Effect of Soil Amendments and Root Containment on Nematode Populations in Organic Greenhouse Tomatoes in the Netherlands

Master Thesis

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Abstract

Root-knot nematodes (*Meloidogyne incognita*) are virulent soil-borne parasites found worldwide in field-grown crops but also in many Dutch organic greenhouses. Due to short crop rotations of susceptible crops, greenhouse producers increase the risk of increasing root-knot nematode (RKN) populations to the point that yield losses can be substantial. Moreover, implementing nematode control techniques such as soil steaming, biofumigation and purchasing rootstocks can increase overall production costs. With the increase in energy costs and the negative impacts on soil ecology, viable alternatives for soil steaming are also required, and the use of soil amendments may hold promise as an effective means for RKN suppression. The goal of the study was to analyze the effects of soil amendments compared to when the soil was not amended or placed in peat pot (root containment). Five different soil amendments (*Nostoc calicola* amended compost, bokashi, biochar, garlic straw, and compost) were applied at 500g/pot, two amendments which were combined (bokashi with garlic straw and compost with garlic straw). Plant performance, and nematode incidence and diversity were analyzed in *Lycopersicon esculentum* cv. *Cappricia*. The results indicated there were no significant differences ($p>0.05$) in plant height, stem diameter, leaf sap analysis (N, K, P, Ca, NO_3, NH_4, Mg, Zn, Mo, Cu, Fe, Mo, Se, Cl, Al, and Mo), tomato fruit production, and RKI among treatments. Root knot index (RKI) values were unexpectedly low (2) which seem to be indicative of relatively low nematode pressure. Significant differences were observed in leaf count, stem final fresh weight, leaf final fresh weight, with the garlic straw treatment having the highest average means. In terms of total nematode populations, bokashi plus garlic straw showed the highest values compared to the other treatments. The only difference in nematode diversity was seen in bacterivorous nematodes with garlic straw having higher counts for *Panagrolaimidae* nematodes. Overall the addition of soil amendments or placing in root containment showed little impact on plant performance or lowering the RKI when compared to control treatment. Additional research is required to assess treatment responses at higher nematode pressures in order to identify soil amendments that require less investment yet can effectively control RKN.

Keywords: Meloidogyne incognita, nematodes, organic greenhouse production, organic soil amendments, nostoc, biochar, bokashi

1 Introduction

1.1 Greenhouse Systems

1.1.1 Conventional Vegetable Greenhouse Production in the Netherlands

The Dutch greenhouse industry in 2010 represented 39% of agricultural production, and aiding the increase in GDP by accounting for 19% of the Dutch trade (Nieuwenhuijse, 2010). Success in this industry is not only credited to the mild sea climate, but also the importance of knowledge sharing and innovation in the field. The use of innovative technology and state-of-the art control systems is integral to obtaining high production. Methods such as CO_2 enrichment, artificial lighting, and improved greenhouse coverings support higher yields. Furthermore, most conventional producers use rock-wool slabs as their growing medium for production, which reduces likelihood of soil-borne diseases. Currently, the total area dedicated to conventional greenhouse production is 9,490 ha. One half of the total area is devoted to floriculture crops (4660 ha), and the remainder to vegetable production (4830 ha). The two main crops with the largest area of production are tomatoes (1780 ha) and peppers (1160 ha). The average family farm income per unpaid average working unit for vegetable production is 62,500 € in 2013 and is estimated to increase by 1,700 Euros in 2014 (Bedrijven-Informatienet, 2014).

1.1.2 Organic Greenhouse Production in the Netherlands

Organic greenhouse production only accounts for 3.2% of the total greenhouses producing vegetables in 2011 (LEI, 2015). The area designated to organic greenhouse production is 92% (116 ha), which focuses on vegetable production. The top two activities include propagation material (22.3 ha) and tomatoes (31.4 ha) (CBS-Statline, 2015). However, despite the relatively small production area, tomatoes are ranked third among the highest exported organic product (Netherlands, 2015). The latest figures show a growth in export sales from 43 to 50 million Euros from 2008-2013 (Netherlands, 2015). Exports to international markets such as United States and Asia provide opportunities for further growth of the organic greenhouse sector as well. Additionally, demand for organic products is slowly increasing within the Dutch market, in 2011 Dutch consumers spent 817.3 million but this amount increased to 934.3 million Euros the following year (Ecology&Farming, 2014). The increase in demand is linked to consumers requesting products that provide benefits in terms of animal welfare, human health and the environment based on use of ecological techniques that are conserving the environment.

1.1.3 Organic Greenhouse Tomato Production

Tomato production accounts for 27% of the total organic greenhouse vegetables. Truss tomatoes account for the majority of total production (26 ha), while cherry tomatoes amount to 10% (3.1 ha) and only 5% (1.6 ha) is planted with hand-picked large tomatoes (CBS-Statline, 2015). Due to current regulations and certification requirements, tomatoes cannot be grown on soil-less media, which reduces average yields by 15% compared to rock-wool production (Gravel et al., 2010). The drop in yields is mainly due to the availability of nutrients and fertilizer sources and effect on microbial population activity. Growing tomato under soil conditions also poses a risk of soil-borne diseases that reduce yield. To maintain high yields and profit margins, producers have year-round crop production and very short fallow periods between crop rotations (1 day to 1 week). A survey conducted in 2007 of organic greenhouse producers showed that *M.*

incognita still is being perceived as the greatest potential threat to production (van der Wurff, 2010).

Yield loss from RKN on tomatoes can range from 50% up to 85% depending on the tomato cultivar, and may lead to substantial economic loss (Kamran et al., 2011; Nicol et al., 2011). The primary cause of this is by second stage juveniles penetrating the root meristem causing swelling, which in turn reduces nutrient and water uptake (Nobre et al., 1995). Their long dormancy (30 years) and quick reproductive cycle (30 days at 25°C) are hard to overcome without the use of synthetic chemicals which is prohibited in organic agriculture (Taylor & Sasser, 1978; Winslow et al., 1972). Such restrictions require producers to utilize costly preventative measures to reduce the build-up of root-knot nematodes (RKN). Overall, there continues to be a lack of easy applicable and cost-effective methods to lower RKI populations, which will be further discussed in the next section.

1.2 Soil Quality Management

The following section will focus on the different strategies applied by organic greenhouse producers to protect plants roots from RKN. Many of the techniques rely on preventive measures as a form or protection. These preventive methods cover a wide range of control approaches and application measures throughout the crop production cycle.

1.2.1 Steaming

Steaming is quite effective in terms of reducing RKN populations but it also negatively influences soil biota and soil nutrient dynamics. Sheet steaming was a method developed in the Netherlands, and is a common technique used in Dutch organic greenhouse systems because it allows producers to sterilize large areas (Runia, 2000). With this method, the soil is covered with high-grade plastic to create a sealed environment and steam is then injected underneath the plastic. The effectiveness of steaming relies heavily on the physical properties and moisture content of the soil (Gay, 2010; Runia, 2000). By raising soil temperatures above 45˚C the survival of RKN is greatly reduced by increasing metabolic rates and draining their energy reserves (Tsai, 2008; K. H. Wang & McSorley, 2008). However, steaming also negatively impacts other (beneficial) soil biota by eliminating all organisms within the top 10-15cm. Additionally, steaming affects nutrient dynamics by releasing large amounts of soluble nutrients (K^+, Mn^{2+}, NH_4^+), which can cause manganese (Mn) toxicity especially after repeated applications (A. Gelsomino, 2010). Both effects result in increased production challenges and extra costs for producers to restore soils to optimal levels. The cost of restoration is only additional to the initial costs of steaming which cost up to 30,000 euros/ha. Steaming rates will likely rise in the future considering the continual raise of global energy costs.

1.2.2 Biofumigation

An alternative technique for controlling RKN is biofumigation, a process which focuses on incorporating plant biotoxins. Families such as the cruciferae (brassicas) are known to contain glucosinolates, which are associated with plant protection (del Carmen Martínez-Ballesta, 2013). When cells are damaged, glucosinolates leak into the cytoplasm and undergo enzymatic hydrolysis by myrosinase, which releases isothiocyanates (ITC). ITCs are known to be toxic to RKN and other soil-borne diseases (Lazzeri et al., 2004). Application method requires

incorporation into the soil using a cultivator, and the soil can then be covered to enhance efficacy. Biofumigation requires a high attention to detail due to phytotoxic effects that may occur if plants are transplanted too early; a ten-day waiting period is therefore usually recommended to insure plant safety (Handiseni, 2009; van der Wurff, 2010). The effectiveness of biofumigation is still inconsistent due to different biological and environmental factors that play a role in the process, notably its application timing, plant type, harvesting time, and if the material is covered or uncovered.

1.2.3 Rootstocks

The most commonly used plant materials within organic greenhouse production are grafted tomatoes. Producers are able to choose from a wide selection of rootstocks that fit their situation; such as saline or drought conditions for example (Eastburn, 2010; Schwarz, 2010). They also have the option of choosing particular varieties with increased tolerances and/or resistance to different pathogens and plant parasites such as RKN (van der Wurff, 2010). However, excessive use of similar rootstock may result in the predominance of more virulent RKN population and possible break-down of root-stock resistance (Ibáñez, 2014). Thus, rotating rootstocks and crops is an important strategy to reduce the buildup of parasitic organisms such as RKN.

1.2.4 Crop Rotation

Crop rotation is an important practice utilized by all organic farmers of field-grown crops. Different crops rotated into the system can improve soil quality by adding nitrogen, increase organic matter content, and reduce pathogens (Henriette C., 2012). Although organic greenhouse producers are required to implement a crop rotation, many greenhouse producers favor rotating cucumbers, tomatoes and peppers due to their high productivity, profitability and market demand. The drawback of these particular rotation systems is an increase in nematode pressure in the following years (VerdejoLucas, 2009). Alternatively, producers may also practice an intercropping system by growing two crops simultaneously. Intercropping marigold within the greenhouse can reduce RKN infections due to them releasing sulfur-containing compounds that are toxic to RKN (van der Wurff, 2010; K. Wang, Hooks, C., & Ploeg, A., 2007). Yet, not all producers can apply an intercropping system because of limited space and light. Similar to the previous technique mentioned above, timing and variety are also important parameters that alter the effectiveness of this option (Piedra Buena et al., 2008; van der Wurff, 2010).

1.2.5 Other (Root Containment)

While growing plants in biodegradable peat-moss pots is not a new concept, it has been overlooked as a method to reduce RKN. Many organic greenhouse producers order tomato transplants grown in grow blocks, where roots are easily accessible to RKN at the start of the season. Growing tomatoes in a peat-moss pot provides a boundary layer that can reduce the number of cysts on newly formed roots. However, more information is needed since there is a lack of information on the effectiveness of this technique in terms of reducing root RKN infection.

1.3 Soil Amendments

Using natural solutions to solve soil health issues, including the effective use of soil suppressiveness, which was discussed and presented by Van Bruggen and Semenov (2000), may provide a viable alternative for managing RKN. Soil amendments can alter soil properties; thereby the potential for prevalent soil-borne parasites (such as RKN) within the soil to cause economic damage to commercial crops may be decreased. This theory can be explained by general soil suppressiveness, which has been linked to these different control methods such as antagonism, substrate (nutrient) competition, production of antibodies, enhancement of plant resistance, and release of bio-toxic compounds (Eastburn, 2010; Sullivan, 2004; van der Wurff, 2010). The addition of soil amendments provides the soil with nutrients while also improving soil physical properties (Giotis et al., 2009). For this experiment, four different soil amendments will be considered in greater depth.

