PSNN sufficiency concentrations were in the range 1500 to 2000 mg·L$^{-1}$at weeks 2 and 3 (Figure 2-12 a, b), and 1000 to 1500 mg·L$^{-1}$ from week 4 until harvest (Figure 2-12 c, d).

PSNN concentrations for conventional pac choi treatments from greenhouse experiment 1 followed the same trend (Figure 2-13). Nitrate concentration significantly increased in leaf petioles of pac choi conventional treatments with increasing N rate up to 225 mg. L$^{-1}$ for weeks 2 and 3, , and up to 150 mg. L$^{-1}$ in week 4 and 5. At the optimum yield, PSN sufficiency concentrations were in the range of 1000-1500 mg·L$^{-1}$ at weeks 2 and 3 (Figure 2-13 a, b), and 800-1000 mg·L$^{-1}$ from week 4 until harvest (Figure 2-13 c, d).

***Nutrient tissue concentrations***

Total N in leaf tissue organic treatments increased significantly with an increasing rate of N up to 32 mg·L$^{-1}$ at week 2, 75 mg·L$^{-1}$ at week 3, 150 mg· L$^{-1}$ at week 4, and 225 mg· L$^{-1}$ at harvest (Figure 2-14). The significant increase in total leaf N for the conventional treatments was up to 32 mg· L$^{-1}$ at weeks 2 and 3, and up to 75 mg·L$^{-1}$ at weeks 4 and 5, while for organic treatment was up to 32 mg· L$^{-1}$ at week 2, 75 mg· L$^{-1}$ at week 3, 150 mg· L$^{-1}$ at week 4, 225 mg· L$^{-1}$ at harvest.

Although total leaf N concentration was less sensitive in showing fertilizer rate effect and did not correspond to optimal yield, it was highly correlated with PSNN concentrations (Figure 2-15). The linear relationship between PSNN and total N measurements indicated that N management criteria based on dry tissue total N can be readily converted to criteria based on PSNN, making it possible to adopt the Cardy meter as a tool for managing N in pac choi. Dry tissue total leaf N can be converted to PSNN readings using the regression equation given. Equations were calculated for week 3 when supplemental fertilizer might be needed and could still be applied.

In greenhouse experiment 1, total leaf N results for conventional pac choi treatments were increased up to 150 mg $^{.}$ L$^{-1}$ at weeks 2, 3, and 4, and up to 225 mg $^{.}$ L$^{-1}$ at harvest (Figure 2-16).

## Outdoor Study

### *Field yield*

A highly significant fertility effect was seen for fresh weight at harvest as the optimum yield was obtained at high N fertility rate (Figure 2-17) and (Table 4). There was also significant system effect for fresh weight at harvest for weeks 3, 4, and 5 in pac choi field plots, as organic treatments had higher yield than conventional.

For the high-tunnel plots there was a significant fertility effect in weeks 3, 4, and 5 (Figure 2-18), as well as system fertility interaction in week 5. System effect was only significant at week 4. Organic plot yield was higher than conventional.

Dry weight for field and high-tunnel, for both organic and conventional treatments followed the same trend as the fresh weight (analysis is shown in table 4).

### *Sufficiency petiole sap NO3-N ranges*

Consistent with the greenhouse studies results, organic and conventional treatments showed similar trends for PSNN concentration during the growing season. Field organic sufficiency ranges were 800-1200 mg.L$^{-1}$ at weeks 2 and 3, then levels dropped as the plants grew and ranged between 600-800 mg.L$^{-1}$ at weeks 4 and 5 (Figure 19, a). As for field conventional treatments, sufficiency ranges were between 800-1000 mg.L$^{-1}$ at weeks 2 and 3, dropped down to 500-700 mg.L$^{-1}$ at weeks 4 and 5 (Figure 19, b)

For high-tunnel conventional treatments, sufficiency ranges were 900-1100 mg. $L^{-1}$ at weeks 2 and 3, and dropped down to 500-700 mg. $L^{-1}$ at weeks 4 and 5 (Figure 20, a).

For high-tunnel organic treatments, sufficiency ranges were between 800-1000 mg. $L^{-1}$ for weeks 2 and 3, dropped to 500-700 mg. $L^{-1}$ at weeks 4 and 5 (Figure 20, b).

As for total leaf N, there was significant system effect at week 3, fertility effect at week 2 for field plots, and system effect at week 2 for high-tunnel plots.

### *Standards validation based on field data*

Sufficiency ranges of PSNN in the greenhouse studies followed the same trend when compared to high-tunnel and field studies. Sufficiency ranges gleaned from the literature for similar vegetable crops are presented in (Table 5).

Differences in sufficiency ranges of PSNN among the greenhouse studies compared to high-tunnel and field studies could be a result of many factors:

1.) Low light levels during the growing period were associated with $NO_3$-N accumulation. Parks, Huett, Campbell, and Spohr (2008) reported that a reduction of light level was associated with reduced nitrate reductase activity and increased nitrate accumulation in lettuce and spinach. Proietti, Moscatello, Leccese, Colla, and Ballistelli (2004) showed that spinach grown at a light intensity of 200 µmol/ $m^2$.s had a higher nitrate concentration than those plants grown at 800 µmol/ $m^2$.s.

2.) Short photoperiod has also been reported to increase nitrate accumulation (Cantliffe, 1972a). Nitrate is translocated to the leaves where it is reduced and incorporated into proteins, and has an essential role in photosynthesis as a component of chlorophyll. Reduced irradiance has been associated with reduced photosynthetic rates and growth.

3.) Leafy vegetables grown in a protective environment might contain higher $NO_3$-N levels. Lyons, Rayment, Nobbs, and McCallum (1994) reported that hydroponically grown head lettuce contained twice the $NO_3$-N concentration of field-grown lettuce, although the N forms in the fertilizers were not specified. These interactions can result in the $NO_3$-N concentration accumulating in pac choi leaves.

### *Petiole sap nitrate-nitrogen in greenhouse vs. field*

In our efforts to answer some of the questions motivated by comparisons of the greenhouse, high-tunnel and field studies, and also to be sure that excess or deficient nutrient levels of P or K were not confounding our results, a saturated media extract (SME) analysis for N-P-K showed the following:

Phosphorus accumulation could happen in organic treatments at 225 $mg.L^{-1}$ and higher N rates; this is due to the nature of the organic fertilizer, as increasing the N rate will eventually increase the P rate (Nelson, 2007). Potassium concentration may have been deficient in conventional treatments and some organic treatments compared to desirable levels of K concentration (SME) in soil-less media (59 to 85 $mg.L^{-1}$) (Clark, Smith, Gornforth, and Prasad, 1986), and (60-149 $mg.L^{-1}$) (Warncke & Krauskopf, 1983), and for lettuce (50 $mg.L^{-1}$) (Maynard, 2007) . $NO_3$-N concentration in SME of conventional treatments increased with increasing fertilizer concentration (Figure 2-21).

K dry-tissue analysis of pac choi showed that K concentrations in greenhouse (2.46-3.08%), high-tunnel (2.41-3.49%), and field (3.43-3.91%) experiments were within sufficiency ranges of similar crops, spinach (2.5-4%),Chinese cabbage (3.0-6.5%), and cabbage (1.2-3%) (Maynard, 2007; Jones, Wolf, & Mills, 1991), which indicates the pac choi plants in the greenhouse studies were not K deficient. This is supported by not having any K deficiency symptoms on pac choi plants.

### *New methodology*

Nitrate sufficiency ranges can be a useful tool to give a grower a range to monitor his or her crop. We are faced with the fact that these ranges could vary based on the variety of the crop, the fertility of soil, and certain environmental factors such as photoperiod, light intensity, and temperature. Hartz (2007) has also raised concerns related to the accuracy of tissue sufficiency ranges using Cardy meter. However, we found a closer relationship between the cardy meter petiole nitrate and yield as compared to whole leaf tissue N. We also found that petiole sap nitrate always increased to the point associated with the maximum biomass, followed by a plateau where sap nitrate remained constant as seen in Figure (2-12, d) and yield in Figure (2-2), for organic and conventional treatments in greenhouse experiment 2 and in Figure (2-13, d) and yield in Figure (2-3), for conventional treatments in greenhouse experiment 1.

Taking this characteristic of the Cardy meter data analysis into consideration we can suggest a new methodology for the practical application in a growers' field. Rather than researchers generating many curves under many different conditions, we can use the plateau to simplify the test, allowing growers to implement cardy meter testing in their fields using the following suggestions. First, they use the recommended level of N fertilization for most of the field production area. They will also demarcate two plots, one to apply N rate higher than recommended and the other plot to apply N rate lower than the recommended rate. The purpose of doing that is to find whether the majority of the pac choi in the field is at or above the plateau level regarding Cardy meter sap nitrate. For example if the rate of N fertilizer recommended for Crucifers was 100-150 lb/acre (Maynard, 1997), the grower can apply this amount to most of the field, apply 10-25% higher than the recommended rate to a plot, and 10-25% less than this amount to the other plot.

A weekly measurement of the nitrate concentration using the Cardy meter throughout the production period for samples from the field, as well as samples taken from the higher N plot and lower N plot can determine if the N status of the crop is above or below the critical threshold level, or the point at which we find the sap nitrate plateau. The recommended rate as applied might be just right, higher or lower than what the pac choi actually requires. If the three points are in the range of expected sufficiency and all in a straight line, or similar, we would assume that even the lower N rate would have been sufficient. The following year, the grower could reduce the N application and re-test. If the "low" fertilizer plot has a low sap nitrate level but the other two areas; the main field and the high fertilizer plot are both higher than the low, but similar to each other, then we might assume that the application rate was adequate, and no change in pre-plant fertilizer needs to be made. In scenario 3, the three sap nitrate levels might go from low in the low N test plot, medium in the main field, and then higher in the high N test plot. In this case, the sap nitrate indicates that the pac choi needs more nitrogen for the highest yield. If this determination is made in week 2 or 3 of the crop growth cycle, additional N can be applied at this time as liquid fertilizer as we did in the experiment, or top-dressed in a soluble form.

The sufficiency ranges associated with the optimal yield as determined by our experiment can be used as starting point for a pac choi standard, but the method described above will adapt it for the specific location. This could be the easiest and fastest way to deal with the variation of standards available in literature without a major loss to the grower's crop. This has not yet been evaluated in our experiments, but is a subject of future research interest for pac choi and other vegetables.

## Conclusions

Results from our studies concur with previous research, and what growers know is that sufficient nitrogen needs to be available in the growing root media to achieve optimum growth. The Cardy meter petiole sap $NO_3$-N gave results that correlated with the laboratory method of total leaf N. Pac choi yield can be optimized at a 150 mg.$L^{-1}$ N fertility rate. Sufficiency ranges of PSNN are varied based on growing period, light intensity, photoperiod, and whether the plants are grown in greenhouse, field, or high tunnel conditions. PSNN sufficiency ranges established in the greenhouse study were consistent with the sufficiency ranges in field and high-tunnel studies, highlighting the higher ranges in the summer greenhouse experiment. PSNN were ranges between 800-1200 mg. $L^{-1}$ at weeks 2 and 3, and dropped down to 500-800 mg.$L^{-1}$ at weeks 4 through harvest. Organic and conventional fertilizers were within similar ranges.

**Table 1: Fertilizer salts in mM used to formulate conventional fertilizer at different nitrogen fertility rates.**

| | Nitrogen fertility rate mg.L$^{-1}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 32 | 75 | 150 | 225 | 300 | 450 |
| | inorganic nutrients (mM) | | | | | | |
| KNO3 | 0 | | | 0.83 | 0.83 | 0.83 | 0.83 |
| NH4H2PO4 | 0 | 0.44 | 0.89 | 1.72 | 1.72 | 1.72 | 1.72 |
| NH4NO3 | 0 | | 0.18 | 0.42 | 1.5 | 2.58 | 4.68 |
| Ca(NO3)2 | 0 | 0.88 | 2.05 | 3.65 | 5.27 | 6.85 | 10.1 |
| MgSO4 | 0 | 2 | 2 | 2 | 2 | 2 | 2 |
| CaCl2 | 0 | 2.54 | 0.95 | | | | |
| KH2PO4 | 0 | 0.83 | 0.83 | | | | |
| CaHPO4 | | 0.45 | | | | | |

**Table 2: Nutrient levels in mM resulted from fish hydrolyzate dilution to obtain different nitrogen fertility rates.**

| | Nitrogen fertility rate mg.L$^{-1}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 32 | 75 | 150 | 225 | 300 | 450 |
| | organic nutrients (mM) | | | | | | |
| N | 0 | 2.29 | 5.36 | 10.71 | 16.07 | 21.43 | 32.14 |
| $P_2O_5$ | 0 | 1.75 | 4.61 | 9.21 | 13.82 | 18.43 | 27.64 |
| $K_2O$ | 0 | 0.94 | 2.20 | 4.39 | 6.59 | 8.79 | 13.18 |

**Table 3: Micronutrients and ammonium (ppm) levels measured in leachate extraction of fallow pots at specific dates (GH Experiment 1- Diagnostics).**

| | Mixed root medium | | | | | SunGro sunshine | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Cu ppm** | **Mn ppm** | **Zn ppm** | **Al ppm** | **NH4-N ppm*** | **Cu ppm** | **Mn ppm** | **Zn ppm** | **Al ppm** | **NH4-N ppm** |
| 3-Sep | 0.2 | 2..9 | 1.7 | 0.1 | 41.1 | 0.2 | 0.0 | 0.4 | 0.1 | 21.4 |
| 10-Sep | 0.0 | 0.3 | 0.4 | 0.1 | 78.0 | 0.1 | 1.5 | 0.6 | 0.1 | 26.3 |
| 17-Sep | 0.2 | 1.5 | 0.2 | 0.0 | 129.7 | 0.0 | 0.3 | 0.5 | 0.2 | 23.0 |
| 29-Sep | 0.2 | 0.8 | 0.1 | 0.0 | 146.7 | 0.0 | 0.9 | 0.4 | 0.5 | 17.4 |

*significant at α= 0.05

**Table 4: Pac choi growth significance from fall 2008 field and high-tunnel studies taken throughout the production period.**