1.3.1 Garlic Straw

The bio-toxic chemicals mentioned above (such ITCs) can also be produced by *Allium sativum*. The addition of garlic straw directly into the soil lowered RKN damage by 72% compared to the control (Gong et al., 2013). In addition to releasing toxins, the application of garlic straw can modify the soil physical properties rendering it less habitable for RKN (van der Wurff, 2010). Furthermore, the Netherlands has many garlic producers who are looking for outlets for their garlic by-products. While greenhouse producers can make use of these outlets and provide environmental benefits by effectively recycling nutrients.

1.3.2 Compost

Compost is commonly used by all organic greenhouse producers because it enhances soil physical properties and adds nutrients (Barbosa, 2004). Additionally, compost has been shown to suppress particular diseases such as *Pythium*, *Phytophthora*, *Fusarium* and *Rhizoctonia* (Hoitink, 1997; Oka, 2010). However, when analyzed for its effectiveness against RKN, with different compost types (tomato, rice hull, rice straw, potato, guava, citrus, and city waste) all evaluated materials resulted in low soil suppression. In most cases, eggplant roots had a root-knot index (RKI) of five, which is indicative of heavy infestation (Doaa, 2012). For compost to be an effective amendment against nematodes, very high application rates of organic matter are needed, which may hamper it's practical use in greenhouse settings (van der Wurff, 2010).

1.3.3 Bokashi

Bokashi is the Japanese word for fermentation, and bokashi product have been used as a fertilizer in Japan since 1935 (R.E.A.P.Canada). To produce bokashi, an anaerobic environment is required with the addition of effective microorganisms or EM (Burt, 2009; Roldi et al., 2013). EM are naturally occurring microorganisms such as lactic acid bacteria, yeasts, and actinomycetes (Burt, 2009). The large amount of microorganisms present in the EM solution will further enhance soil suppression while they also may suppress pathogens such as E. coli (Burt, 2009). Different formulations of bokashi have been shown to be effective against RKN. Experiments on tomatoes showed a 72% decrease in galls compared to the control (Roldi et al., 2013). Adding bokashi to bananas resulted in a 77% decrease in nematode populations, with only the addition of 3.8 kg/plant (Nevárez, 2003). However, similar to composting, there are many forms and preparations of bokashi, which may alter their effectiveness in terms of controlling

RKI.

1.3.4 Biochar

Biochar has also been recognized as a promising soil amendment with RKN suppressive properties. The process of biochar production begins with slow pyrolysis of organic matter in the inner chamber of a two-chamber oven. Adding biochar to the soil stimulates microbial activity and nitrogen supply over time (Clough, 2010; Lehmann, 2011). Furthermore, experiments have shown that the addition of biochar to a grape vineyard reduced the incidence of plant parasitic nematodes by a factor of eight compared to the control (Rahman, 2014).

1.4 Biological Control Agents

Specific suppression via applying specific (beneficial or predatory) organism is another form of controlling soil-borne parasites and this technique may also be referred to as application of biological control agent (BCA). Combining BCA, such as *Trichoderma* with carrier agents, such as compost is currently being used commercially; but their effectiveness on RKN depends on soil properties (Harman, 2000). Furthermore, other BCAs have been identified that suppress RKN such as cyanobacteria (blue green algae). The addition of cyanobacteria strains such as *Anabaena oryzae*, *Nostoc calcicola, and Spirulina sp.* all reduced galling of cowpea when inoculated with *M. incognita* (M. Youssef & Eissa, 2014). However, they lack commercial production protocols that can be applied to the field (Holajjer et al., 2013).

1.5 Knowledge Gaps and Goals

Dutch organic greenhouse production relies heavily on soil amendments, and some of which may provide a solution for reducing the RKI. Currently, new methods of composting (bokashi, for instance) have gained some attention in reducing plant RKI, but lacks standardization. Additionally, inoculating composts with BCA has been tried before with algae strain *Nostoc calicola*, but the effectiveness in controlling RKN of this BCA when added to compost directly has not been evaluated. Moreover, different by-products (such as garlic straw) are readily available within the Netherlands but their effect on crop growth and suppression of RKN must be verified under Dutch greenhouse conditions. Lastly, the combination of two forms of soil amendments can help shed light on when different composts and plant matter effect plant development and soil nematode diversity.

The goal of this thesis thus is to examine nematode diversity and population as influenced by soil amendment. Moreover if these potentially can lower RKI compared to a non-amended control or when roots are contained. Furthermore, the thesis will analyze how the growth of tomato plants is affected by different soil amendments and root containment (use of Jiffy peat pots).

1.6 Research Questions and Hypothesis

The following questions are being addressed in this thesis:

1. Will crop performance be negatively or positively influenced by different treatments compared to the control?
2. What are the effects (negative or positive) of different treatments (alone or mixed) on

nematode populations and diversity compared to the control?
3. How do different soil amendments affect crop nutrient status and plant health?

It was hypothesized that:

1) The addition of soil amendments should positively influence crop performance with an addition of an amendment (Chavarria-C.l, 1998).

2) Additions of organic amendments are expected to increase bacterivorous nematodes compared to when no amendment is added. (Thoden, 2011)

3) In the case of bokashi, due to composting process for a number of weeks, the high bacterial populations should increase bacterivorous and thus predatory nematode populations (Burt, 2009).

4) The addition of BCAs and bio-toxic chemicals from garlic straw and cyanobacteria amended composts should effectively reduce RKN and therefore RKI scores. (Gong et al., 2013; Holajjer et al., 2013)

5) The addition of root containment should reduce RKI compared to the control.

1.7 Thesis Structure

In the second section materials and methods are presented. The third section will focus on the results and discussion based on the data obtained which is followed by some concluding remarks regarding the experiment. The final section will provide a short synthesis of how these initial findings may guide future research for organic greenhouse producers in the Netherlands.

2 Materials and Methods

2.1 Experimental Design

A randomized complete block design (RCBD) was used for this experiment at the experimental greenhouse at BioVerbeek B.V. located in Velden, Netherlands (51˚25'11 N, 6˚ 11'46 E). This thesis will focus on nine out of the twelve treatments that were included in the experimented (Table 1). Due to confidentiality the remaining three treatments were not included. Treatments were replicated seven times with each of the seven different blocks. To reduce any border effects, one or two plants were placed at the end and beginning of each rows. The experimental layout can be found in Appendix A.

Table 1 Experimental treatments tested for this experiment, a three-letter abbreviation and application rate.

	Treatment	Code	Application rate
1	Biochar	BIO	500g/pot
2	Bokashi	BOK	500g/pot
3	Bokashi+garlic straw	BKG	250g/pot of each
4	Compost	COM	500g/pot
5	Compost+Cyanobacteria	CYB	500g/pot
6	Compost+garlic straw	CMG	250g/pot of each
7	Control	NUL	0 g/pot
8	Peat-pot	PPT	0 g/pot
9	Garlic straw	GST	500g/pot

2.2 Crop Management

The tomatoes (*Lycopersicon esculentum* cv. *Cappricia*) were grown by GrowGroup in grow blocks, except for the peat pot treatment. The size of the grow block was 10x10x10cm (1000 ml) while the size of the peat pot was 11x10x7.5cm (680ml). Plants were grown at the nursery for four weeks before being transplanted into 5-L PVC containers with a diameter of 26 cm and a height of 19cm.To reduce cross contamination 30x30cm agriculture plastic was placed on the bottom along with 250grams of hydroton pellets. The remaining space within the pot would be filled with 7.5 kg soil obtained from 'Fensland' one of the greenhouses at BioVerbeek. The soil was steamed in the winter of 2012-2013, afterwards sweet pepper was planted. In 2014 tomatoes were grown and had a RKI of 3.2 at the end of the growing season, which is considered to be normal value by BioVerbeek. Soil type was a sandy soil with 7% clay and a pH of 6.8 (Table 2).

Table 2 Soil analysis from Fensland (BioVerbeek B.V.), performed by BLGG AgroExperts.

	Unit	Value
Nitrogen-total	mg N/kg	2870
C:N Ratio		13
P-Al	mg P_2O_5/100 g	143
pH		6.8
Organic matter	%	6.3
Clay	%	7
C-inorganic	%	0.14
Calcium Carbonate	%	0.6
Clay-humus (CEC)	mmol+/kg soil	162

Containers were placed on a plastic gutter that was elevated 35cm. Two gutters were positioned 50cm apart and ran parallel to each other. Every container was placed 40cm apart. Plants were watered using a spaghetti system with an automatic timer. For every increase of 150 w/m2, plants were watered with 50mL per pot. An additional 500mL of water was also given weekly with a watering hose.

Plants were fertilized with organic chicken manure pellets, a detailed chemical composition is provided in Table 3. Fertilizer was applied twice; the first dose of 150g of organic chicken pellets was applied at 21DAT while 250g was added on 56DAT. Tomato plants were trained manually every week while lateral shoots ("dieven") were also removed weekly. The first five leaves of the tomatoes were pruned along with the first and second cluster at 56DAT. The remaining clusters and leaves were removed at the end of the experiment.

Table 3 Chemical composition of organic chicken manure pellets analyzed by Agro Experts B.V.

	Unit	Value
C:N Ratio		8.00
Nitrogen	g N/kg	32.7
Phosphorous	g P_2O_5/kg	25.2
Potassium	g K_2O/kg	22.5
Organic matter	g OM[1]/kg	577

[1] OM: Organic matter

To reduce potential pest damage organic techniques were used. Mycotol and Spiderx (Koppert) were applied three times (7DAT, 42DAT, 70DAT) during the experiment to control of *Tetranychus urticae* (red spider mite). For *Tuta absooluta*, pheromones were placed in delta traps throughout the greenhouse. Plants were grown for a period of 12 weeks (105 days). All treatments were grown in a greenhouse environment with temperature settings of 16˚C during the day and 18˚C during the night with no supplemental lightning.

2.3 Treatments

An overview of treatments along with treatment codes is provided in Table 1. A more detailed description of the specific treatments is provided below.

Control was prepared by first mixing soil from the BioVerbeek greenhouse with shovels three times to create a homogenous mixture. The mixed soil was then added to the containers.

Compost was prepared by mixing green compost made from greenhouse tomato leaves and prepared using the windrow method. Compost was rotated six times and reached a temperature of 70˚C (Appendix B). Compost was applied at 500 grams per pot. The required amount of compost was mixed thoroughly with soil obtained from BioVerbeek greenhouse with shovels then the mix was placed in the containers.

Bokashi was prepared by obtaining fresh tomato leaves from BioVerbeek, which were shredded to 5-10 cm lengths using a leaf shredder (ATX2000 Bosch, Germany). The shredded leaves were then mixed with horse manure at a ratio of 6:4. The final weight of all ingredients was 10 kg, which was placed in 40x60x20cm hole dug with a shovel. The mixture was layered at depth increments of 5cm. Between each layer was an application (spread equally) of 12 grams Edasil clay minerals, 12 grams of Ostrea sea shells, and 30mL of Microfern diluted with distilled water at a ratio of 1:100mL (total of 60g edasil , 60g ostrea and 150mL of diluted microfern), which were all obtained from Agriton B.V. (http://www.agriton.nl/homeeng.html) After layering, a plastic sheet was used to cover the freshly mixed bokashi in order to maintain an anaerobic environment. The mixture was compressed by adding 15 kg of soil above the plastic film to ensure compaction. The temperature inside the heap was also measured every week by placing a thermometer in three locations of the heap and the average was taken. Holes were then sealed with tape to maintain the anaerobic environment. Samples were also taken every two weeks and placed in a freezer kept at -10˚C. At the end of the experiment, the samples were removed from the freezer and were analyzed for their nitrogen and carbon content (%). A graph is presented in Appendix C showing the different temperatures and C:N ratio over the duration of the composting process. After eight weeks the heap, was uncovered and the material was vacuum-sealed in an impermeable plastic bag for three weeks. At the beginning of the experiment the bags were opened. Bokashi was applied at 500g per pot. The bokashi was first mixed with soil using shovels three times, and then incorporated into each pot marked for this treatment.