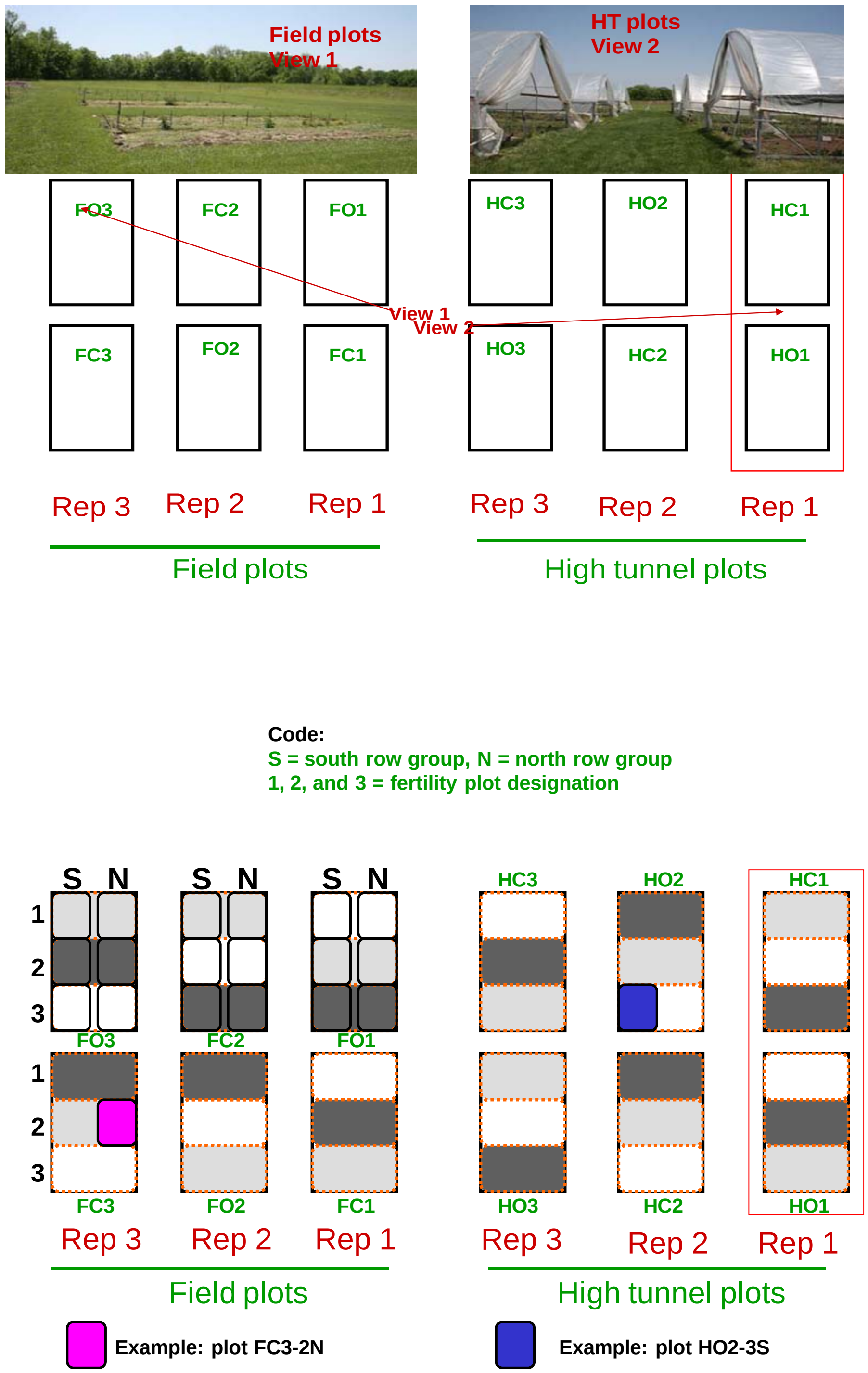
| | **Field** | | | **High tunnel** | | |
|---|---|---|---|---|---|---|
| | **System** | **Fertility** | **Sys* Fert.** | **System** | **Fertility** | **Sys* Fert.** |
| **Fresh weight** | | | | | | |
| **week 2** | NS | NS | NS | NS | NS | NS |
| **week 3** | ** | *** | NS | NS | * | NS |
| **week 4** | **** | **** | NS | * | * | NS |
| **week 5** | ** | **** | NS | NS | ** | *** |
| **Dry weight** | | | | | | |
| **week 2** | NS | NS | NS | *** | ** | NS |
| **week 3** | * | * | NS | * | NS | NS |
| **week 4** | ** | ** | NS | NS | NS | NS |
| **week 5** | * | * | NS | NS | * | * |
| **Petiole sap $NO_3$-N** | | | | | | |
| **week 2** | NS | ** | NS | NS | * | NS |
| **week 3** | * | **** | **** | NS | * | NS |
| **week 4** | NS | *** | NS | NS | NS | NS |
| **week 5** | NS | **** | **** | * | NS | NS |
| **Total tissue N** | | | | | | |
| **week 2** | NS | NS | NS | NS | NS | NS |
| **week 3** | NS | * | NS | * | NS | NS |
| **week 4** | **** | NS | NS | NS | NS | NS |
| **week 5** | NS | NS | NS | NS | NS | NS |

NS, *, **, ***, **** Nonsignificant, or significant at α= 0.05, 0.01, 0.001, and 0.0001 respectively.

**Table 5: Comparison of petiole sap nitrate-nitrogen PSNN for pac choi obtained from greenhouse studies, field and high-tunnel, with published sufficiency ranges for comparable vegetable crops.**

| | Source | wk 2 or early production | wk 3 or mid-production | wk 4 or pre-harvest | wk 5 or harvest |
|---|---|---|---|---|---|
| **Chinese cabbage** | | | | | |
| *summer* | **(Matthaus, D. 2001) Field** | 1469 | 1243 | 904 | NA |
| *Fall* | | 1356 | 1130 | 904 | 678 |
| **Broccoli & Collards** | **Hochmuth, G. 2007) Field** | 1000 | 800 | 500 | 300 |
| **Broccoli** | **(Schulbach, 95 CalDeptAg) Field** | 1600 | 1200 | 800-1000 | 600 |
| **Cabbage** | | 1500 | 1200 | 1000 | 900 |
| **Lettuce** | | 600 | 500 | 400 | 350 |
| **Pac choi / summer** | **Greenhouse study1** | 1000-1500 | | 800-1000 | |
| **Pac choi / fall** | **Greenhouse study 2*** | 1500-2000* | | 1000-1500* | |
| **Pac choi / fall** | **Field study** | 800-1200 | | 500-800 | |
| **Pac choi / fall** | **High tunnel study** | 800-1100 | | 500-700 | |

*Sufficiency ranges are higher than obtained from GH 1. Back to the text for explanations.



**Code:**
S = south row group, N = north row group
1, 2, and 3 = fertility plot designation

**Figure 2-1: Diagram showing the latin square design layout of the organic and conventional plots (system), split plot design of fertility levels (N fertility rates), non-statistical comparison (field and high-tunnel).**

Source: Powerpoint presentation prepared by Dr. kim Williams.

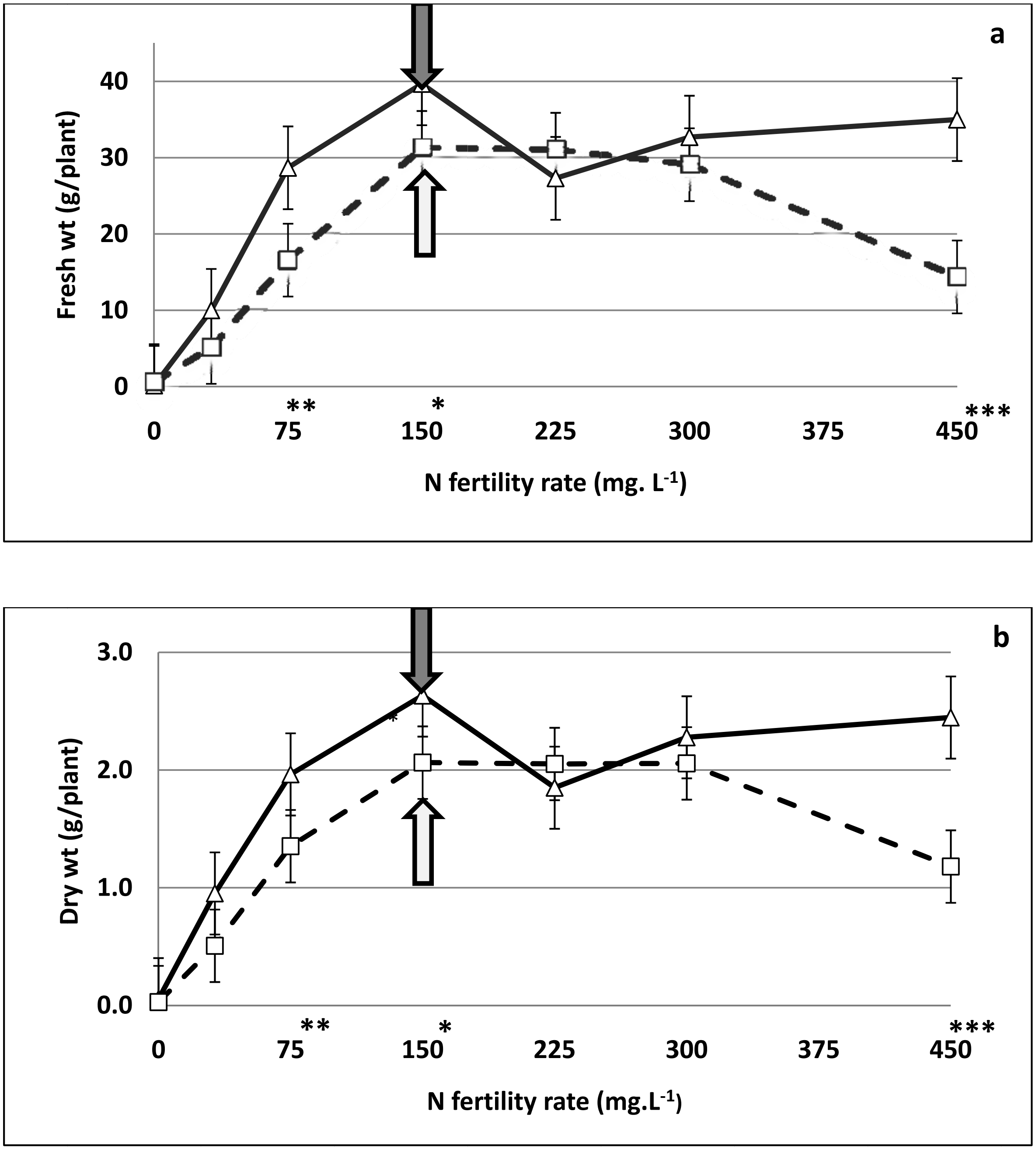


**Figure 2-2: Pac choi fresh (a) and dry (b) weight (g/plant) at harvest for greenhouse study 2 at different nitrogen fertility levels for conventional (∆) and organic ( ) treatments. Arrows show the point at which there were no significant differences between N fertility rate and the next higher nutrient level for conv ⇩ and org ⇧ treatments. *, **, ***, and **** show significant differences between conv, and org at α=0.05, 0.01, 0.001, and 0.0001, respectively.**

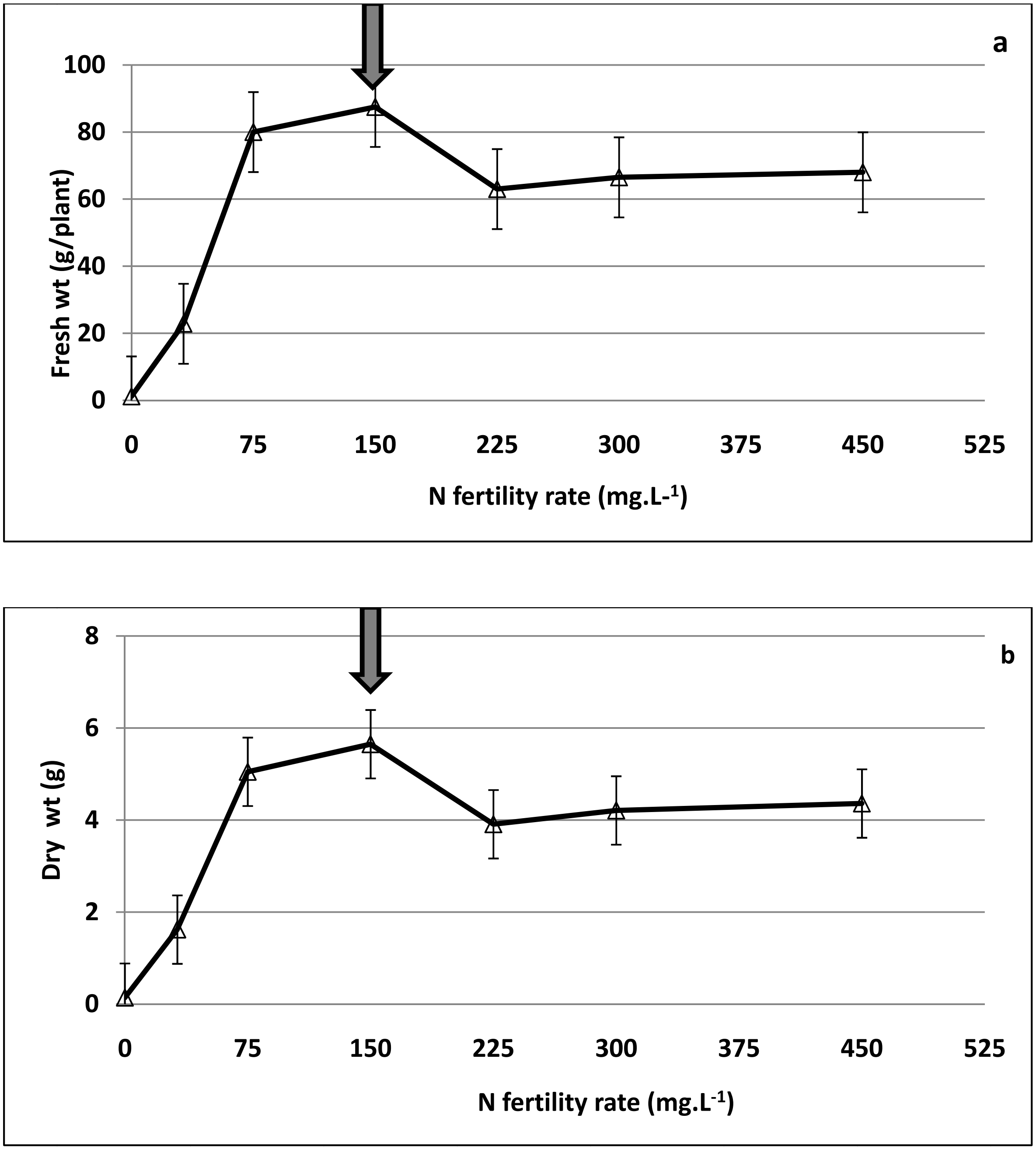


**Figure 2-3: Pac choi fresh (a) and dry (b) weight (g/plant) at harvest for greenhouse study 1 at different nitrogen fertility levels for conventional (∆) treatments. Arrow shows the point at which there were no significant differences between N fertility rate and conv ⇩ treatments.**

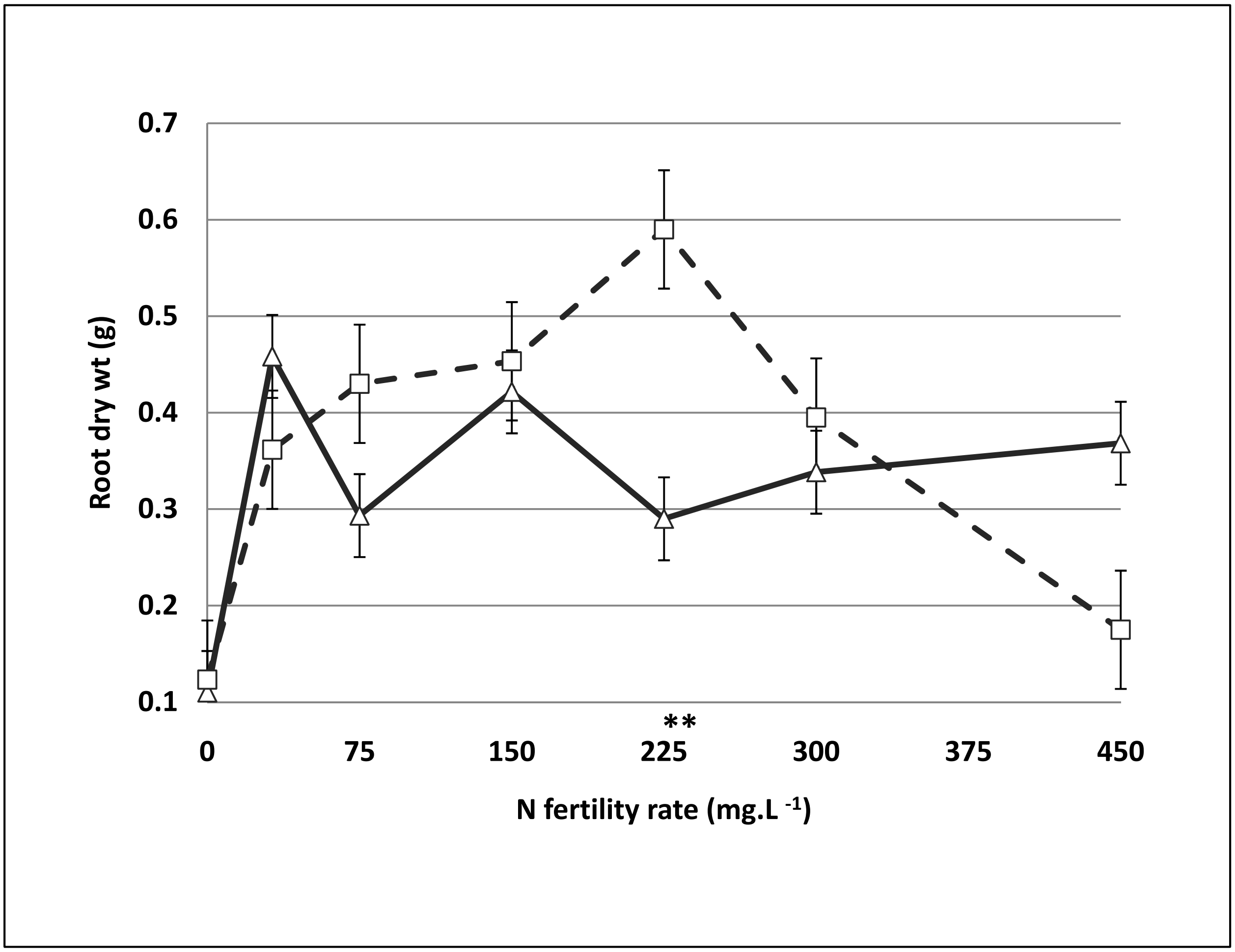


**Figure 2-4: Pac choi root dry weight (g/plant) for greenhouse study 2 at harvest for conventional (∆) and organic (   ) treatments. No significant differences among N fertility rates. *, **, ***, and **** show significant differences between conv, and org at α=0.05, 0.01, 0.001, and 0.0001, respectively.**

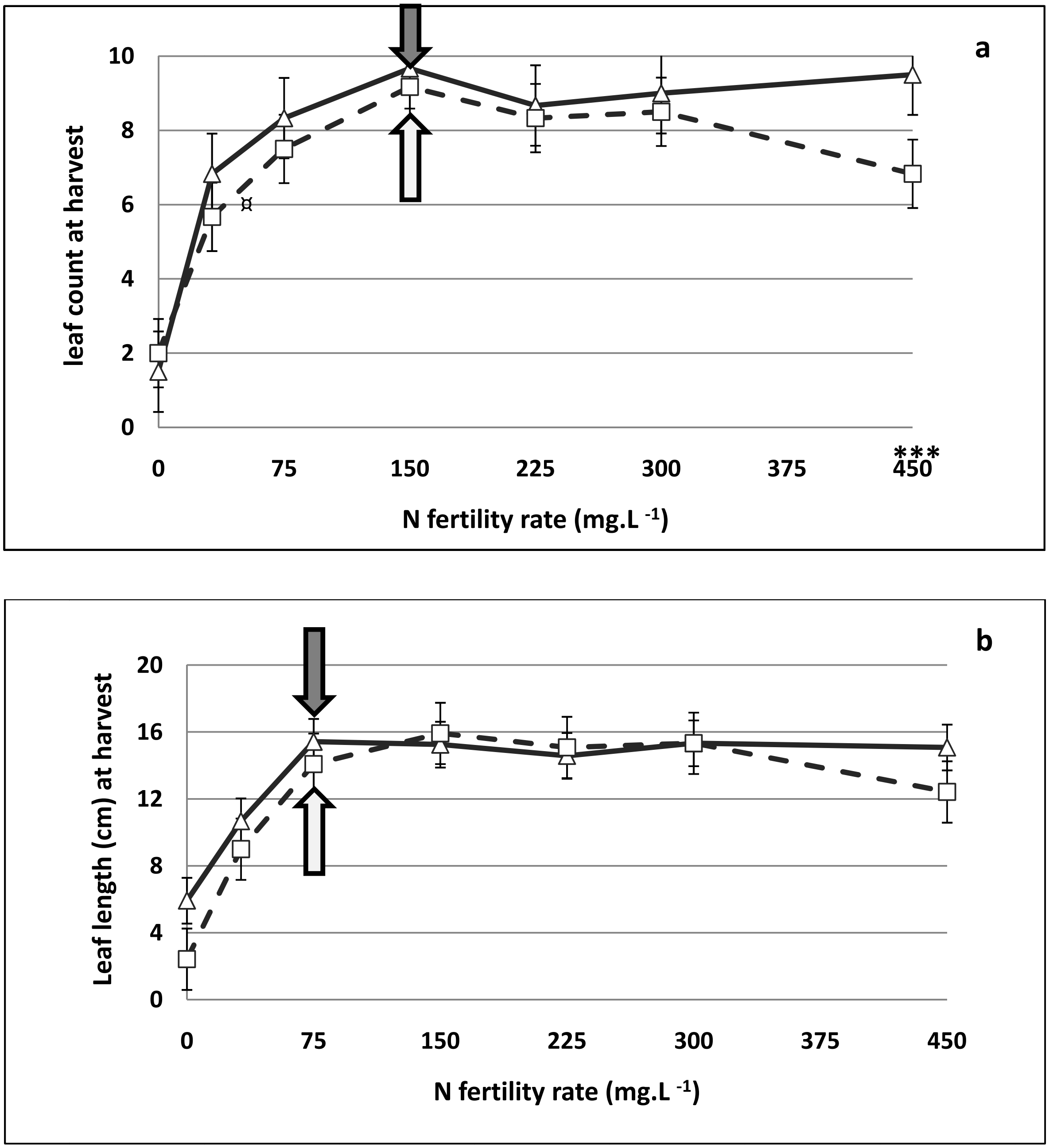


**Figure 2-5: Pac choi count of leaf (a) and leaf length (cm) (b) at harvest for greenhouse study 2 at different fertility levels for conventional (∆) and organic ( ) treatments. Arrows show the point at which there were no significant differences between N fertility rate and the next higher nutrient level for conv ⬇ and org ⇧ treatments. *, **, ***, and **** show significant differences between conv. and org at α= 0.05, 0.01, 0.001, and 0.0001, respectively.**

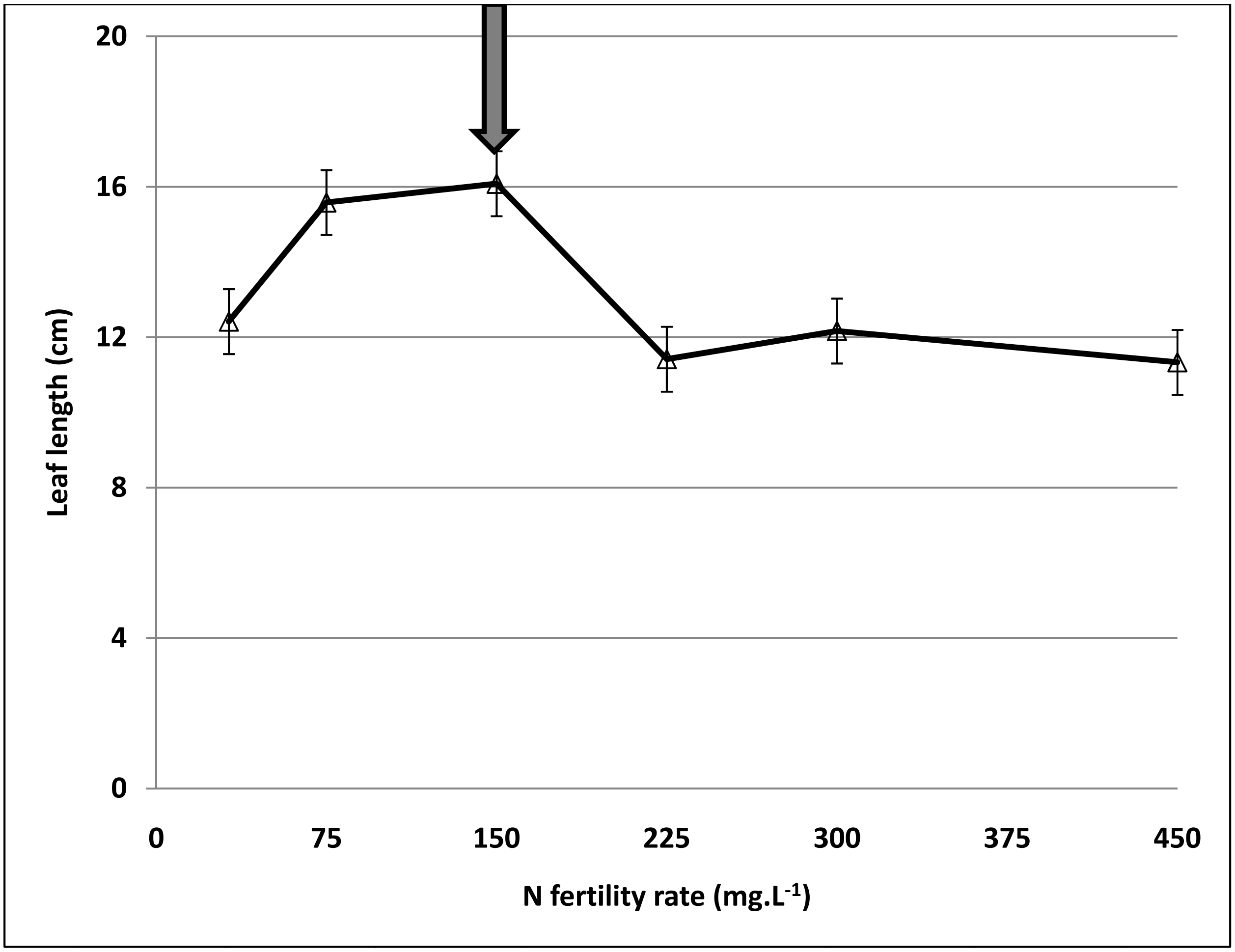


**Figure 2-6: Pac choi leaf count (a) and leaf length (cm) (b) at harvest for greenhouse study 2 at different fertility levels for conventional (Δ) and organic ( ) treatments. Arrows show the point at which there were no significant differences between N fertility rate and the next higher nutrient level for conv ⬇ and org ⇧ treatments. *, **, ***, and **** show significant differences between conv. and org at α= 0.05, 0.01, 0.001, and 0.0001, respectively.**

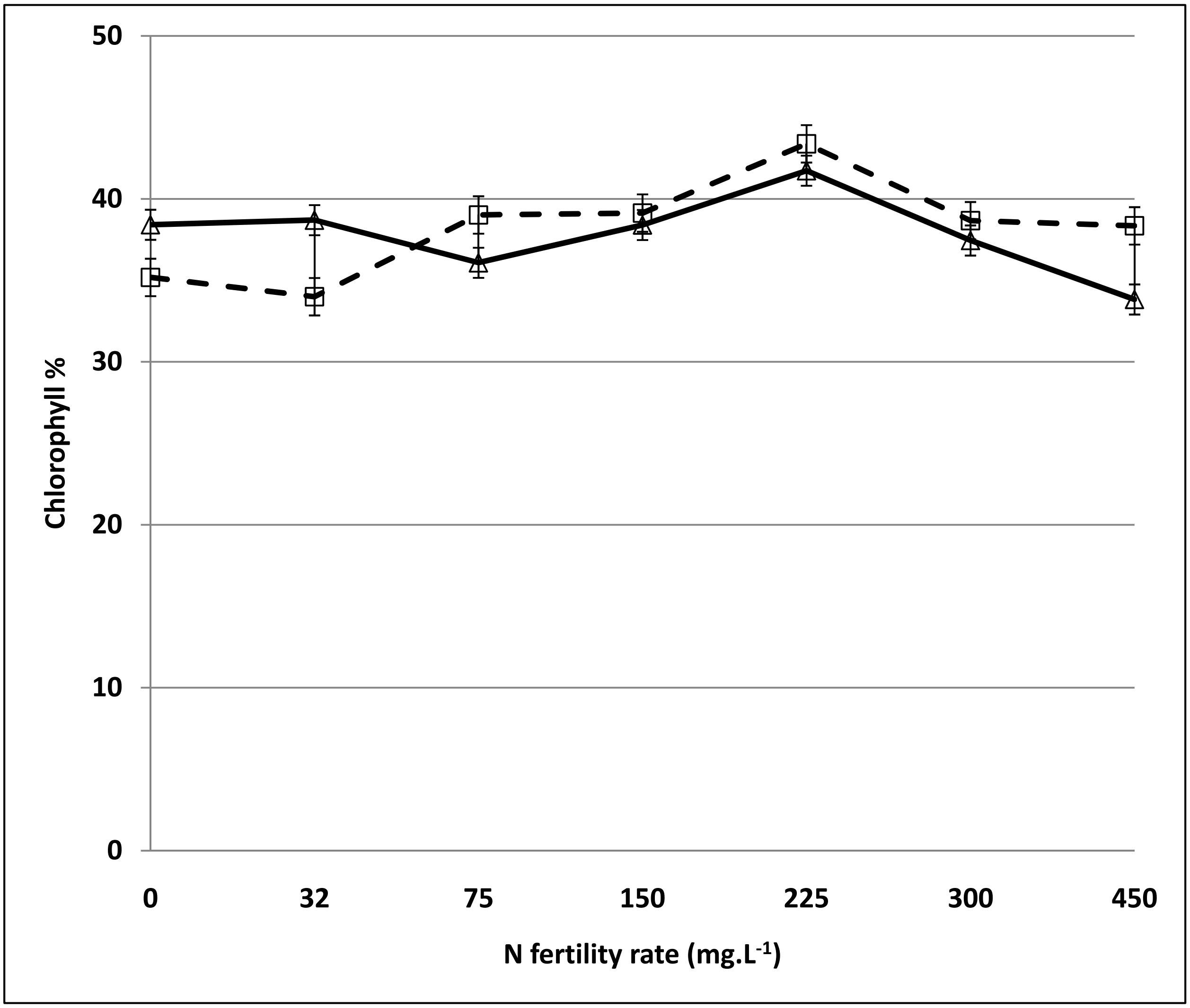


**Figure 2-7: Pac choi chlorophyll % at harvest in greenhouse study 2 at different fertility levels for conventional (∆) and organic ( ) treatments. No significant differences among N fertility rates. *, **, ***, and **** show significant differences between conv, and org at α= 0.05, 0.01, 0.001, and 0.0001, respectively.**

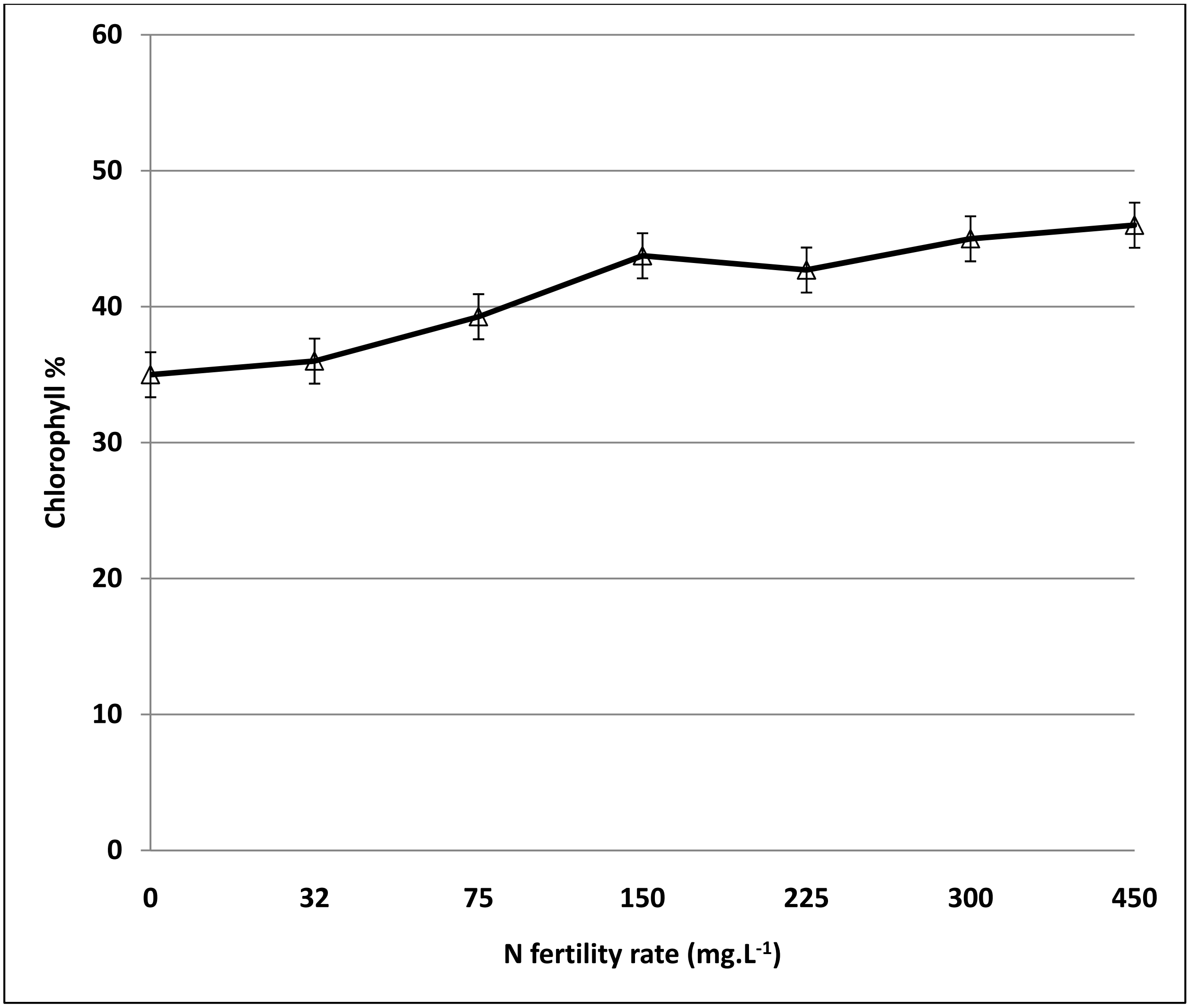


**Figure 2-8: Pac choi chlorophyll % at harvest in greenhouse study 1 at different fertility levels for conventional treatments (∆). No significant differences among N fertility rates.**