Compost+Cyanobacteria *Nostoc calicola* were obtained from Culture Collection of Algae and Protozoan (CCAP, http://www.ccap.ac.uk/). Cultures were then transferred to five 500 mL polypropylene bottles, which contained a mixture of distilled water and Laworski's Medium as advised by CCAP. Algae culture was placed in a metal cupboard with three fluorescent lights (T5 with ballast Philips, Netherlands) connected to a timer set to 16hours of light. Daily agitation was done by hand. Three weeks before beginning the trials, algae was strained with 1.25 m mesh to remove contaminants. The remaining algae were diluted in distilled water with a ratio of 25mL: 1gram fresh algae. The mixture was then applied to 6kg of compost at a rate of 10% v/w with a hand sprayer and the material was then mixed by hand. The compost was wrapped in landscape plastic and placed indoors at room temperature. The compost plus algae treatment was maintained at 25% moisture content for three weeks to insure high amount of algae growth within the compost, as described by El-Gamal (2011). A total of 500 grams of the compost was

applied per pot. The compost inoculated with algae was first mixed with soil three times with a shovel then put into each pot.

Garlic straw was obtained from an organic garlic grower (http://www.biologischpootgoed.nl/). The straw was delivered by mail and was received after two days. The garlic was put through a juice mixer until grounded to 1-10 cm long strands. A total of 3.5 kg (500 g/pot) was measured and mixed with soil using a shovel three times. The mixture was added to each pot.

Peat-pots were purchased from Jiffy-Tref (http://www.jiffygroup.com/en/substrates/tref-go.html) and used for tomato transplants. Tomato seedlings were placed in 680mL (11x10x7.5cm) pots and grown to GrowGroup specifications (http://www.growgroup.com/). Tomatoes were then placed into a container similar to the control.

Biochar was purchased from Carbon Gold (http://www.carbongold.com/). A total of 3.5 kg (500 g/pot) was measured and mixed with soil using a shovel three times. The mixture was then added to each pot assigned to this treatment.

Compost and Garlic straw was prepared by mixing 1.750 kg of compost and 1.750 kg garlic straw with a shovel three times. The mixture was mixed again with soil using a shovel three times. The final mixture was then placed into each pot.

Bokashi and Garlic straw was prepared by mixing 1.750 kg of bokashi and 1.750 kg of garlic straw (250g/pot of each treatment) with a shovel three times. The mixture was mixed again with soil using a shovel three times. The final mixture was then added into each pot.

2.4 Measurements

2.4.1 Non-destructive plant measurements

In this section the non-destructible measurements are being described that aimed to monitor the effects of treatments on plant growth through the growing season. In most cases measurements were obtained once a week for each experimental unit (plant). Each measurement is described in more detail below.

Plant Height Weekly measurements of plant height were taken throughout the experiment using a tape measure.

Stem Diameter Weekly measurements of plant stem diameter were taken 7cm above the soil using a digital caliper.

SPAD Values Weekly readings were taken using the fifth (most recently matured) leaf counting from the apical meristem using a chlorophyll meter (Konica Minolta SPAD-502, Tokyo, Japan).

Leaf number Leaf counts were taken by counting the last leaf until the first open leaf. Counting began after the first five leaves were removed.

2.4.2 Soil amendment analysis

In addition to plant growth measurements the dry matter, nitrogen and carbon content of each soil amendment were determined by the FSE lab at Wageningen University and Research. The C an N contents were then use to calculate the C:N ratio. The results for each treatment can be found in Table 4.

Table 4 Chemical analysis of different treatments prior to adding into soil, analysis was conducted by FSE group at Wageningen University and Research.

Treatments	%DM	%N	%C	C:N
Biochar	67.5	0.72	45.8	63.6
Bokashi+GS[1]	80.1	0.30	4.5	15.0
Bokashi	30.2	1.82	22.2	12.2
Compost	55.9	0.41	10.1	24.4
Compost+GS[1]	78.9	0.29	4.5	15.5
Cyanobacteria	80.1	0.26	4.1	15.8
Garlic Straw	53.2	1.00	37.4	37.4

[1]Garlic straw

2.4.2 Destructive measurements

The experiment was terminated at 84 DAT by cutting the shoots 7cm above the soil surface and harvesting the above-ground parts.

Stem and leaf final fresh weight On the final day of the experiment, all leaves were removed and weighed on a scale. Stems were cut 7cm from above the soil surface and cut into 10cm pieces and weighed on a scale.

Fruit production Tomato fruits were harvested on 54 DAT (cluster 1 and 2) and 84 DAT (remaining clusters) and fruits were weighed on an UWE electronic scale (HGW-6000, Taipei, Taiwan). Fruit number per cluster and average fruit weight along with total fruit number and yield were determined as well.

Nematode Count Four 200g soil samples were taken from different replicates of each treatment using a hand soil sampler and placed in paper bags. Nematodes were then extracted using an Oostenbrink elutriator in the WUR Nematology lab. A total of 100 g soil was placed into the Oostenbrink elutriator (Oostenbrink, 1960), and placed in 100mL glass jars. The jars were left to settle for one hour before using an aspirator (Eyela A-3S, Tokyo, Japan) with a glass tip to remove excess water. The nematode solution was then placed in a 100mL-graduated cylinder. Solutions were lowered to 40, 50, or 60mL, depending on the initial amount placed and recorded. The solution was stirred for a few seconds and 2mL and 15 ml were placed in a 50mL-graduated cylinder. The newly diluted solution was stirred for a 10 seconds and 1mL of the stirred solution was placed on a counting tray and was measured three times using a stereomicroscope at 50x (Van Bezooijen, 2006).

Nematode functional diversity After counting nematodes, the stock solution was transferred to 20mL glass jars, after allowing for the samples to set for one hour. To preserve nematodes, Formalin was placed in wash bottle then placed in a 70˚C hot water bath for several minutes. First, a small amount of warm formalin was added followed instantly by cold formalin to prevent nematodes from getting damaged. Solutions were stirred for a few seconds before a small drop was placed on a glass slide; this was repeated three times. Using a microscope at a magnification of 100x, 150 nematodes were randomly selected from the three different slides (Bhusal, 2014). Nematodes found were identified to the genus level using the identification key of Bongers and Vereniging (1988). The nematodes were then divided based on functional diversity classes according to Yeates (1993).

Root Knot Index Soil was shaken off the roots and rinsed with water to remove soil. A standard RKI chart was used to determine the severity of the damage, which can be found in Appendix D.

Leaf-Sap Analysis One petiole from the fifth leaf from the apical meristem was taken from every replicate and petioles were placed in a zip-locked bag at biweekly intervals. Leaf sap analysis was thus determined every two weeks and this analysis was conducted by Nova Crop Control (www.novacropcontrol.nl). Leaf-sap analysis began 14DAT and the last measurement occurred at 70DAT. The following nutrients are routinely measured by Nova Crop Control: total nitrogen, phosphorous, potassium, ammonium, nitrate, calcium, magnesium, sulfur, copper, iron, zinc, aluminum, sodium, chlorine, boron, molybdenum, and silicon.

2.5 Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0.0 (SPSS, Inc., Chicago, IL). To see if there were any significant differences among the treatments a one-way Analysis of Variance (ANOVA) was conducted except for leaf sap analysis. Leaf sap analysis data was averaged for all the weeks and a two-way ANOVA was used. Lastly to check if means were significantly different a Tukeys Honest Significant Difference (HSD) was carried out. For leaf fresh weight a lower p-value (0.10) was used to find significant difference between the means.

3 Results and Discussion

3.1 Plant Height

There was a significant difference ($p>0.05$) in tomato plant height from 0DAT until 21DAT (Table 5). Plants grown in peat pot were shorter compared to the control during 0DAT, 7DAT, 14DAT and 21DAT with differences of 27%, 16%, 12%, and 12%, respectively. Biochar plants were significantly taller than the control at 7DAT, 14DAT and 21DAT but differed only 1-2%. After 21DAT, there were no longer any significant differences among the treatments when compared to control.

The results obtained are in contrast with previous scientific findings. Bokashi did not show an increase in growth as witnessed by Lee and Sung (2001) when two different application rates (200g/m2 and 400 g/m2) were used for tomatoes. When garlic straw was applied as a soil amendment in a study by Gong et al. (2013) it was a reduction in plant height by 10cm compared to the control treatment, when more than 2% garlic straw is added to the soil. When compost that was inoculated with cyanobacteria *Nostoc calicola*, the lack of a clear growth response were in contrast with findings by Al-Khiat (2006) who showed that adding *Nostoc ellipsoidum* directly into sandy soil at 2% w/w increased plant height.However, in other instances results matched reports in the scientific literature. Reduced plant height with peat pot may be explained by the initial pot size that had 32% less volume compared to the growblock. Poorter (2012) noted doubling the pot size could increase biomass by up to 43%. In the case of compost, our results were in agreement with Arthur (2012), who observed no difference in tomato plant height between different types of green compost. For biochar, the results were also similar to Nzanza (2012), who observed no benefit to plant growth. Inconsistency of results across studies may be related to differences in nematode pressure which was relatively low in the current experiment and results will be discussed in Section 3.11.

Table 5 Tomato height (cm) as influenced by different treatments at different sampling dates.

	Plant height (cm)												
Treatments	0 DAT[1]	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	49 DAT	56 DAT	63 DAT	70 DAT	77 DAT	84 DAT
Biochar	45.7 a[3]	60.7 a	82.1 a	100.1a	111.1	124.9	138.6	150.1	154.0	166.6	177.0	202.0	213.3
Bokashi	45.7 a	59.7 ab	81.1 ab	100.9a	114.4	125.0	136.6	151.6	161.9	173.9	189.3	209.3	225.0
Bokashi+GS[4]	44.3 a	58.7 ab	79.4 ab	97.7ab	112.4	124.9	139.0	156.1	164.3	178.0	193.9	210.9	217.3
Compost	45.4 a	59.4 ab	81.6 ab	101.8a	118.2	131.2	146.7	158.0	166.2	175.8	189.0	209.2	228.8
Compost+GS[4]	45.6 a	59.4 ab	81.1 ab	97.4ab	116.0	126.4	140.7	156.3	164.6	178.4	181.7	212.9	224.0
Control	46.1 a	59.7 ab	81.1 ab	99.3ab	114.0	124.0	140.6	152.9	159.3	168.1	184.9	206.9	237.1
Cyanobacteria	46.7 a	60.3 a	81.1 ab	99.1ab	117.7	127.0	141.6	152.0	157.7	166.9	185.1	200.4	220.4
Garlic Straw	43.0 a	57.1 ab	77.7 ab	93.6ab	111.6	125.4	139.7	156.0	170.1	187.7	199.7	218.5	231.5
Peat pot	33.6 b	50.1 b	70.6 b	87.3b	105.1	124.9	137.3	153.0	162.6	172.4	187.9	206.6	218.3
Sig.[2]	***	*	*	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] Days After Transplanting

[2] (***): P < 0.001; (**) P < 0.01; (*) P < 0.05; (n.s). P > 0.05

[3] Letters indicate that means differed significantly (P<0.05) as established by Tukeys HSD Test.

[4] Garlic Straw (GS)

3.2 Stem Diameter

Tomato stem diameter was only significantly influenced ($p<0.05$) by treatments at 0, 7 and 14 DAT. Peat pot treatments had smaller steam diameter compared to all other treatments (Table 6). When compared to the control, its stem diameter was 14.5%, 14% and 20% lower on 0, 7 and 14 DAT, respectively. The small stem diameter for the peat pot treatment is due to the smaller volume in the pot compared to the growblock. These results match findings by Marr and Jirak (1990) who observed that stem size was reduced when plants were being grown in trays. There was no significant difference ($p>0.05$) after 14DAT among all the treatments.

Based on overall numeric values, the compost treatments consist of the thinnest stems throughout most of the growing season. Although values were consistently lower compared to other treatments, differences were not significant. These observations were in par with those reported from compost by Walker (2007), who showed a lower stem diameter compared to the control or the addition of grassy weed residues, barley residues, lucerne pellets, and molasses. In agreement with the current study, Lima (2012) also reported that use of bokashi did not affect stem diameter when compared to the control, or when fertilized with NPK. However, results for the garlic straw treatment are in contrast with those of Gong et al. (2013) who reported a decrease in stem diameter of 2.3 mm with an application 3% raw garlic straw compared to the control. The lack of response of biochar , is in contrast with the results of Vaccari et al. (2015). Moreover, it also appears to contradict that biochar positively influences nitrogen supply (Clough, 2010). Despite the rather high C:N ratio's for biochar, the carbon present in this material tends to be rather stable and its biological degradation is rather (s)low. Its main function may thus be nutrient retention rather than being biologically readily degradable an providing net mineralization as noted by (Bruun & Luxhoi, 2008).

Table 6 Changes in stem Diameter (mm) of tomato plants as influenced by different treatments.

	Stem Diameter (mm)												
Treatments	0 DAT[1]	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	49 DAT	56 DAT	63 DAT	70 DAT	77 DAT	84 DAT
Biochar	27.8 a[3]	30.0 ab	36.3 ab	35.7	35.7	44.3	44.9	43.4	45.8	46.9	48.0	48.6	48.4
Bokashi	27.1 a	29.9 ab	32.9 bc	37.3	37.3	39.6	43.2	42.6	45.3	47.1	48.2	48.4	48.5
Bokashi+GS[4]	26.9 ab	30.6 a	32.3 ab	35.5	35.5	43.4	43.2	44.7	45.1	47.0	48.1	47.2	48.4
Compost	26.7 ab	30.7 a	34.3 ab	33.5	33.5	35.9	35.6	36.3	37.1	38.1	38.4	39.7	39.9
Compost+GS[4]	26.1 ab	30.8 a	35.7 ab	35.7	35.7	41.4	41.9	41.7	43.1	44.8	45.3	46.2	47.3
Control	26.8 ab	29.8 ab	35.6 ab	36.3	36.3	41.7	42.2	41.7	44.4	45.1	46.4	47.0	47.1
Cyanobacteria	27.0 ab	30.8 a	35.6 ab	34.0	34.0	43.4	43.0	42.8	44.9	45.5	47.4	46.4	46.9
Garlic Straw	26.0 ab	29.7 ab	33.7 ab	33.8	33.8	43.3	42.5	43.3	44.0	46.2	46.4	48.4	48.8
Peat pot	22.9 b	25.5 b	28.0 c	28.7	30.1	37.4	39.4	40.0	41.1	42.6	43.5	44.0	44.2
Sig.[2]	**	**	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] Days After Transplanting

[2] (***): $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; (n.s). $P > 0.05$

[3] Letters indicate that means differed significantly ($P<0.05$) as established by Tukeys HSD Test.

[4] Garlic Straw (GS)

3.3 SPAD Readings

Based on SPAD readings, which are indicative of leaf greenness (chlorophyll content, which accounts for most of the N in leaves), it appears that treatments had no significant effect ($p>0.05$) on leaf N content, throughout the experiment (Table 7). A clear increase in chlorophyll levels occurred 2-3 weeks after the application of chicken manure pellets. However, chlorophyll levels started to decline again at 49 DAT possibly due to continuous growth and dilution of nutrients in the dry matter. Biochar and bokashi were the first treatments to express low chlorophyll levels 63DAT, though difference was not significantly different. It may thus be possible that available nutrients at that point in time no longer matched plant growth. Readily available nutrients (such as Nmin) in the soil amendment were already taken up. Furthermore the slow release of nutrients from the chicken pellets lagged behind with crop N demand.

Overall, the range of SPAD values observed throughout the study were typically consistent with SPAD values provided by Fontes and Ronchi (2002), which are between 41.6-44.4. However, both starting 63 DAT the observed values in the current study started to drop, possibly due to lack of nitrogen. SPAD readings trend was also comparable to critical (target) values suggested for different plant physiological stage by Fontes and de Araujo (2006). It is evident that it took approximately 3 weeks for SPAD values to recover after the 2^{nd} application of chicken manure pellets. This underlines that management of (solid) organic amendments is more complex and requires timely interventions. In contrast with the use of chemical fertilizer where a crop response (greening of the crop) may be observe within one week after fertilizer application event.

Table 7 Mean leaf chlorophyll concentrations from SPAD-502 as influenced by different treatments.

	SPAD[1] reading												
Treatments	0 DAT[2]	7 DAT	14 DAT	**21 DAT[3]**	28 DAT	35 DAT	42 DAT	49 DAT	**56 DAT[3]**	63 DAT	70 DAT	77 DAT	84 DAT
Biochar	51.67	50.77	47.66	48.29	45.67	48.87	53.77	45.53	40.66	26.43	26.57	35.93	35.69
Bokashi	49.81	53.41	50.30	48.00	48.36	51.10	53.51	50.24	40.91	25.34	25.29	33.86	35.67
Bokashi+GS[4]	48.40	52.13	48.97	47.11	49.01	52.89	54.03	51.54	39.80	38.80	23.89	28.07	29.71
Compost	47.83	51.99	48.47	47.55	47.38	50.08	52.10	50.75	41.05	29.92	25.98	32.62	34.92
Compost+GS[4]	48.93	52.79	48.21	45.87	47.57	52.00	51.09	50.44	42.24	29.64	30.60	32.04	31.09
Control	49.07	51.06	49.99	48.54	47.87	49.86	52.90	51.04	40.74	29.26	25.24	33.07	31.67
Cyanobacteria	46.29	49.51	48.30	47.31	47.00	53.04	54.07	51.03	42.49	28.60	26.64	33.11	34.67
Garlic Straw	49.33	54.13	48.84	45.61	46.20	52.26	50.55	51.13	39.83	32.12	32.22	35.00	34.58
Peat pot	49.64	50.89	46.56	48.26	45.90	52.23	52.06	51.50	43.10	30.13	26.77	28.91	30.70
C.V.[5]					**45.90**		**43.60**		**41.20**		**38.8**		**36.4**
Sig.[6]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] SPAD values measure leaf chlorophyll levels which are indicative of the nitrogen concentration within the leaf.

[2] Days After Transplanting

[3] Fertilizer application.

[4] Garlic Straw (GS)

[5] Critical Values set by Fontes and de Araujo (2006)

[6] (***): $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; (n.s). $P > 0.05$

3.4 Leaf Count

Leaf counts per plant were significantly different ($p<0.05$) on 56, 63 and 84DAT. Garlic straw was significantly different during those particular sampling date, but overall it maintained the highest amount of leaves throughout the experiment. While the peat pot and Cyanobacteria treatments had the lowest values at 63 and 84 DAT, respectively (Table 8). The garlic straw treatment had 8-10% higher leaf number counts compared to the control (Table 6). Compost, on the other hand, had a similar leaf counts to the control during the duration of the experiment. The results obtained are similar to the findings by Lindani and Brutsch (2012), who reported that there were no pronounce and/or consistent difference in leaf counts between compost and control treatments. It was reported that higher leaf numbers may be related to increased nitrogen as shown by Aloni (1991) for peppers, when nitrogen was increased from 0-200 mg liter^{-1}. In the current study it is of interest to note that despite it's relatively high C:N ratio (Table 4), garlic straw had the highest leaf counts and also average or above-average SPAD readings towards the end of the growing season. It thus appears that it may decompose relatively easy. In terms of biochar, despite it's very high C:N ratio, it does not seem to negatively affect plant growth, probably because the carbon is mostly in an inert form an therefore will not induce microbial immobilization (Rondon et al., 2007).

Table 8 Leaf count of Tomato plants as influenced by different treatments at different sampling dates.

	Leaf Count (no. /plant)					
Treatments	49 DAT[1]	56 DAT	63 DAT	70 DAT	77 DAT	84 DAT
Biochar	17.4	19.9 ab[3]	22.0 ab	24.1 ab	25.1	26.0 ab
Bokashi	17.2	19.9 ab	22.3 ab	23.6 ab	25.1	26.7 ab
Bokashi+GS[4]	18.2	22.0 ab	24.4 ab	25.1 ab	26.9	27.6 ab
Compost	18.1	20.3 ab	22.5 ab	23.8 ab	26.0	26.2 ab
Compost+GS[4]	18.1	20.6 ab	22.4 ab	24.4 ab	26.7	27.1 ab
Control	17.3	20.1 ab	22.1 ab	24.0 ab	26.3	26.9 ab
Cyanobacteria	17.6	20.0 ab	21.9 ab	23.7 ab	25.1	25.1 b
Garlic Straw	19.3	22.7 a	24.5 a	26.5 a	28.0	29.3 a
Peat pot	17.3	20.1 ab	21.1 b	23.4 ab	25.4	26.2 ab
Sig.[2]	n.s.	**	**	n.s.	n.s.	*

[1] Days After Transplanting

[2] (***): $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; (n.s). $P > 0.05$

[3] Letters indicate that means differed significantly ($P<0.05$) as estabisheded by Tukeys HSD Test.

[4] Garlic Straw (GS)

3.5 Nematode functional diversity

In terms of soil nematode functional diversity, the biggest differences ($p<0.05$) were observed in *Panagrolaimida* counts, which is a bacterial feeding nematode (Table 9). Use of bokashi plus garlic straw had the lowest results due to a higher percentage of *Rhabditida.* Garlic straw on alone had the highest percentage of *Panagrolaimidae* with 94%. The only plant parasitic nematode found was *M. incognita*, though values were relatively low and not significantly different ($p>0.05$) among the treatments. Moreover, parasitic nematode populations accounted for 0.1- 0.3% (100-500 nematodes) of the total observed population counts.

The results obtained were somewhat expected. An increase in bacterial feeders when adding compost to soil on rye fields was also noticed by Nair and Ngouajio (2012). Additionally, Porazinska (1999) also noticed an increase in bacterial feeding nematodes with the addition of organic inputs. With the addition of garlic straw the increase of *Panagrolaimidae* could be due to specific food choice, (which was abundant in the garlic straw).

3.6 Nematode Counts

Different soil treatments showed a significant effect on nematode populations (Table 9). The highest total nematode population in 100g of soil, which was dominated by non-parasitic nematodes, occurred with the use of bokashi plus garlic straw. In this case, the nematode population was 35% higher than the control. Surprisingly, the control had comparable populations to garlic straw; and higher populations than for the compost, bokashi, cyanobacteria, compost plus garlic straw, peat pot and biochar treatments. The biochar treatment had 82.5% less nematodes compared to the highest population achieved and it may be that it contains bio-toxic compounds.