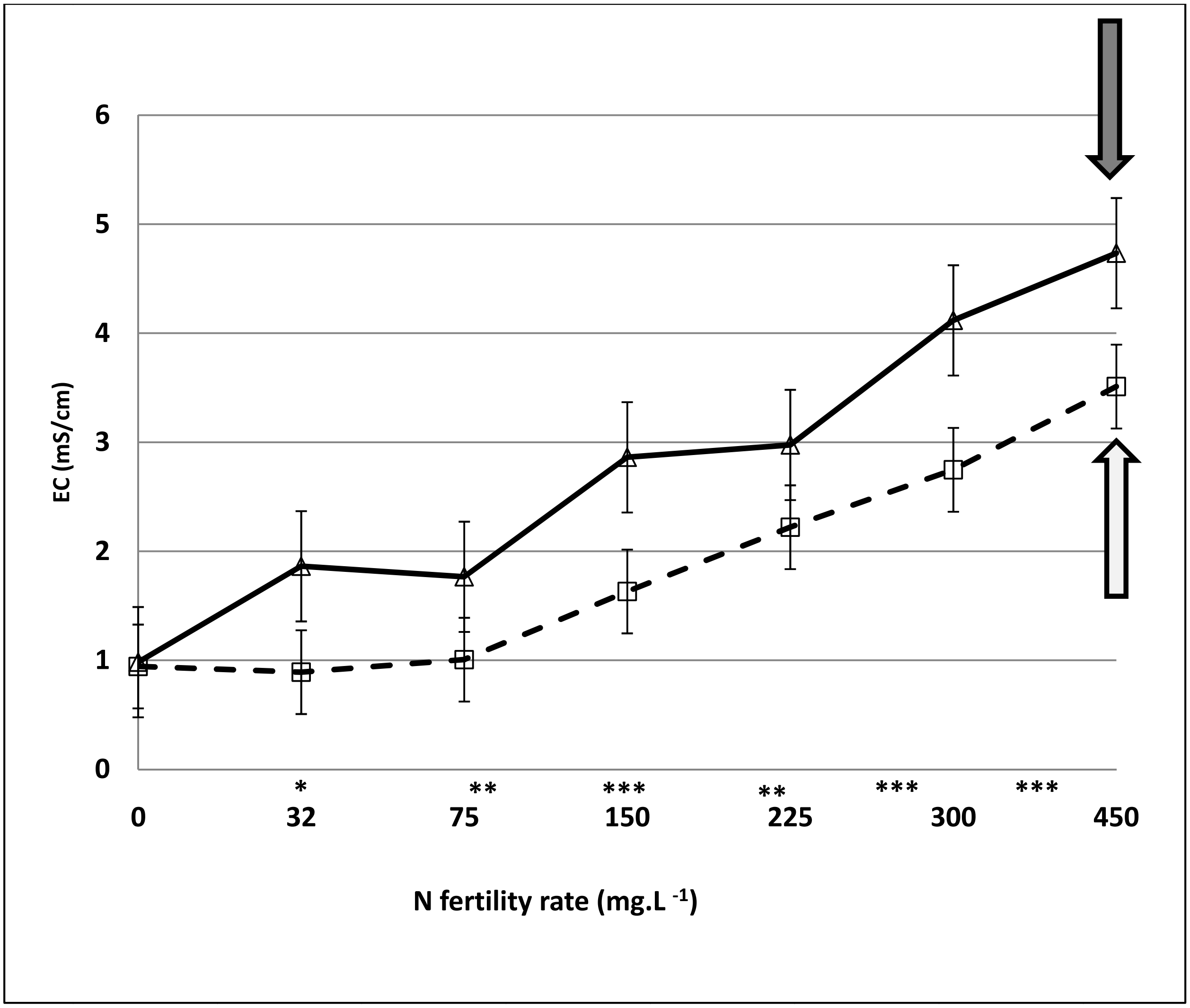


**Figure 2-9: Pac choi electrical conductivity (mS/cm) measured on saturated media extract at harvest for greenhouse study 2 for conventional (∆) and organic ( ) treatments. Arrows show the point at which there were no significant differences between N fertility rate and the next higher nutrient level for conv ⇩ and org ⇧. *, **, ***, and **** show significant differences between conv. and org at α= 0.05, 0.01, 0.001, and 0.0001, respectively.**

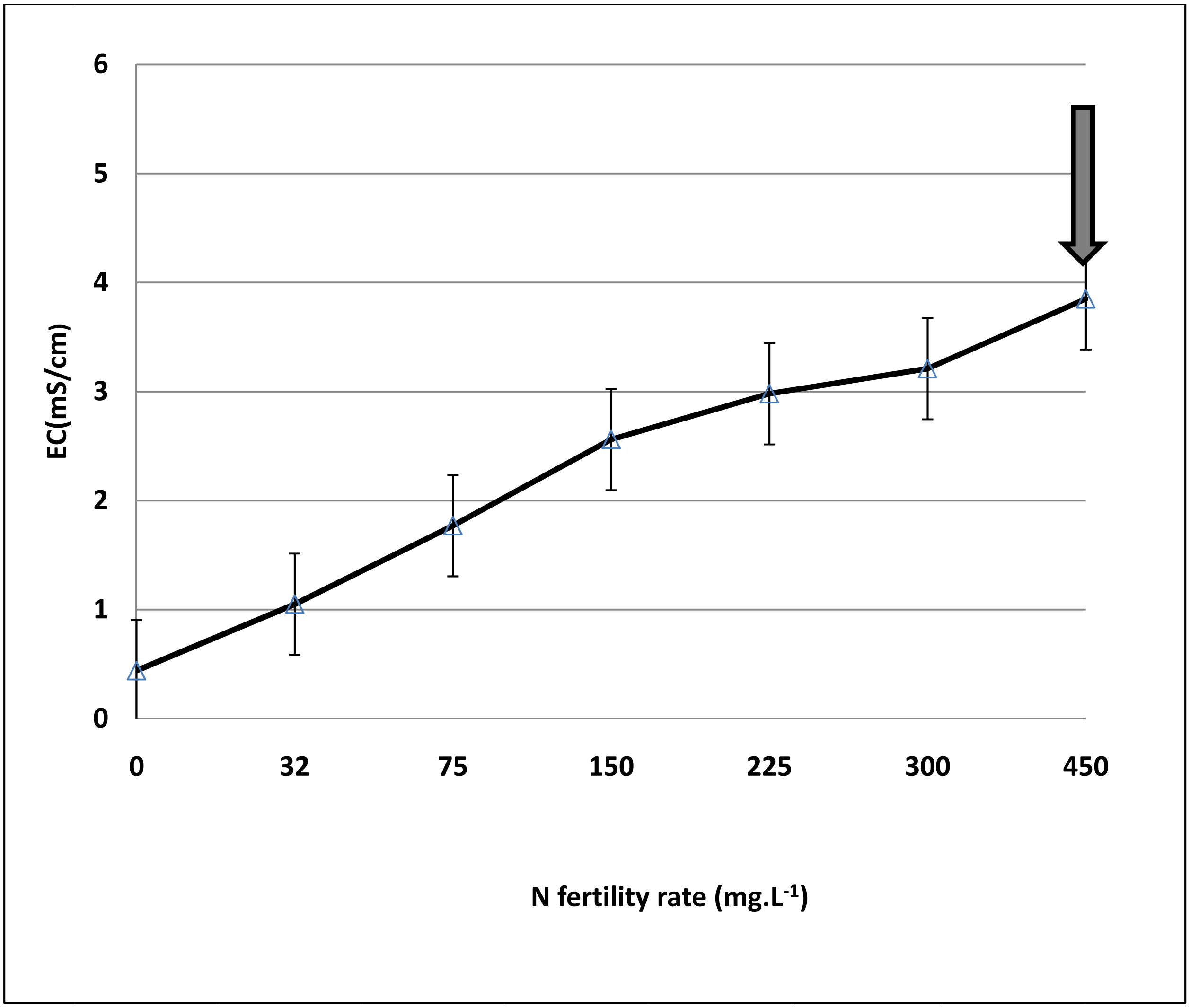


**Figure 2-10 Pac choi electrical conductivity (mS/cm) measured on saturated media extract at harvest for greenhouse study 1 for conventional (Δ) treatments. Arrow shows the point at which there were no significant differences between N fertility rate and the next higher nutrient level for conv ⇩ treatments.**

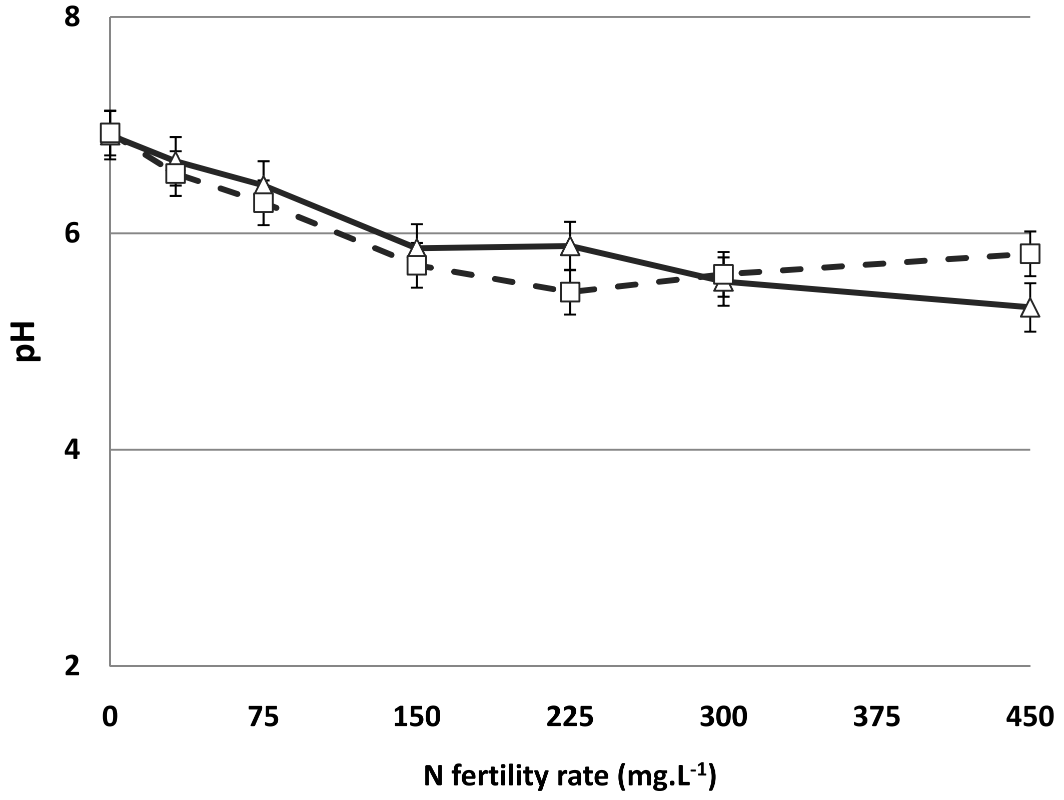
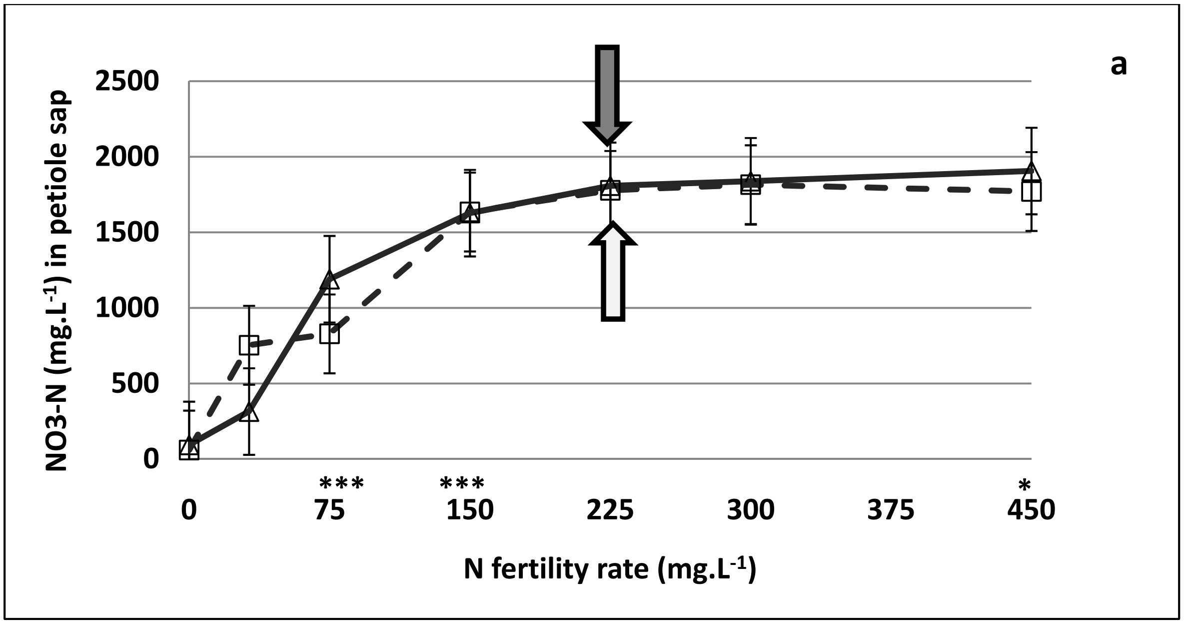
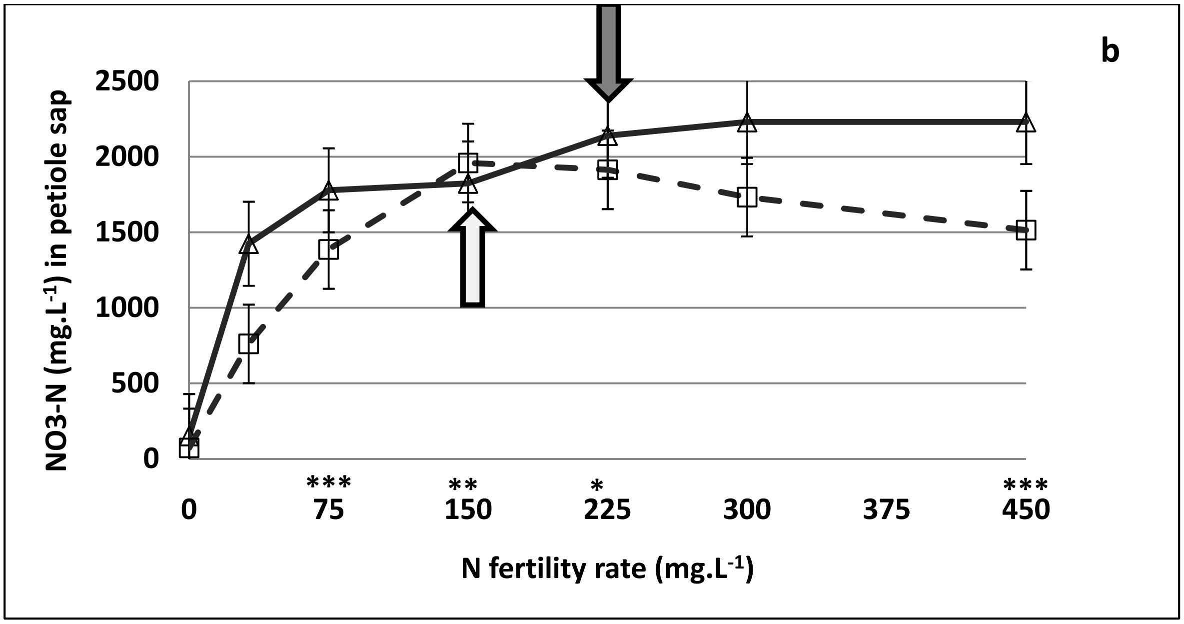


**Figure 2-11: Pac choi pH measured on saturated media extract at harvest for greenhouse study 2 for conventional (∆) and organic (□) treatments. No significant differences among N fertility levels.**


a
NO3-N (mg.L-1) in petiole sap
2500
2000
1500
1000
500
0
***
***
*
0
75
150
225
300
375
450
N fertility rate (mg.L-1)


b
NO3-N (mg.L-1) in petiole sap
2500
2000
1500
1000
500
0
***
**
*
***
0
75
150
225
300
375
450
N fertility rate (mg.L-1)

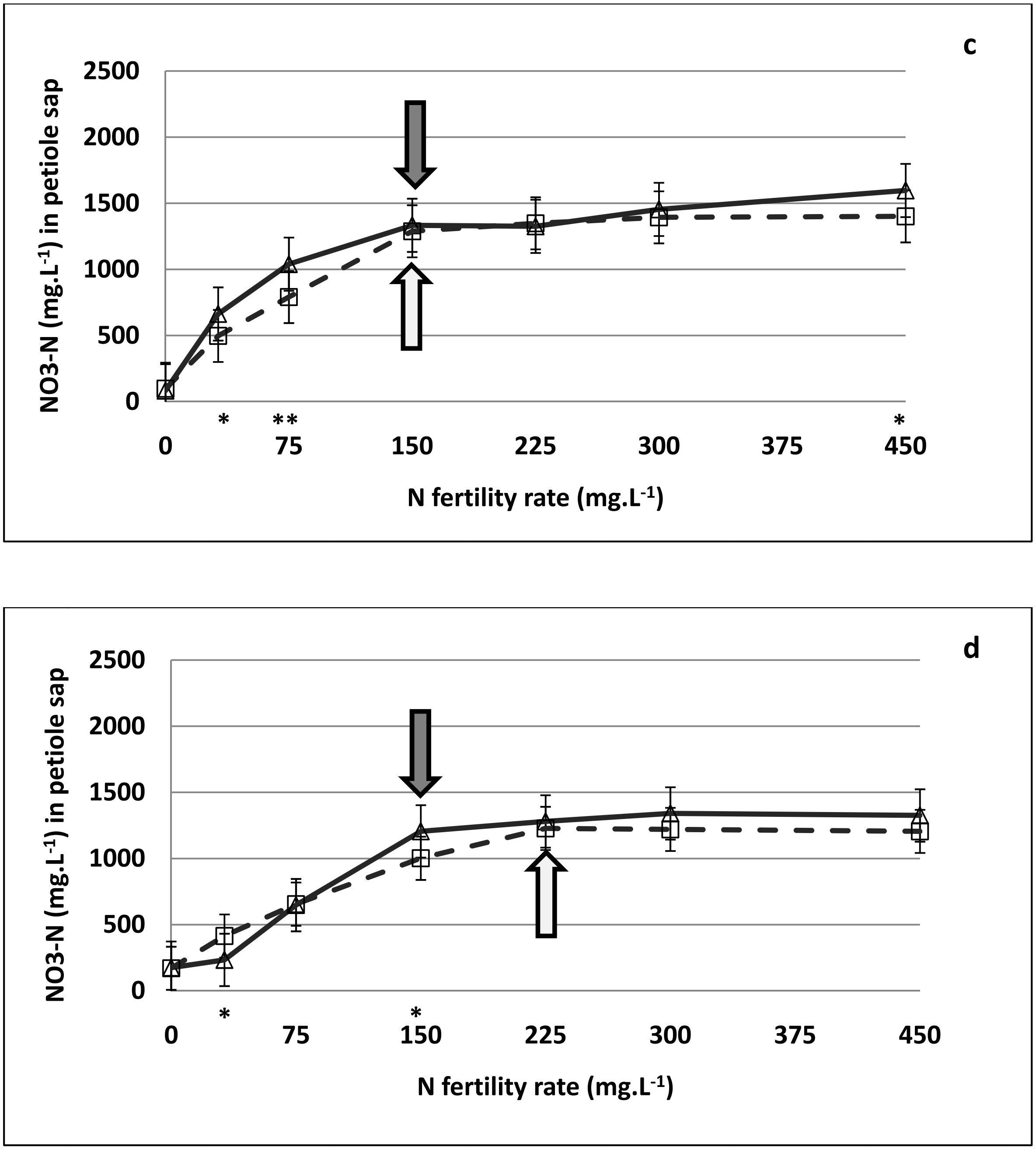
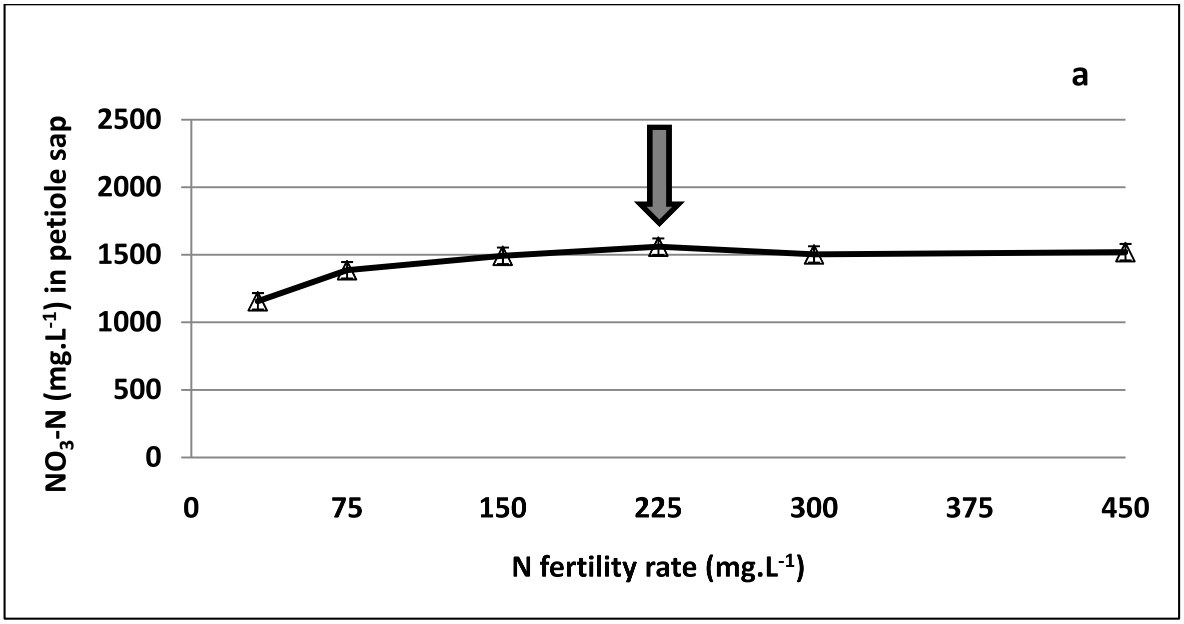
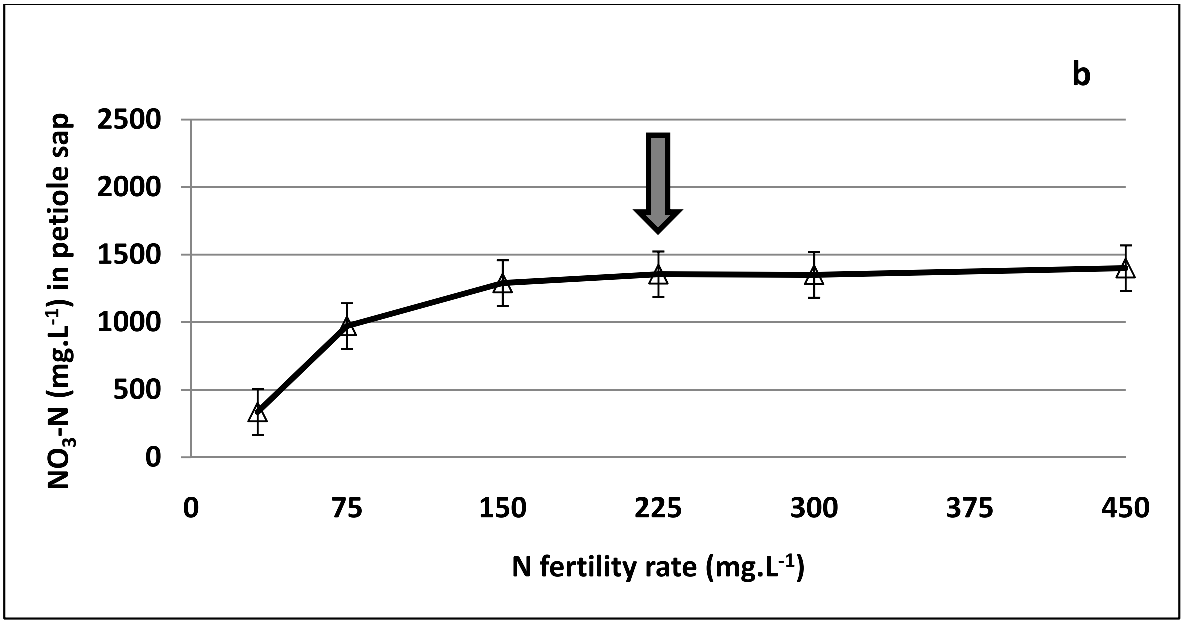


**Figure 2-12: Pac choi petiole sap nitrate-nitrogen levels (mg.L$^{-1}$) in greenhouse study 2 for conventional (∆) and organic ( ) treatments at week 2 (a), week 3 (b), week 4 (c), and week 5 (d). Arrows show the point at which there were no significant differences between N fertility rate and the next higher nutrient level for conv ⬇ and org ⇧. *, **, ***, and **** show significant differences between conv, and org at α= 0.05, 0.01, 0.001, and 0.0001, respectively.**


a
2500
2000
1500
1000
500
0
0
75
150
225
300
375
450
N fertility rate (mg.L-1)
NO3-N (mg.L-1) in petiole sap


b
2500
2000
1500
1000
500
0
0
75
150
225
300
375
450
N fertility rate (mg.L-1)
NO3-N (mg.L-1) in petiole sap

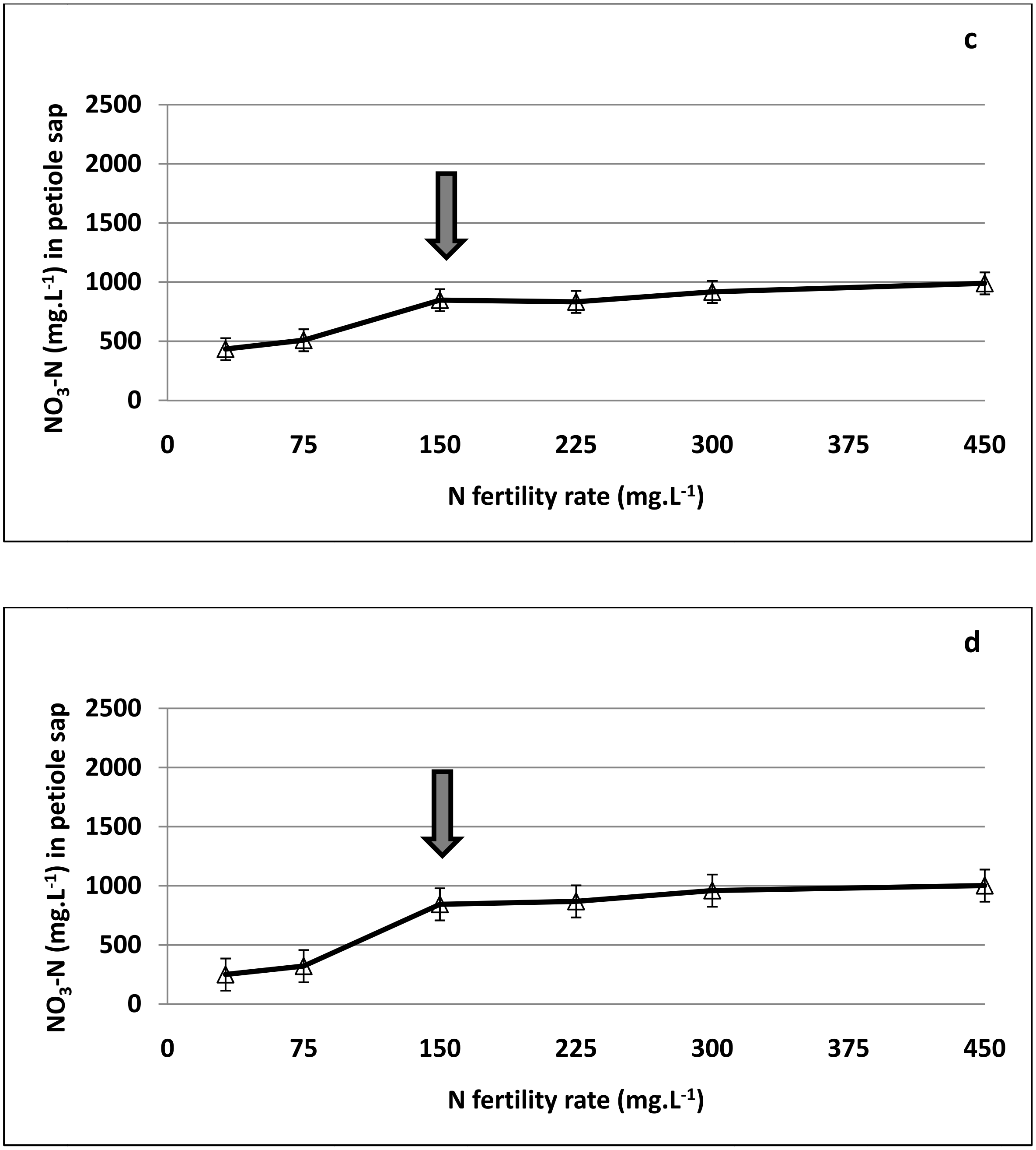
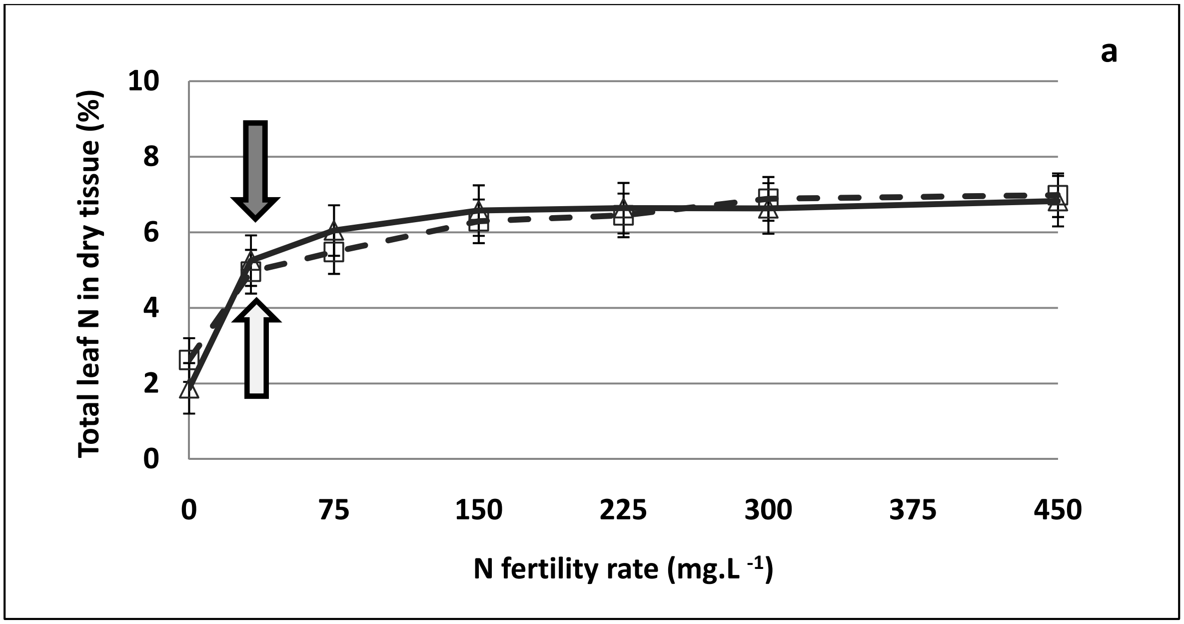
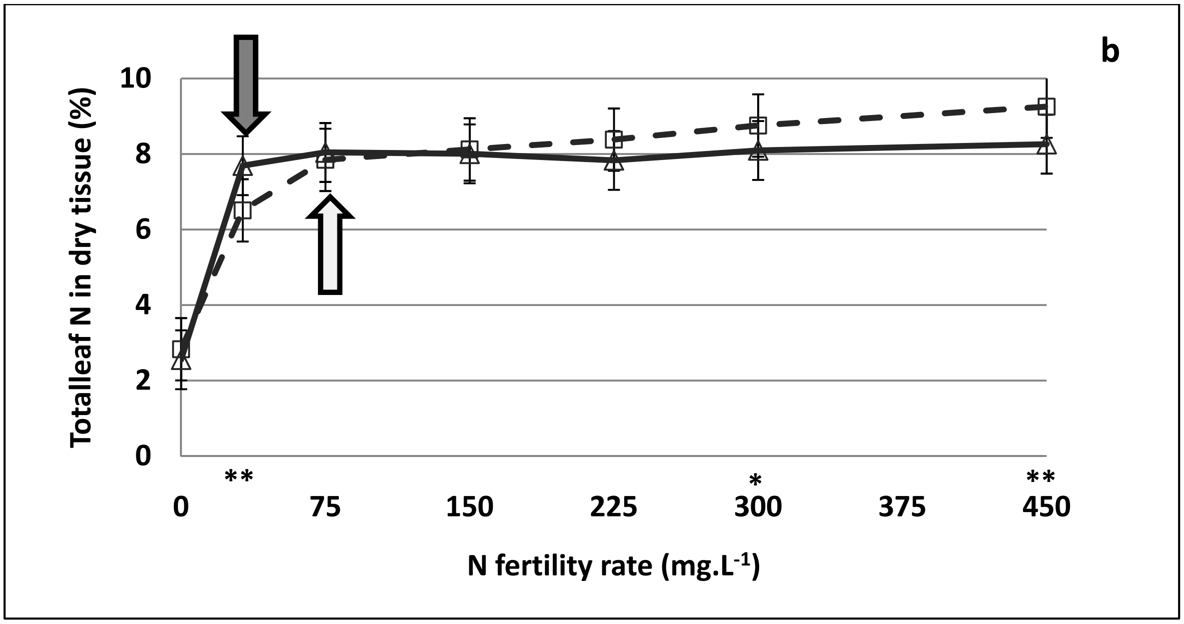