In terms of the data, these results were somewhat unexpected. In their review of nematode population dynamics, Thoden (2011) noted a typical increase in free-living nematode populations with the addition of soil amendments. Furthermore, Zelenev (2004) found that nematode populations, specifically bacterial feeders, explode within the first few days of incorporation of organic amendments. They also noted that bacterial numbers are related to bacterial feeding nematodes growth rates. Populations should have been highest for bokashi due to the high population of bacteria. However, in the case of combining bokashi and garlic straw, different C:N ratios may have provided different feeding options for the bacterivorous nematodes (Korthals et al., 2014). Moreover, the C:N ratio of the used soil amendment also play a crucial role in nematode diversity. In the case of biochar with a high C:N ratio, Zhang et al. (2013) observed there was no change in nematode abundance at different biochar rates. However, in the current study, there was a sharp drop in total nematode population compared to the control or compost.

Table 9 Population diversity of different nematode functional groups in 100g of soil as influenced by different treatments.

	Functional Diversity															
	Bacterial Feeding										Fungal Feeding		Plant feeding		Predator		
Treatments	Rhabditida		Diplogasteridae		Panagrolaimidae		Cephalobidae		Prismatolaimidae		Aphelenchidae		M. Incognita		Mononchidae		Total Pop.
Biochar	11129	(11%)	14119	(14%)	72757 b[2]	(73%)	664	(0.67%)	166	(0.17%)	332	(0.33%)	498	(0.50%)	0.00	(0.00%)	65888 b
Bokashi	3614	(5%)	8260	(11%)	65820 ab	(85%)	258	(0.33%)	129	(0.17%)	0	(0.00%)	0	(0.00%)	0.00	(0.00%)	97559 b
Bokashi+GS[3]	70767	(23%)	39933	(13%)	187027 ab	(62%)	3538	(1.17%)	0	(0.00%)	505	(0.17%)	1516	(0.50%)	0.00	(0.00%)	376966 a
Compost	8467	(6%)	16698	(12%)	116417 ab	(83%)	235	(0.17%)	706	(0.50%)	0	(0.00%)	470	(0.33%)	235.19	(0.17%)	101726 b
Compost+GS[3]	11153	(6%)	9392	(5%)	153788 ab	(87%)	1174	(0.67%)	0	(0.00%)	0	(0.00%)	587	(0.33%)	0.00	(0.00%)	124351 b
Control	4217	(2%)	26923	(14%)	161539 ab	(83%)	649	(0.33%)	0	(0.00%)	0	(0.00%)	324	(0.17%)	0.00	(0.00%)	244711 ab
Cyanobacteria	8649	(5%)	21912	(13%)	142137 ab	(82%)	1730	(1.00%)	288	(0.17%)	0	(0.00%)	288	(0.17%)	0.00	(0.00%)	160214 b
Garlic Straw	6166	(3%)	5441	(3%)	204581 a	(94%)	725	(0.17%)	363	(0.00%)	0	(0.17%)	363	(0.00%)	0.00	(0.17%)	248766 ab
Peat pot	7966	(5%)	19418	(13%)	120492 ab	(81%)	747	(0.83%)	249	(0.17%)	249	(0.17%)	0	(0.33%)	248.95	(0.00%)	170637 b
Sig.[1]	n.s.		n.s.		**		n.s.		n.s.		n.s.		n.s.		n.s.		***

[1] (***): P < 0.001; (**) P < 0.01; (*) P < 0.05; (n.s). P > 0.05

[2] Letters indicate that means differed significantly (P<0.05) as established by Tukeys HSD Test.

[3] Garlic Straw (GS)

3.7 Stem Fresh Weight

There was a significant difference in stem fresh weight among treatments (Table 10). Use of garlic straw resulted in 20% greater stem fresh weight compared to the control. Biochar and cyanobacteria treatments had the lowest weights comparable to that of the non-amended control.

Results that were obtained did not match those from previously published data. When increasing fertilizing rates on pepper plants under a tunnel house in Spain Flores (2007) witnessed a significant increase in stem fresh weight. Since bokashi had the highest percent of nitrogen, it was expected to have the highest stem fresh weight. In the case of biochar, Vaccari et al. (2015) documented an increase in plant growth and dry weight with the application of two different types of biochar that had undergone different pyrolysis processes. However, in the current study use of biochar did not provide a significant increase in stem growth compared to the non-amended control. Differences in outcome may be related to differences in growth environment as the study by Vaccari et al. (2015) was a field study with processing tomatoes grown on a fertile soil where inorganic fertilizer was applied. In this context, use of biochar enhanced plant available $NH_4^{+,}$ K^+, and P values via enhancing soil nutrient retention while also improving soil moisture supply thus reducing crop water stress. In the current study, on the other hand, plants were grown in a protected environment and under optimal growth conditions without pronounced water stress while nutrients were applied as organic amendments. It may be possible that under near-optimal growth conditions benefits of biochar on plant growth may be less articulated.

3.8 Leaf Fresh Weight

Similar to results for stem weight, fresh leaf weight was increased with the application of garlic straw, with values being 12% higher when compared to the control (Table 10). The three lowest results were obtained by biochar, cyanobacteria and peat pot treatments, with numeric values being about 5% lower compared to the control. However, these difference were not significant ($p>0.05$).

The results obtained although consistent with those for stem weights did not match previous scientific findings. Eggplant biomass was reported to increase with increasing compost rates to the soil ((Taguiling, 2013). In the case of biochar and through a meta-analysis on processing tomatoes, Vaccari et al. (2015) found that the use of biochar on tomatoes increased plant growth compared to that of non-amended control for processing tomatoes. But this may be related to differences in production environments as discussed in the previous section. Dao (2013) noted an increase in growth when adding biochar to fertile soils but not under sandy soils. Plant growth for the peat pot treatment, on the other hand, was expected to be low because of the initial slower growth due to root constraint and this finding was consistent with reduced initial plant height and stem diameter (Tables 5 and 6).

Table 10 Final fresh weights of tomato leaves and stem as affected by different treatments at end of the experiment.

Treatments	Stem (g)	Leaf (g)
Biochar	285 b[2]	176 b
Bokashi	307 ab	204 ab
Bokashi+GS[3]	341 ab	194 ab
Compost	329 ab	196 ab
Compost+GS[3]	300 ab	194 ab
Control	302 ab	189 ab
Cyanobacteria	277 b	177 b
Garlic Straw	375 a	215 a
Peat pot	297 ab	176 b
Sig.	*	x

[1] (***): P < 0.001; (**) P < 0.01; (*) P < 0.05; (x) P<0.10; (n.s). P > 0.05

[2] Letters indicate that means differed significantly (P<0.05) as established by Tukeys HSD Test.

[3] Garlic Straw (GS)

3.9 Fruit production

There were no significant differences (p>0.05) in tomato fruit counts and weights for none of the fruit clusters (Table 11-12). When comparing treatments based on total production, there were also no significant difference, between total fruit number, average fruit weights and total fruit yield expressed in kg m^{-2}. Garlic straw though not significantly different had the highest production compared to the control or compost.

The results we achieved were unexpected compared to results observed by other experiments. In many instances the addition of a soil amendment such as composts was reported by Abbasi (2002) increased total yield of organic grown tomatoes by 10 tons ha^{-1} even with application rates of only 12-15 tons ha^{-1} which is low compared to standard practices in Dutch organic greenhouses. An increase in cherry tomatoes was also seen with the addition of biochar, which increased production by 64% compared to control (Hossain et al., 2010). This was also the case when bokashi was applied as Jahja (2002) documented that there was an increase in production of 12% with the addition of bokashi applied at 10 ton ha^{-1}.

Table 11 Mean number of fruits per plant on different clusters as influenced by different treatments.

	Fruit Cluster Number							
Treatments	No. 1	No. 2	No. 3	No. 4	No.5	No. 6	No. 7	No. 8
Biochar	9.3	10.6	7.3	8.1	6.6	2.1	2.1	0.0
Bokashi	10.3	8.1	7.0	8.7	5.9	3.9	3.9	0.0
Bokashi+GS[3]	8.3	8.6	8.4	7.7	7.6	4.4	2.7	0.6
Compost	6.7	8.0	7.3	6.1	5.4	3.6	2.3	1.4
Compost+GS[3]	7.4	8.7	8.1	6.9	6.9	3.6	3.3	0.7
Control	7.9	8.1	8.1	7.1	6.0	2.7	3.6	0.0
Cyanobacteria	8.0	8.7	6.4	7.3	4.1	2.4	1.3	0.0
Garlic Straw	8.3	8.0	8.3	8.7	7.3	4.6	5.1	0.7
Peat pot	6.6	7.7	8.7	8.6	5.9	5.9	3.4	2.6
Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] (***): $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; (n.s). $P > 0.05$

[2] Letters indicate that means differed significantly ($P<0.05$) as established by Tukeys HSD Test.

[3] Garlic Straw (GS

Table 12 Average fruit weight on different clusters as influenced by different treatments.

	Fruit Cluster Number								Total Production		
Treatments	No. 1	No. 2	No. 3	No. 4	No.5	No. 6	No. 7	No. 8	Count	Avg Wt. (g)	Kg^{3} m^{-2}
Biochar	63.0	35.1	33.0	36.5	20.3	1.3	0.5	0.0	46.1	189.7	1.39
Bokashi	65.9	41.4	34.1	39.3	12.0	4.9	0.3	0.0	47.7	206.0	1.50
Bokashi+GS[3]	69.3	40.0	37.5	37.5	20.8	8.4	0.6	0.0	48.3	214.1	1.46
Compost	65.5	32.6	34.1	39.9	18.0	9.8	2.7	0.2	40.9	202.9	1.86
Compost+GS	75.6	33.1	48.1	36.8	22.2	7.6	0.9	0.1	45.6	224.5	1.48
Control	60.1	46.5	36.0	44.7	25.5	3.6	0.4	0.0	43.6	216.8	1.42
Cyanobacteria	77.0	39.1	54.2	33.2	12.6	4.4	0.1	0.0	38.3	220.5	1.46
Garlic Straw	75.8	44.1	47.0	31.6	13.9	0.1	0.2	0.0	51.0	237.8	1.90
Pet pot	43.6	48.7	33.7	32.7	24.9	4.3	0.7	0.0	43.4	200.4	1.43
Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] (***): $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; (n.s). $P > 0.05$

[2] Garlic Straw (GS)

[3] Accounts for all tomatoes and not marketable tomatoes.

3.10 Leaf-Sap Analysis

3.10.1 Macronutrients

There were no differences in time-averaged (across the sampling dates) for macronutrient concentrations in the petiole extracts among any of the treatments (Table 13). Specific temporal patterns for each nutrient are provided in Appendix E.

Total nitrogen levels in the petiole sap extract were below the target levels included in Table 13. These values are based on long-term observations for tomato for the BioVerbeek operation that routinely and frequently monitors plant nutritional status via Nova Crop Control petiole sap analysis support program. The highest nitrogen levels were achieved by bokashi, though values were not significantly different from the other treatments. The Biochar treatment, on the other hand, had the lowest nitrogen levels. Ammonium levels within the plant leaf were also not significantly different among the treatments. Leaf ammonium levels were also relatively low but not below the set concentration limit. Surprisingly the peat pot treatment values were relatively high considering the poor initial start. In the case of nitrate, all plants were below the target level except for bokashi plus garlic straw. However bokashi plus garlic levels were only slightly above the minimum limit, though it was not significantly different among other treatments.

The results obtained in the current study were contradictory to those published by many other researchers. In many cases when tomatoes were placed in a hydroponic system, increasing the nitrogen application rates correlates with increasing leaf nitrogen levels (He, 1999; Huett & Rose, 1988; Wira, 2013). Ozores-Hampton (2005), also observed higher leaf petiole nitrate levels with addition of biosolids on field grown tomatoes from 1998-99. However, it may be possible that with the addition of organic soil amendments requires time before nitrogen is released thus making nitrogen the most limiting factor (Clark et al., 1999).