**Figure 2-13: Pac choi petiole sap nitrate levels (mg.L$^{-1}$) in greenhouse study 1 for conventional (∆) treatments at week 2 (a), week 3 (b), week 4 (c), and week 5 (d). Arrow shows the point at which there were no significant differences between N fertility rate and the next higher nutrient level conv ⇩ treatments.**


a
Total leaf N in dry tissue (%)
0
2
4
6
8
10
0
75
150
225
300
375
450
N fertility rate (mg.L -1)


b
Totalleaf N in dry tissue (%)
0
2
4
6
8
10
**
*
**
0
75
150
225
300
375
450
N fertility rate (mg.L-1)

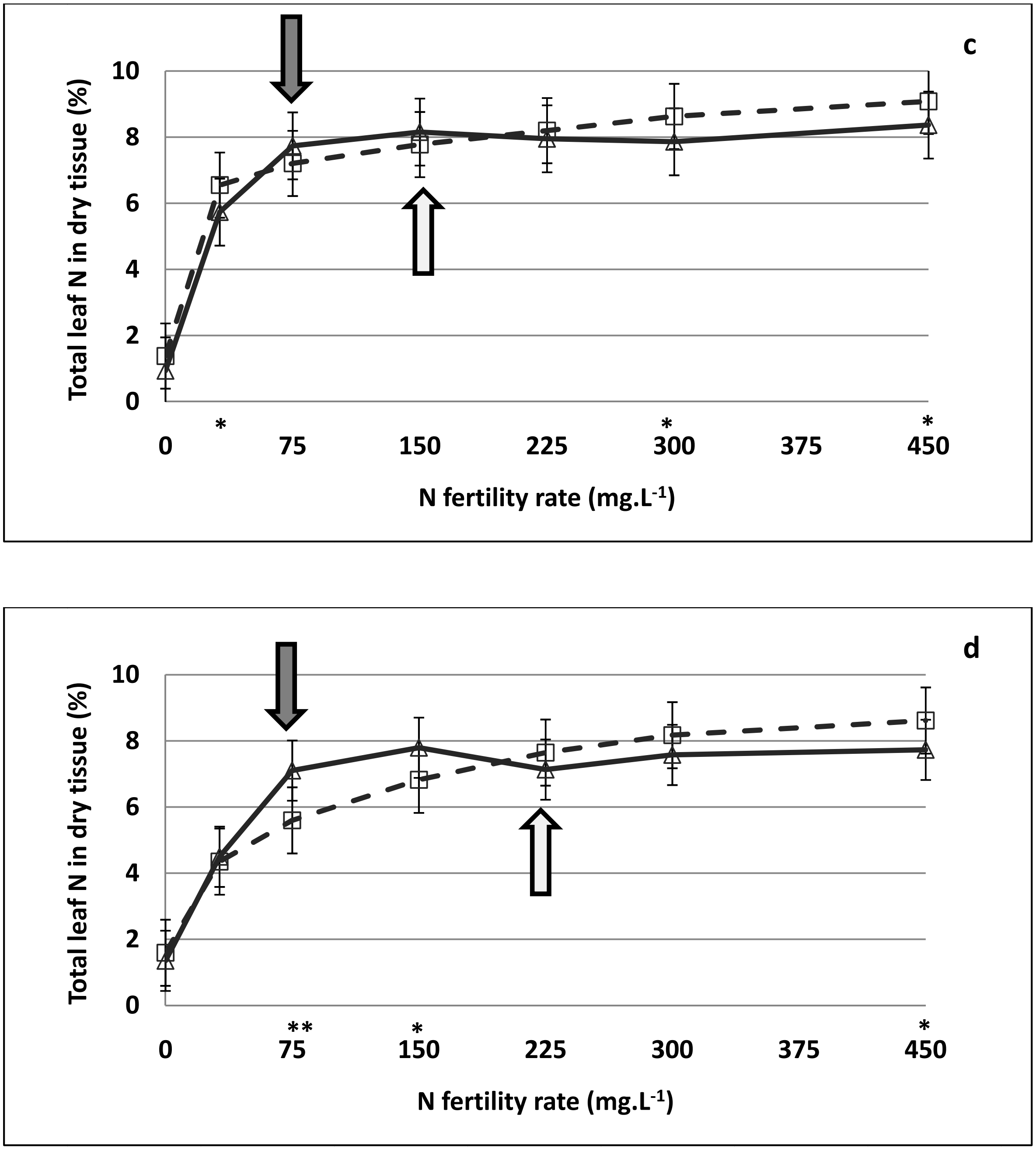


**Figure 2-14: : Total N % in pac choi leaves in greenhouse study 2 for conventional (∆) and organic ( )treatments at week 2 (a), week 3 (b), week 4 (c), and week 5 (d). Arrows show the point at which there were no significant differences between N fertility rate and the next higher nutrient level for conv ⇩ and org ⇧. *, **, ***, and **** show significant differences between conv. and org at α= 0.05, 0.01, 0.001, and 0.0001, respectively.**

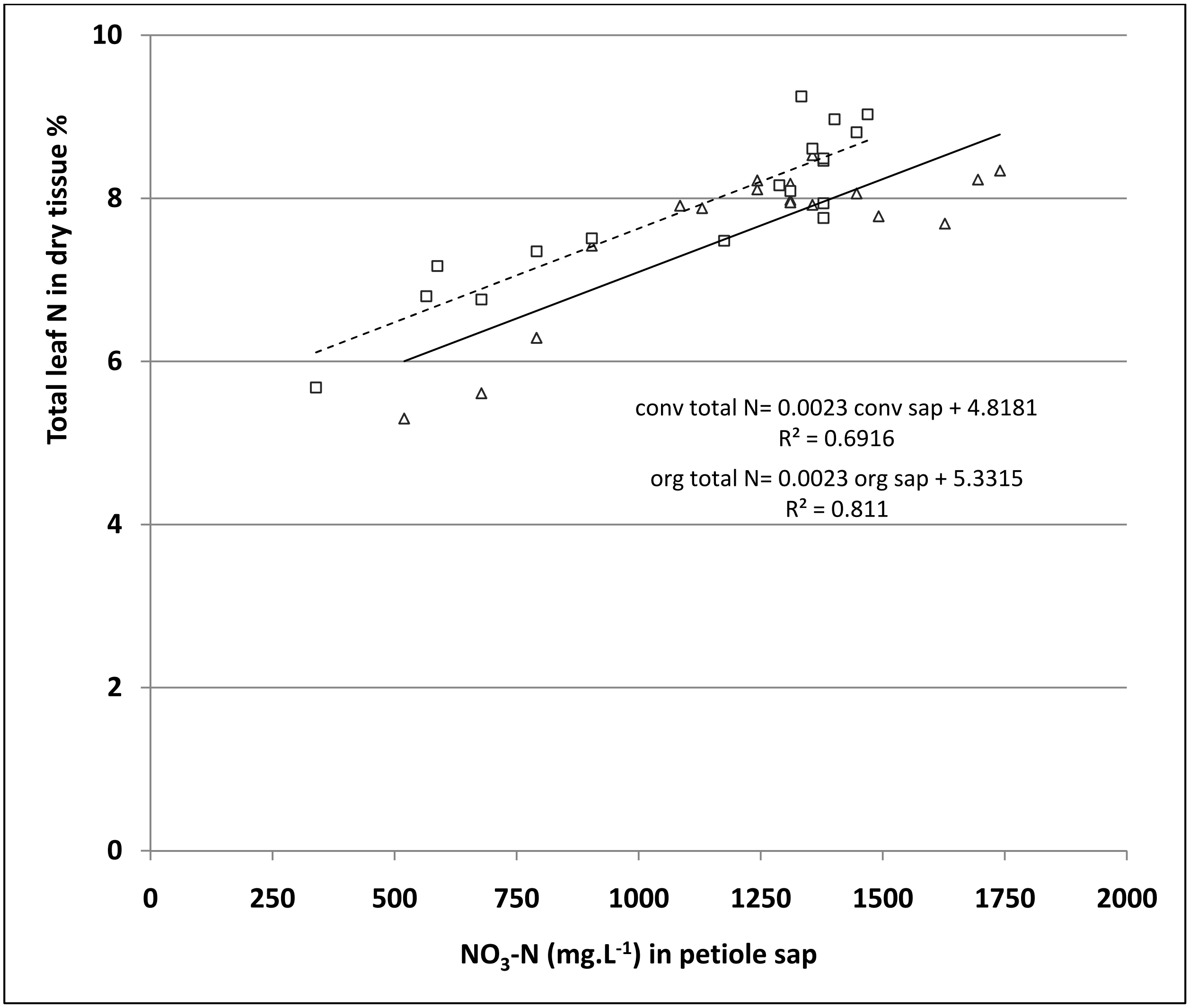
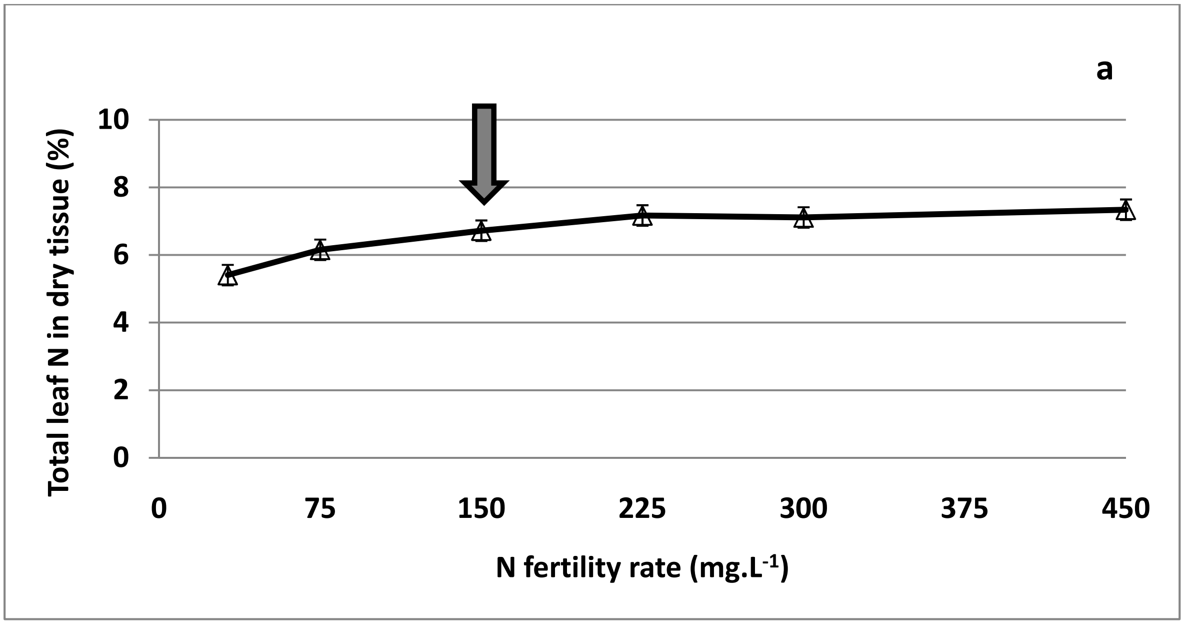
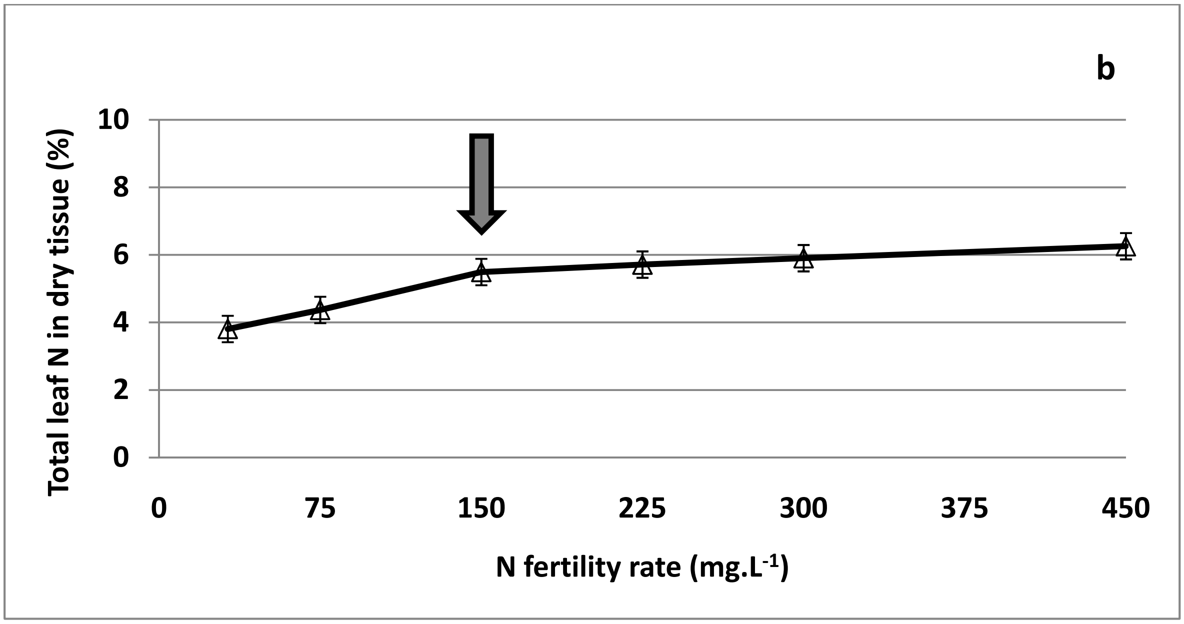