In the case of phosphorous leaf concentrations, levels were moderate-high, though there were no differences among the treatments. The lowest value was observed for biochar, which had 20% lower phosphorus values compared to the control. Potassium concentrations on average were within the range, and most treatments had values between 4000-5000 mg/l. Again, there were no significant differences among the treatments, although biochar had the lowest levels compared to the other treatments.

Phosphorous in organic farming systems often can be a limiting nutrient as observed by Barker (2012) for different soils. Furthermore the type of soil amendment used can also impact the effectivity of adding P. However, in the current study observed values were indicative of adequate P supply as petiole samples showed no deficiency (Båth & Otabbong, 2013). In the case of potassium Wira (2013) also observed an increase uptake with increasing fertigation concentrations of nitrogen from 100-220ppm when grown in coco-peat media. Ozores-Hampton (2005) observed lower potassium levels when peppers were amended with biosolids. The relatively low P values with biochar are in contrast with reports by Vaccari et al (2015) for a field study with processing tomato where the use of biochar enhanced plant available NH_4^+,' K^+,

and P values.

Calcium levels were on the low end of the spectrum of the typically observed (targeted) values, though there was no significant among the treatments (Table 13). The lowest value was achieved with biochar which was 6% lower than the control compared to garlic straw which achieved the highest value. The results do not match findings by He (1999) who observed that nitrates compete with calcium uptake. In the of case biochar relatively low nitrate levels coincided with a slightly lower level of calcium, the opposite occurred with bokashi, which showed relatively low levels of nitrate while having similar or slightly higher calcium levels compared to the biochar.

Magnesium, there were no significant differences among treatments (Table 13). The lowest value was achieved by biochar which had 10% lower numeric values compared to the control. All treatments had moderate levels of magnesium in the petiole sap. Similar to findings by He (1999) on calcium, magnesium is also in competition with nitrate uptake. But in the current study overall trends and correlation appeared to be less clear and/or inconsistent.

Sulfur also did not show any significant difference between the treatments (Table 13). The highest numeric values occurred with bokashi plus garlic straw, while the biochar treatment again showed the lowest value. In general, sulfur levels appear to fall in the average range for all treatments.

3.10.2 Micronutrients

Copper levels were on the low end of the commonly observed values, and there was no significant difference among the treatments (Table 14). The difference between the highest numeric value occurred with the peat pot treatment while biochar had the lowest values

Iron levels were similar across all treatments (Table 14) while the peat pot treatment had the highest numeric values that were 27% over the maximum value set. The remaining treatments had levels that were on the high end of the previously observed values.

Zinc leaf petiole values also showed no significant difference among treatments (Table 14). All treatments had moderate levels of zinc. In terms of numeric values, bokashi had the highest value of 2.2mg/l, which was 36% higher than the control at 1.4 mg/l.

Aluminum There was no significant difference across treatments for petiole aluminum concentrations (Table 14). The highest values were observed with bokashi, which had a 37% higher aluminum concentration compared to the control.

Sodium levels exceeded the desired values for all treatments, but treatments were not significantly different (Table 14). The highest values were achieved by compost, which was 14% higher than the control. Similar to findings in sulfur, Kirkby (1981) saw no changes in chloride levels with increasing levels of potassium.

Chlorine levels were not significantly different across treatments (Table 14). Mean values of chlorine for all the treatments were between the minimum and maximum observed values. The lowest values were observed for peat pot treatments which were 40% lower than the control.

Results obtained were contradictory to findings of Kirkby (1981) who documented higher chlorine levels with increasing levels of potassium.

Boron levels were moderate to high but not significantly different among all treatment means (Table 14). There was a 1mg/l difference between biochar and garlic straw, which had the highest value.

Molybdenum levels were low to moderate though they were not significantly different (Table 14). Values for bokashi and bokashi plus garlic straw, though not significantly different, were 30% lower than the highest one which occurred with the garlic straw and peat pot treatments.

When relating different nutrients there was a surprising relationship between biochar's low phosphorous and sulfur levels while resulting in a relative increase in molybdenum levels compared to a majority of the other treatments (Table 14). Alhendawi (2005) saw an increase in molybdenum levels when sulfate levels decrease. In another case, Heuwinkel (1992) saw a direct relationship between phosphorus deficiency and molybdenum increase when grown tomato under water culture.

Silicon concentrations were similar across treatments (Table 14). Biochar treatments had the lowest numeric value, although not significantly different from other treatments, while the highest value was observed for the peat pot treatment with overall values being 20% higher than biochar.

Table 13 Seasonal averages for petiole concentrations for macronutrients as affected by different treatments based on Nova Crop Control leaf sap analysis results.

Treatments	Total Nitrogen	Phosphorous	Potassium	Ammonium (NH_4)	Nitrate (NO_3)	Soluble Nitrogen[3]	Calcium	Magnesium	Sulfur
Low	1000	200	2500	40	500	540	1000	225	1250
Moderate	1600	400	4250	80	2250	2300	3500	760	1875
High	2200	600	6000	125	4000	4125	6000	1300	2500
Biochar	680	385	4413	58	260	103	1797	657	1714
Bokashi	788	529	4852	68	478	160	1866	708	1931
Bokashi+GS[2]	727	516	4909	62	508	162	1839	749	1871
Compost+GS	693	490	4960	57	365	126	1938	706	1849
Compost	724	495	4821	64	372	133	1858	730	1718
Control	764	487	4702	62	377	133	1913	727	1898
Cyanobacteria	773	498	4839	69	325	127	1907	731	1776
Garlic Straw	710	483	4724	63	303	117	1979	735	1824
Peat pot	763	537	4717	70	400	798	1961	710	1874
Treatment[1]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Treat x day[1]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] (***): P < 0.001; (**) P < 0.01; (*) P < 0.05; (/) P<0.10; (n.s). P > 0.05

[2] Garlic Straw (GS)

[3] Soluble nitrogen is calculated by adding $N\text{-}NH_4$ and $N\text{-}NO_3$

Table 14 Seasonal averages for petiole concentrations for micronutrients as affected by different treatments based on Nova Crop Control leaf sap analysis results.

Treatments	Copper	Iron	Zinc	Aluminum	Sodium	Chlorine	Boron	Molybdenum	Silicon
Low	0.5	1.5	1		10	425	2.5	0.25	
Moderate	0.8	2.5	2.5		105	1200	3.7	0.5	
High	1.1	3.5	4		200	2000	5	0.75	
Biochar	0.48	3.5	1.2	1.2	210	1144	3.6	0.32	18.0
Bokashi	0.59	3.5	2.2	2.2	221	1102	4.0	0.25	21.9
Bokashi+GS	0.54	3.3	1.4	1.4	213	1137	4.2	0.26	21.5
Compost+GS	0.57	3.7	1.4	1.4	252	963	4.2	0.31	21.9
Compost	0.57	3.7	1.3	1.3	223	1120	4.2	0.32	21.6
Control	0.54	3.6	1.4	1.3	216	842	4.0	0.28	21.4
Cyanobacteria	0.59	3.2	1.5	1.5	230	1037	4.2	0.33	21.5
Garlic Straw	0.59	3.6	1.6	1.6	219	959	4.6	0.36	20.5
Peat pot	0.62	4.8	2.1	2.1	236	691	4.4	0.36	22.6
Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Treat x day	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] (***): P < 0.001; (**) P < 0.01; (*) P < 0.05; (/) P<0.10; (n.s). P > 0.05

[2] Garlic Straw (GS)

3.11 RKI

Soil amendments did not reduce the RKI when compared to the control. Moreover, there was no significant difference ($p>0.05$) across treatments (Table 15). The mean for all treatments was an RKI of two, which is indicative of low incidence of parasitic nematodes. The results obtained were surprising as the soil that was used for the experiment has not been steamed for two years and even with the presence of RKN. Results observed from garlic straw were not similar to findings from Gong et al. (2013) who observed reductions of up to 50% compared to control. *Nostoc calicola* has shown gall reductions on cow peas up to 90% by M. M. A. Youssef and Ali (1998), which may suggest that incubation with compost did not work correctly. The findings from biochar were also not similar to results obtained by Zhang et al. (2013), who noticed a decrease of up to 50% in plant parasitic nematode counts. In the case for compost, results were similar to findings by Walker (2007) who observed an insignificant difference of -0.6 between the control. In light of these consistently contrasting results, it may be possible that effects of soil amendments will only be evident at intermediate high (e.g. RKI=3-4) values. In the absence of high enough nematode numbers, the beneficial effects of soil amendment on nematode suppression thus may be less articulated and differences among treatments in terms of RKI which is a semi-qualitative measurement thus may be less pronounced.

Table 15 Root knot Index (RKI) values based on a visual assesment of different tomato root systems as affected by organic amendments.

Treatments	RKI
Biochar	2
Bokashi	2
Bokashi+GS[2]	2
Compost	2
Compost+GS[2]	2
Control	2.2
Cyanobacteria	2
Garlic Straw	2
Peat pot	2
Sig.[1]	n.s.

[1] (***): P < 0.001; (**) P < 0.01; (*) P < 0.05; (/) P<0.10; (n.s). P > 0.05

[2] Garlic Straw (GS)

4 Conclusion

Several of the proposed hypotheses that were stated earlier thus cannot be affirmed (Table 16). In the case of adding soil amendments, plant growth was not positively enhanced compared to the control. Only in some cases and for a few measurements there were significant differences compared to the control. The only distinct difference was between the leaf counts and stem/leaf fresh weights. The additions of garlic straw positively influenced those particular parameters compared to compost or bokashi.

One of the most significant differences on crop performance was found in the peat pot treatment. The smaller pot size hampered plant especially during the first few weeks. However, within a few weeks peat pot had similar heights and stem diameters as other treatments, indicating a higher growth rate possibly due to lower light conditions. Overall, the use of soil amendments, or the use of root containment, did not positively influence growth parameters nor did it reduce the RKI of tomatoes.

When considering if plants were deficient in terms of either macro or micro-nutrients, there were no pronounced visual differences that could be spotted when comparing the treatments. Based on SPAD readings, crop nitrogen status was relatively similar across all treatments throughout the experiment. Although nitrogen supply may have been rather low around the time of the second application as shown by declining SPAD values around 63-70 DAT for all the treatments, still there were no significant difference among treatments. Phosphorus and potassium levels were adequate and there was no sign of any differences between the treatments either. Overall, biochar had the lowest values for majority of the nutrients that were analyzed.

In terms of tomato production, there wasn't any significant benefit gained from the addition of any of the included soil amendments. The control treatment did fairly well since it was supplemented with chicken manure. However, addition of garlic straw showed some benefits as numerically it had the highest production compared to all the treatments. Therefore, it may hold promise as a soil amendment especially since it can easily be found locally.

In the case to nematode population and diversity, the hypothesis stated in the introduction was true in some cases since there was a positive effect with the addition of certain treatments on nematode diversity. Main effects were only seen among nematode bacterivores. The addition of bokashi plus garlic straw increased *Rhabtidia spp.* populations and nematode populations above other treatments. Interestingly enough, the control treatment had relatively high total nematode numbers, which relates to their quick reproductive behavior. When garlic straw was added alone, it increased the amount of *Panagrolaimidae spp.*, which may have positively influenced plant performance. However the hypothesis regarding an increase in predatory counts had to be rejected.