**Figure 2-15: Linear relationship between total leaf N % and PSNN (mg.$L^{-1}$), and the regression equations for greenhouse study 2 (conventional (Δ) and organic ( ) treatments) calculated at week 3.**


a
Total leaf N in dry tissue (%)
10
8
6
4
2
0
0
75
150
225
300
375
450
N fertility rate (mg.L-1)


b
Total leaf N in dry tissue (%)
10
8
6
4
2
0
0
75
150
225
300
375
450
N fertility rate (mg.L-1)

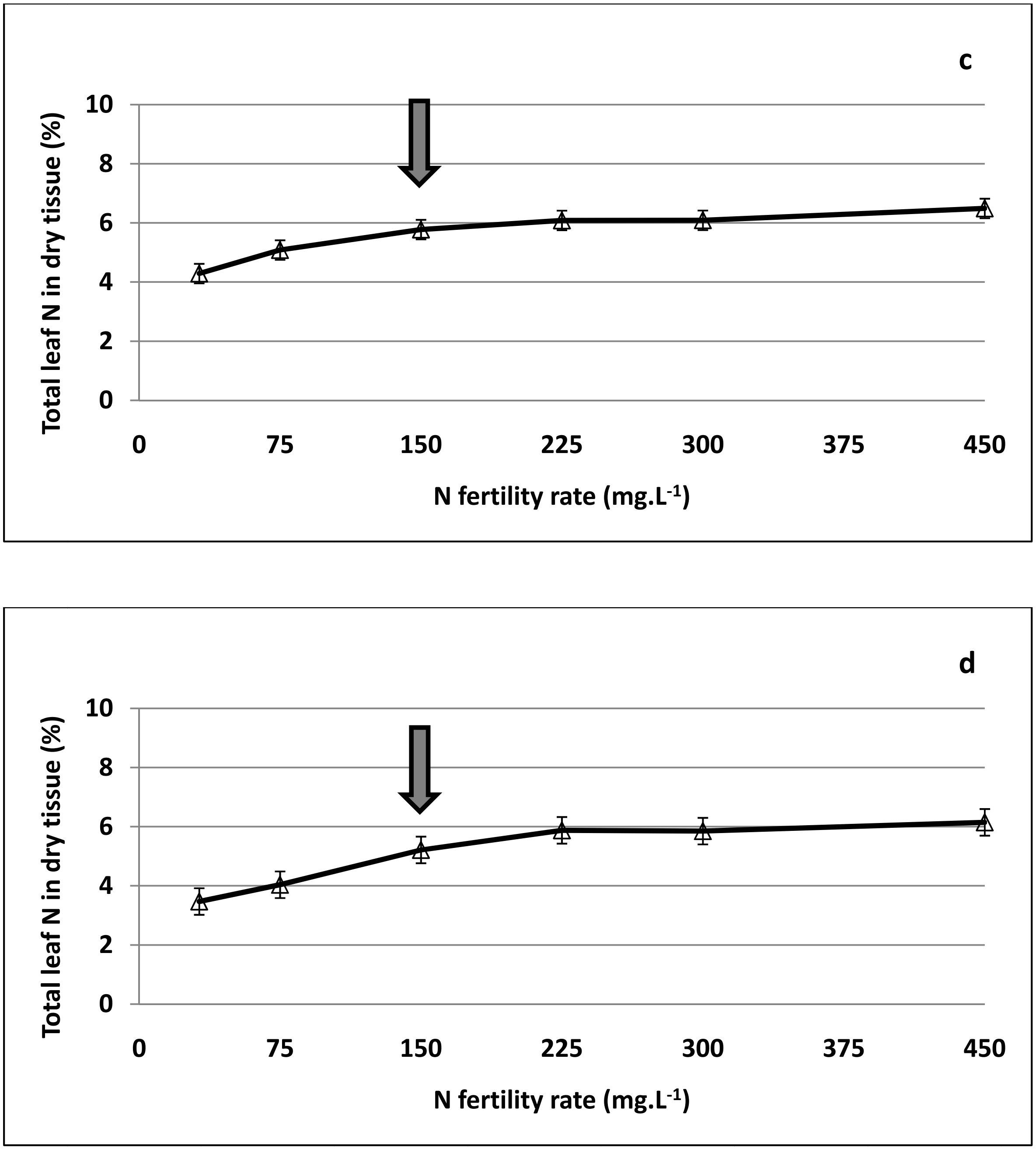


**Figure 2-16: Total N % in pac choi leaves in greenhouse study 1 for conventional (Δ) treatment at week 2 (a), week 3 (b), week 4 (c), and week 5 (d). Arrow shows the point at which there were no significant differences between N fertility rate and the next higher nutrient level conv ⇩ treatment.**

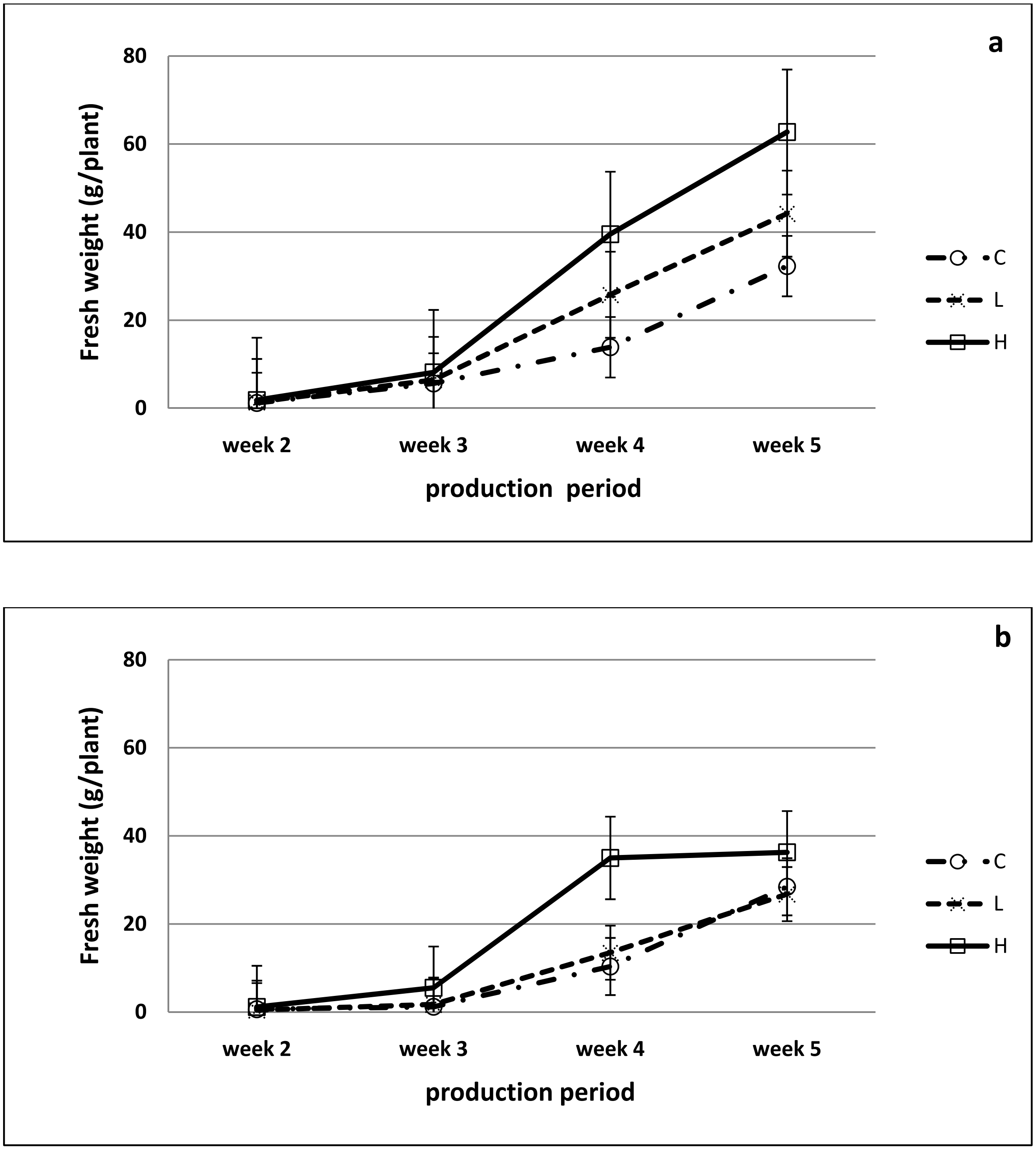


**Figure 2-17: Pac choi fresh weight (g/plant) during production period at field organic (a) and conventional (b) plots for different N fertility rate (control, low, and high) treatments. Fertility effect is significant at α= 0.0001.**

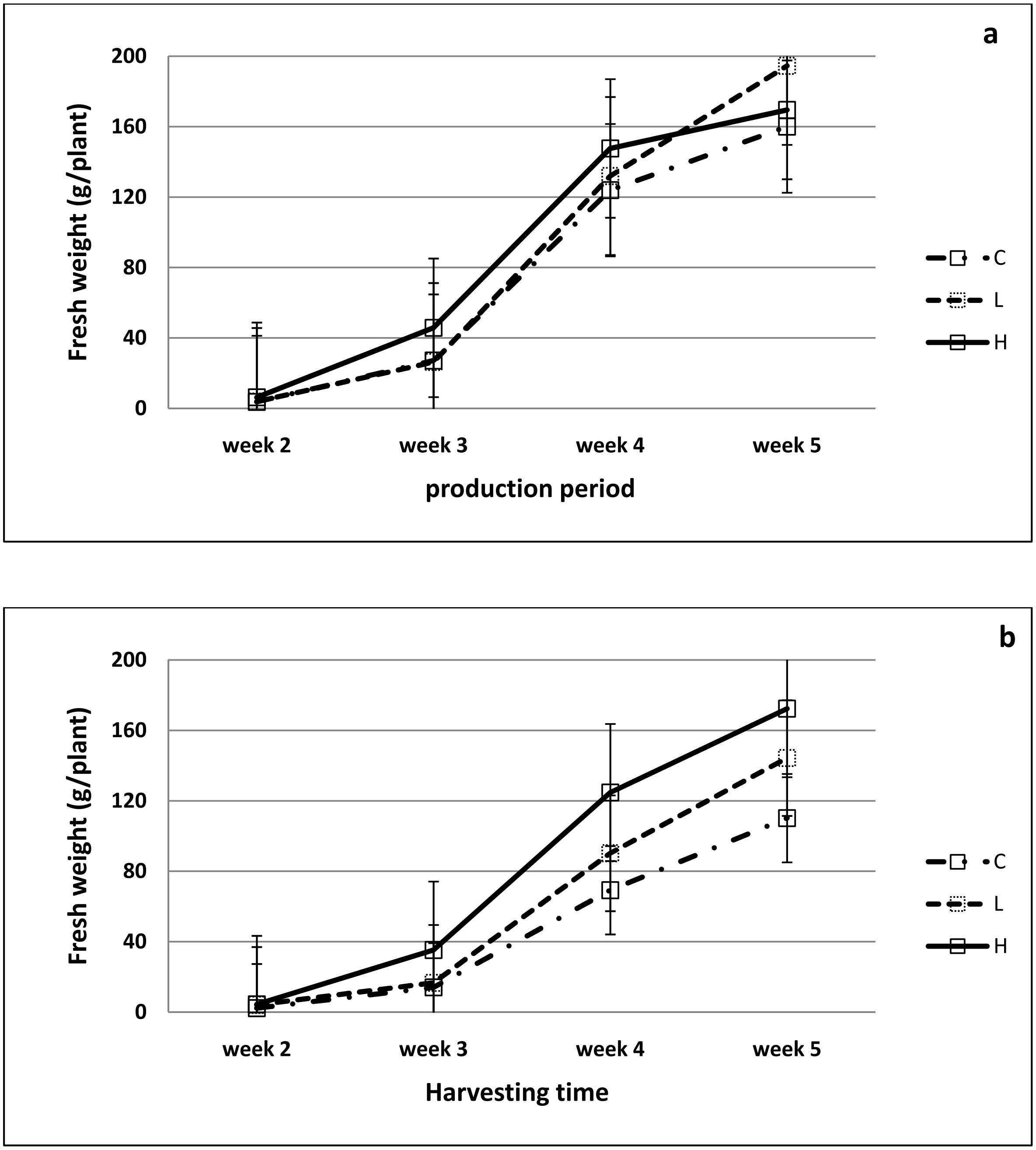


**Figure 2-18: Pac choi fresh weight (g/plant) during production period at high-tunnel organic (a) and conventional (b) plots for different N fertility rate (control, low, and high) treatments. Fertility effect is significant at weeks 2 and 4.**