Lastly, there was no effect seen in reducing the RKI. The values for all the treatments were similar with little or no nodulation being observed through the visual assessment method that was being employed. The time frame (incubation period) may have been too short for the nodules to fully develop or alternatively may be the initial nematode pressure may have been too low. The use of algae was also not effective in reducing RKI values while it also appears to be difficult to inoculate large volumes of compost without proper lighting. As was discussed before, it may well be that effects of different soil amendments may be more pronounced when the incidence of nematodes (parasite pressure) is greater and in that case potential effectiveness and benefits in terms of crop performance may be more articulated as well.

Table 16 Hypothesis set prior to experiment and the outcome.

	Hypothesis	Outcome
1	Soil amendments should positively influence crop performance	Rejected
2	Increase bacterivorous nematodes compared to when no amendment is added	Not Rejected
3	Higher number of predatory nematodes within bokashi treatment	Rejected
4	BCAs and bio-toxic chemicals should reduce RKI	Rejected
5	Reducing RKI when using root containment	Rejected

5 Recommendations

The use of soil amendments for adding vital nutrients and stimulating soil biota is crucial to Dutch organic greenhouse producers. In regards to the current study and treatments that were evaluated it appears that garlic straw and bokashi, although not showing significantly increases in crop growth and nematode suppression, still may hold promise as a viable alternatives to use of standard compost material . The benefits to producers of using garlic straw are that it can be purchased locally, at a low price since it is a by-product. In the case of bokashi, due to the anaerobic environment less labor is needed to turn the heap and it requires only seven weeks to fully ferment. In terms of growing plants in peat pots (soil containment); stronger pots may be needed which are infused with nematocidal properties that are slowly released The use of peat pot may require more attention due to the smaller pot volume, especially when growing tomato transplants dedicated to production. Additionally, the width of the wall may also be altered to modify the life span of the physical barrier it provides within the soil, and this should be tested during subsequent studies. Based on the current study, the use of biochar does not appear to be beneficial to plant growth nor did it show benefits in terms of enhancing crop nutrient status. Moreover, with the application of biochar small amounts of polycyclic aromatic hydrocarbons (PAHs) may be released to the environment. These compounds originate mainly from incomplete combustion of fossil fuels, and pose a significant human health risk (Quilliam et al., 2013). The small amounts of carcinogenic biochemicals may be released, which could cause some problems in terms of perceived health benefits associated with organic products. Lastly inoculating compost with cyanobacteria will be difficult to implement on a large scale due to the amount of energy required to cultivate the algae. Overall many of these treatments require more research to clarify the results found in the current experiment at higher concentrations of RKN nematodes.

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7 Appendices

Appendix A ***Experimental layout***

Figure 1 RCBD layout of nine different treatments.

Appendix B ***2013 BioVerbeek Compost Temperatures, CO_2 and Rotations***

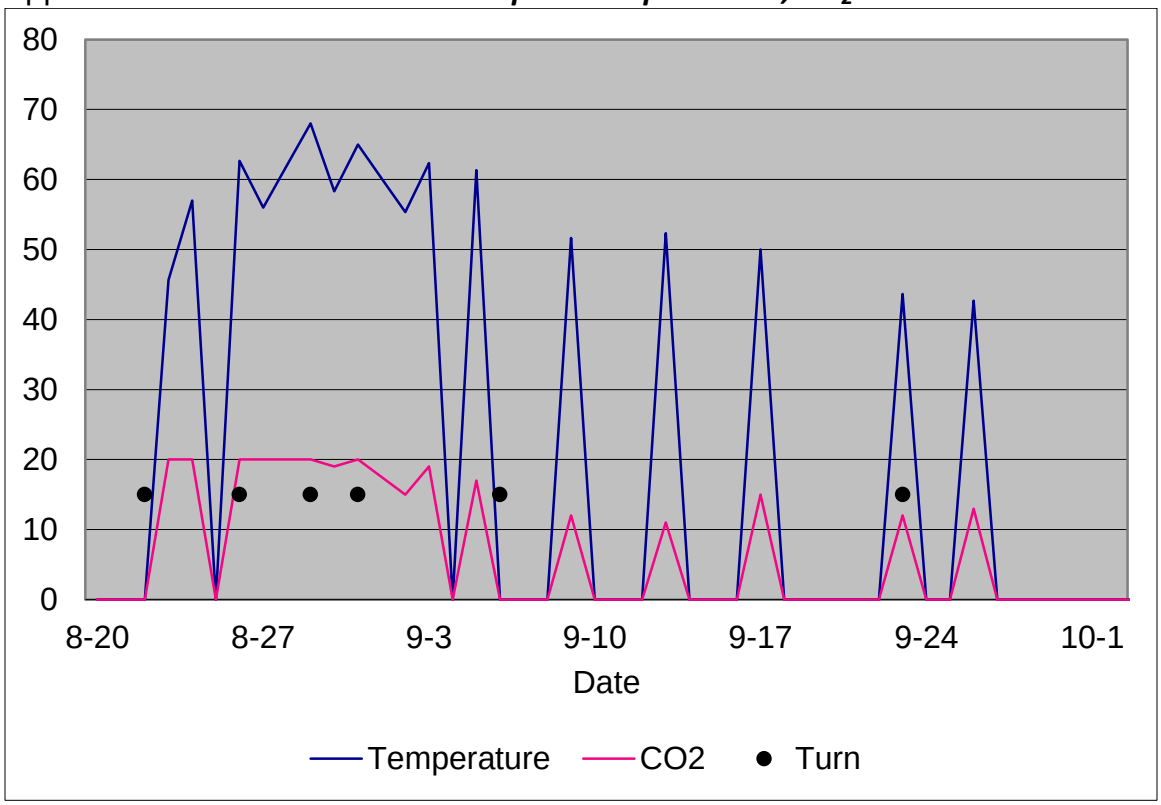


Figure 2 Change in temperature and CO2 and the number of rotations for compost produced by BioVerbeek in 2013.

Appendix C ***Bokashi Temperature and C:N ratio over 7 weeks***

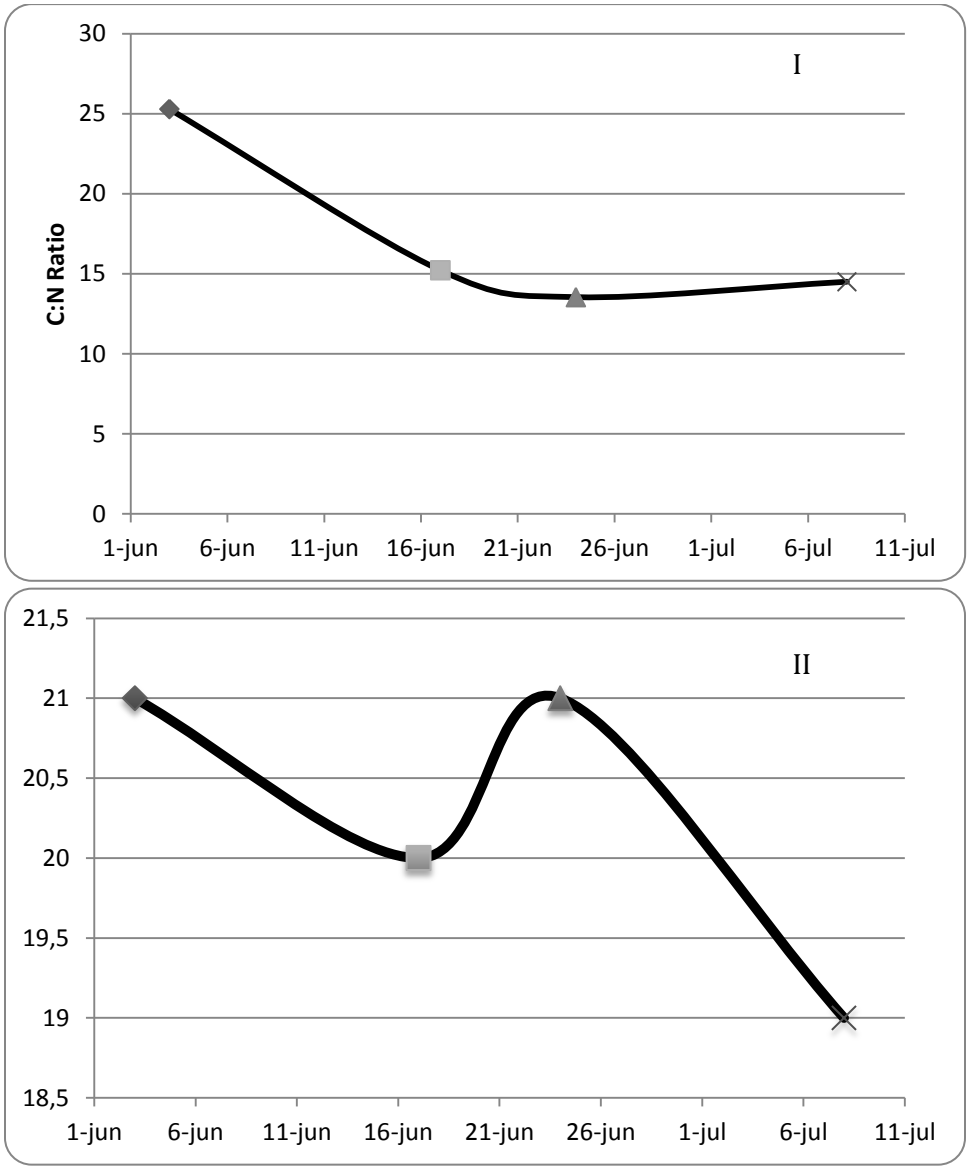


Figure 3 Graph I represents the change of C:N of bokashi during the composting phase. Graph I represents the change in temperature during the composting process.