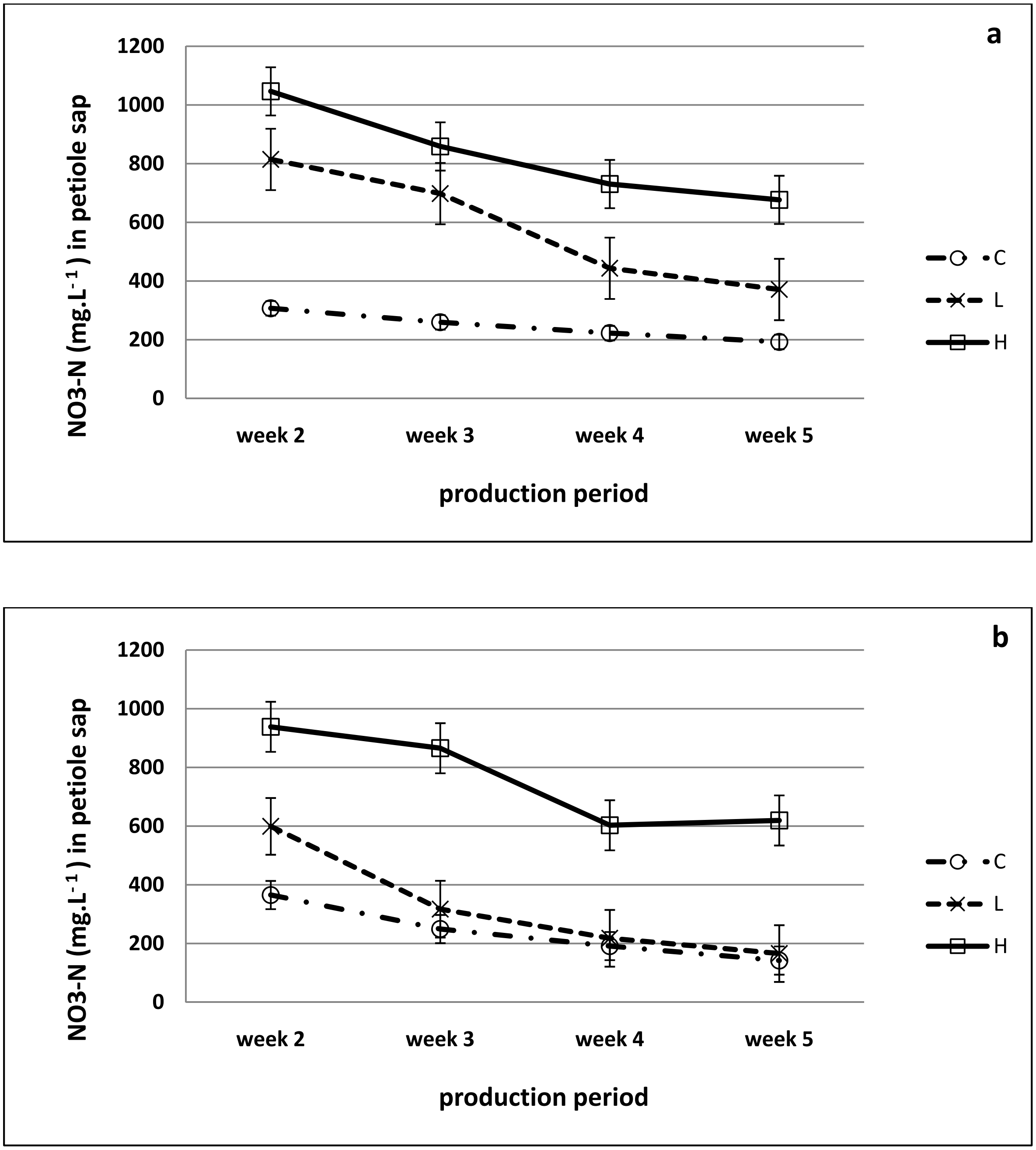


**Figure 2-19: Pac choi petiole sap nitrate-nitrogen level (mg.L$^{-1}$) during production period in field organic (a) and conventional (b) plots for different N fertility rate (control, low, and high) treatments. Fertility effect is significant at α= 0.02.**

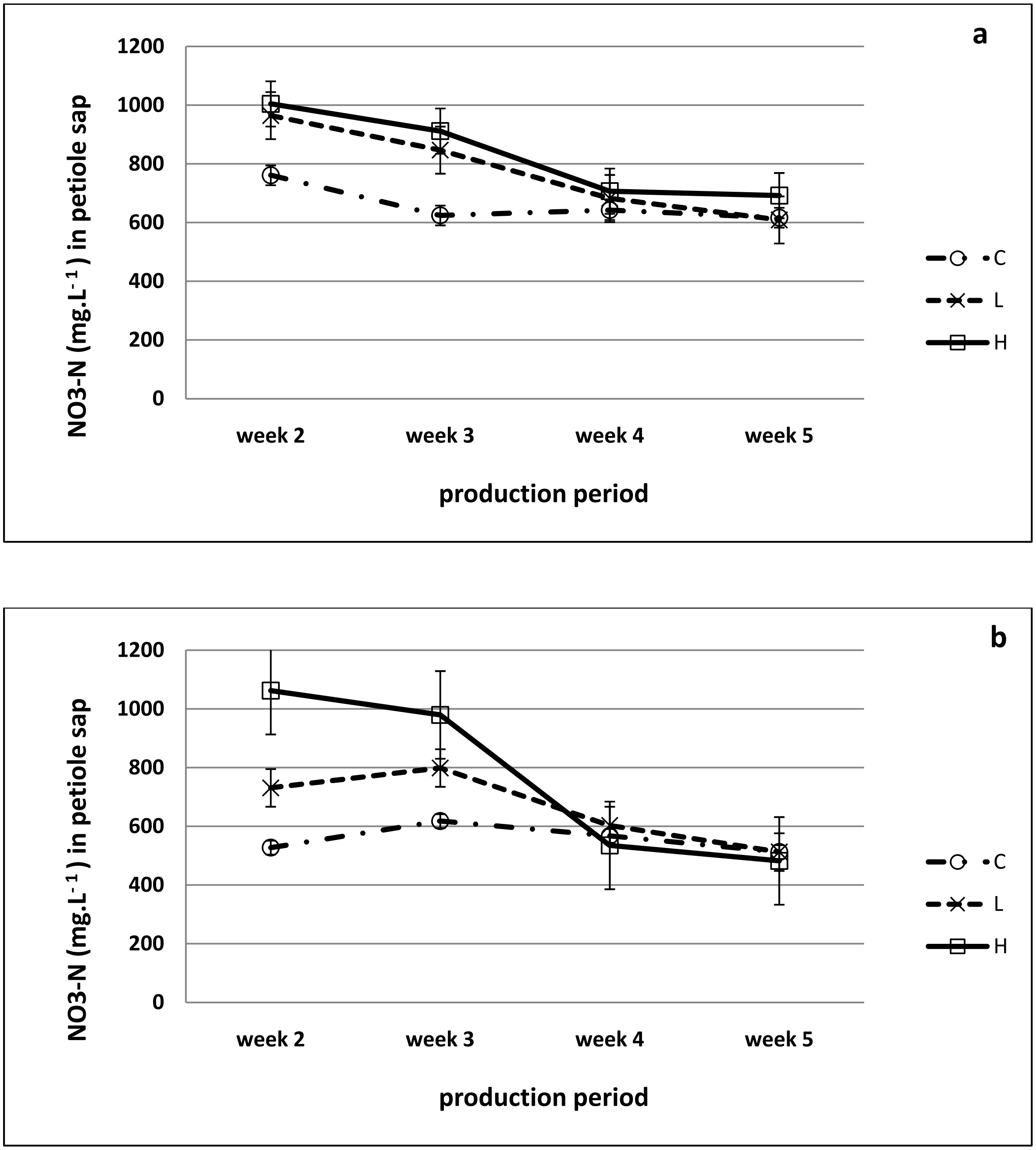


**Figure 2-20: Pac choi petiole sap nitrate-nitrogen level (mg.L$^{-1}$) during production period in high-tunnel organic (a) and conventional plots for different N fertility rate (control, low, and high) treatments. Fertility effect is significant at weeks 2 and 3.**

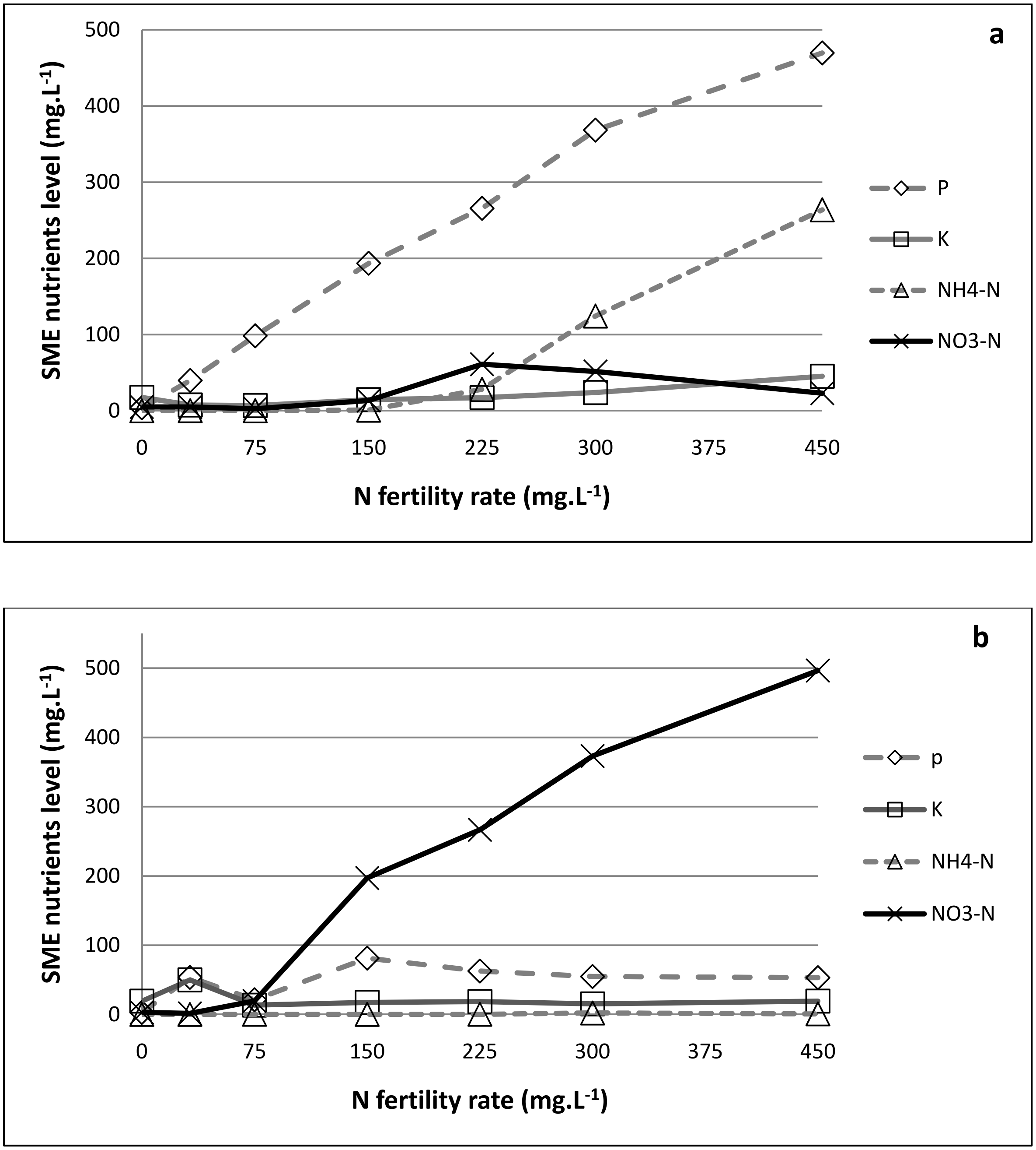


**Figure 2-21: N-P-K level (mg.L$^{-1}$) in saturated media extract at harvest for greenhouse study 2. Organic pac choi (a), conventional pac choi (b)**

## Literature cited

Albright, L. (2010). Pak choi handbook. Cornell University. Biological and Environmental Engineering. http://www.cornellcea.com/Pakchoi_Handbook/pc_biological_significance.htm

Amor, F. M. d., Espinosa, M. F., Molina, S., Varo, P., Camara, J. M., & Lopez, Y. A. (2006). Evaluation of methods for the determination of N in pepper crops. Horticultura, Revista De Industria, Distribucion y Socioeconomia Horticola, (No.196), 14-20.

Berghage, R. D. (1987). Micronutrient testing of plant growth media: Extractant identification and evaluation. Communications in Soil Science and Plant Analysis, 18(10), 1089.

Cantliffe, D. J. (1972a). Nitrate accumulation in spinach grown under different light intensities. J. of American Society for Horticultural Science, 97, 152-154.

Cavins, T. J., Whipker, B. E., & Fonteno, W. C. (2008). Pourthru: A method for monitoring nutrition in the greenhouse. Acta Hort. (ISHS), (779), 289.

Clark, C. J., Smith, G. S., Gornforth, I. S., & Prasad, M. (1986). Fertiliser recommendations for horticulture crops No. 03/96)The Horticulture and Food Research Institute of New Zealand Ltd.

Drews, M., & Fischer, S. (1992). Standards for nitrate and potassium in press-sap analysis of greenhouse tomatoes and cucumbers. Gartenbauwissenschaft, 57(3), 145-150.

Evans, L. (2006). Salinity tolerance in irrigated crops. http://www.dpi.nsw.gov.au/agriculture/resources/soils/salinity/crops/tolerance-irrigated

Gardner, B. R. (1989a). Midrib nitrate concentration as a means for determining nitrogen needs of cabbage. Journal of Plant Nutrition, 12(9), 1073.

Gardner, B. R. (1989b). Midrib nitrate concentration as a means for determining nitrogen needs of broccoli. Journal of Plant Nutrition, 12(1), 111.

Gardner, B. R., & Pew, W. D. (1974). Response of spring grown head lettuce to nitrogen fertilizer.Technical Bulletin, Agricultural Experiment Station, Arizona University.

Hartz, T. K. (2007). Efficient nitrogen management for cool-season vegetables. University of California, Department of plant Science. UC Davis http://vric.ucdavis.edu/pdf/fertilization_EfficientNitrogenManagementforCoolSeasonvegetable2007.pdf.

Hartz, T.K., Johnstone,P.R., Williams, E. and Smith, R.F. 2007. Establishing lettuce leaf nutrient optimum ranges through DRIS analysis. HortScience 42:143-146.

Hochmuth, G. J. (1994). Plant petiole sap testing for vegetable crops. CIR 1144. Florida: University of Florida.

Hochmuth, G. J., Maynard, D., Vavrina, C., & Hanlon, E. (1991). Plant tissue analysis and interpretation for vegetable crops in florida. No. SS-VEC-42)Univ. Florida Special Publication.

Hochmuth, G. J. (1994). Efficiency ranges for nitrate-nitrogen and potassium for vegetable petiole sap quick tests. HortTechnology, 4(3), 218.

Jones, J. B., Wolf, B., & Mills, H. A. (1991). Plant analysis handbook: A practical sampling, preparation, analysis, and interpretation guide. Athens (GA): Micro-Macro Publishing.

Knewtson, J. S. (2008). Studies in vegetables and high-tunnel production on the central great plains. (Doctor of Philosophy, Kansas State University).

Lyons, D. J., Rayment, G. E., Nobbs, P. E., & McCallum, L. E. (1994). Nitrate and nitrite in fresh vegetables from Queensland. Journal of the Science of Food and Agriculture, 64(3), 279.

Marr, C. W., Morrison, F. D., and Whitney, D. A. (1998). Fertilizing gardens in Kansas. MF-2320. Kansas State University Agricultural Experiment Station and Cooperative Extension Service.

Maynard, D. (2007). Knott's Handbook for Vegetable Growers, 5th edition.

Nelson, N.O. and R.R. Janke. 2007. Phosphorus sources and management in organic production systems. HortTechnology 17:442-454.

Parks, S. E., Huett, D. O., Campbell, L. C., & Spohr, L. J. (2008). Nitrate and nitrite in Australian leafy vegetables. Australian Journal of Agricultural Research, 59(7), 632-638.

Proietti, S., Moscatello, S., Leccese, A., Colla, G., & Ballistelli, A. (2004). The effect of growing spinach (spinacia oleracea L.) at two light intensities on the amounts of oxalate, ascorbate, and nitrate in their leaves. The Journal of Horticultural Science Biotechnology, 79(4), 606.

Schulbach, K., Smith, R., Hartz, T., & Jackson, L. (2007). Guide to nitrogee quick-tests for vegetables with 'Cardy' nitrate meter No. 95-0582). California: CDFA.

Scuderi, D., Giuffrida, F., & Noto, G. (2009). Effects of nutrient solution EC on yield, quality, and shelf-life of lettuce grown in floating system. Acta Hort. (ISHS), (807), 221.

Talavera-Bianchi, M. (2009). Sensory alanlysis of pak choi and tomato grown under organic and conventional systems. (Doctor of Philosophy, Kansas State University).

Warman, P. R. and Havard, K. A. 1997. Yield, vitamin, and mineral contents of organically and conventionally grown carrots and cabbage. Agric. Ecosyt. Environ. 61: 155-162.

Warncke, D. D., & Krauskopf, D. M. (1983). Greenhouse growth media: Testing and nutrients guidlines. No. E-1736) Michigan State University.

Westerveld, S. M., McKeown, A. W., Scott-Dupree, C. D., & McDonald, M. R. (2004). Assessment of chlorophyll and nitrate meters as field-tissue nitrogen tests for cabbage, onions, and carrots. HortTechnology, 14(2), 179-188.

Wright,A.F. (2001). Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a leco CN-2000 dry-combustion analyzer. Communications in Soil Science and Plant Analysis, 32(19-20), 3243.