Appendix D ***RKI Chart***

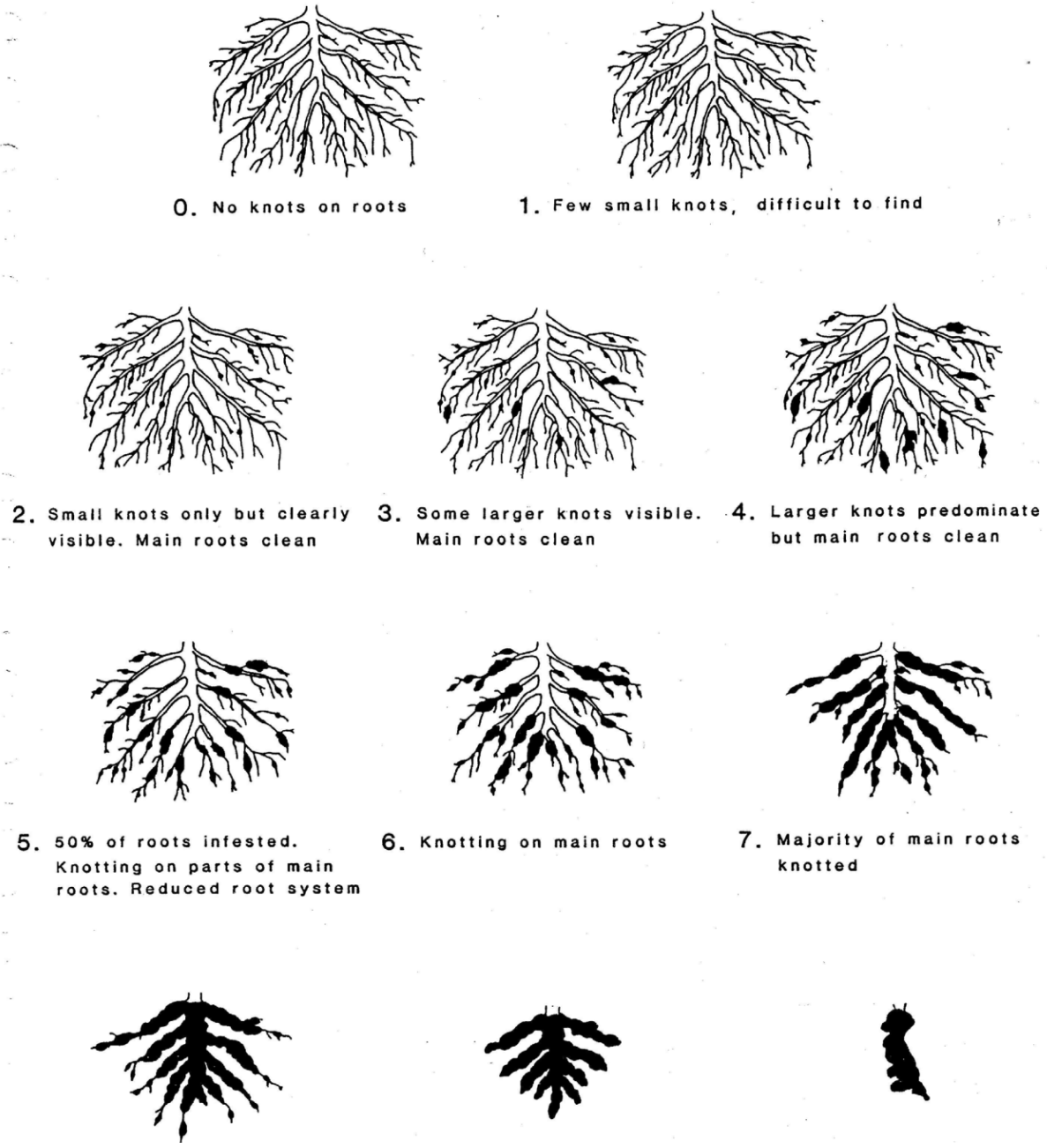
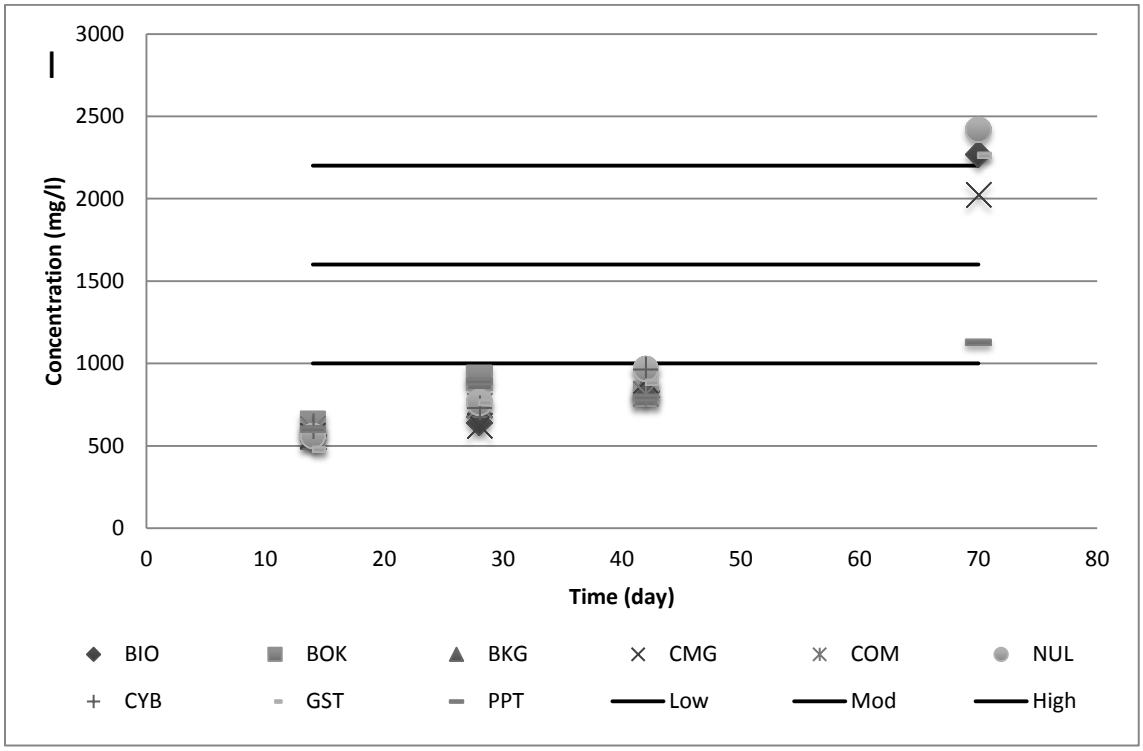


Figure 4 RKI chart use to determine the level of incidence on tomato roots. Taken from van der Wurff (2010).

Appendix E ***Leaf sap analysis specific temportal pattern***



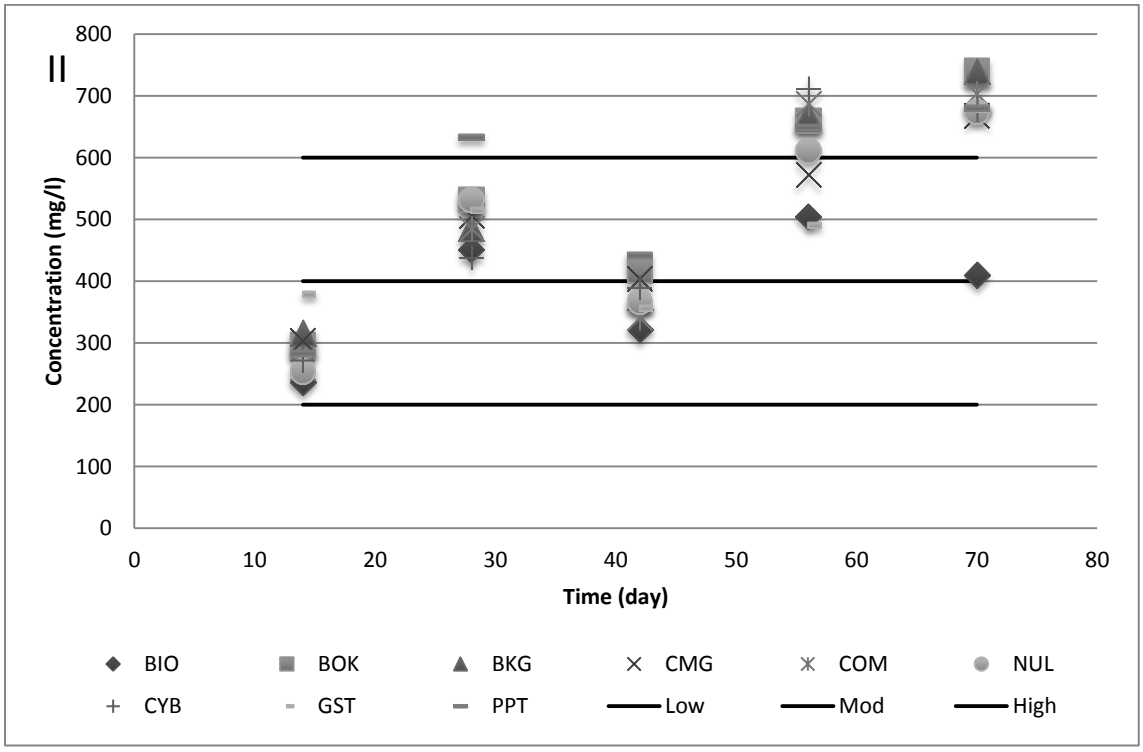


Figure 5 I – Leaf sap analysis of total nitrogen at different periods of the experiment. II – Leaf sap analysis of leaf phosphorus concentrations at different periods of the experiment.

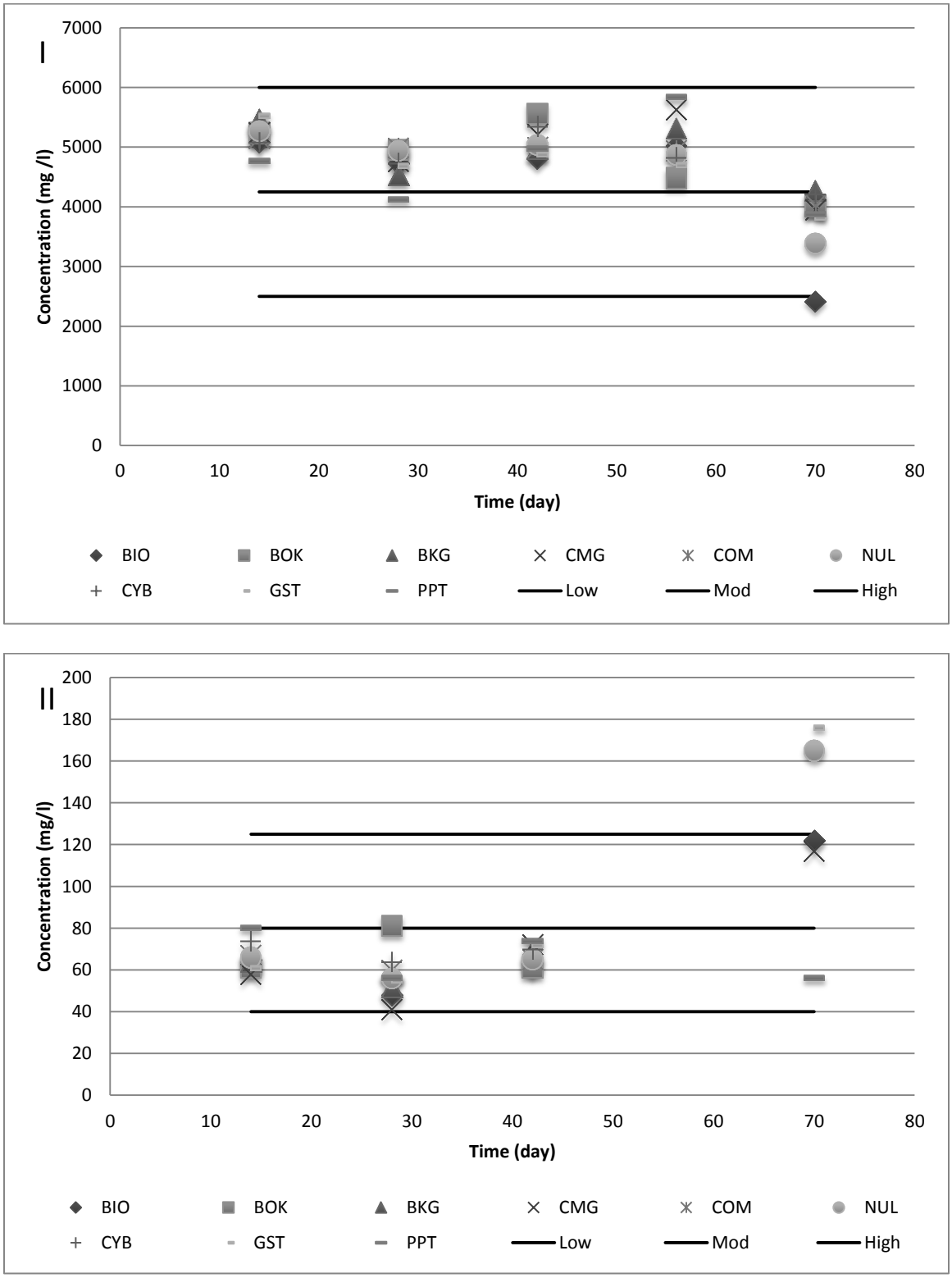


Figure 6 I – Leaf sap analysis of potassium levels at different periods of the experiment. II – Leaf sap analysis of leaf ammonium (NH4) concentrations at different periods of the experiment.

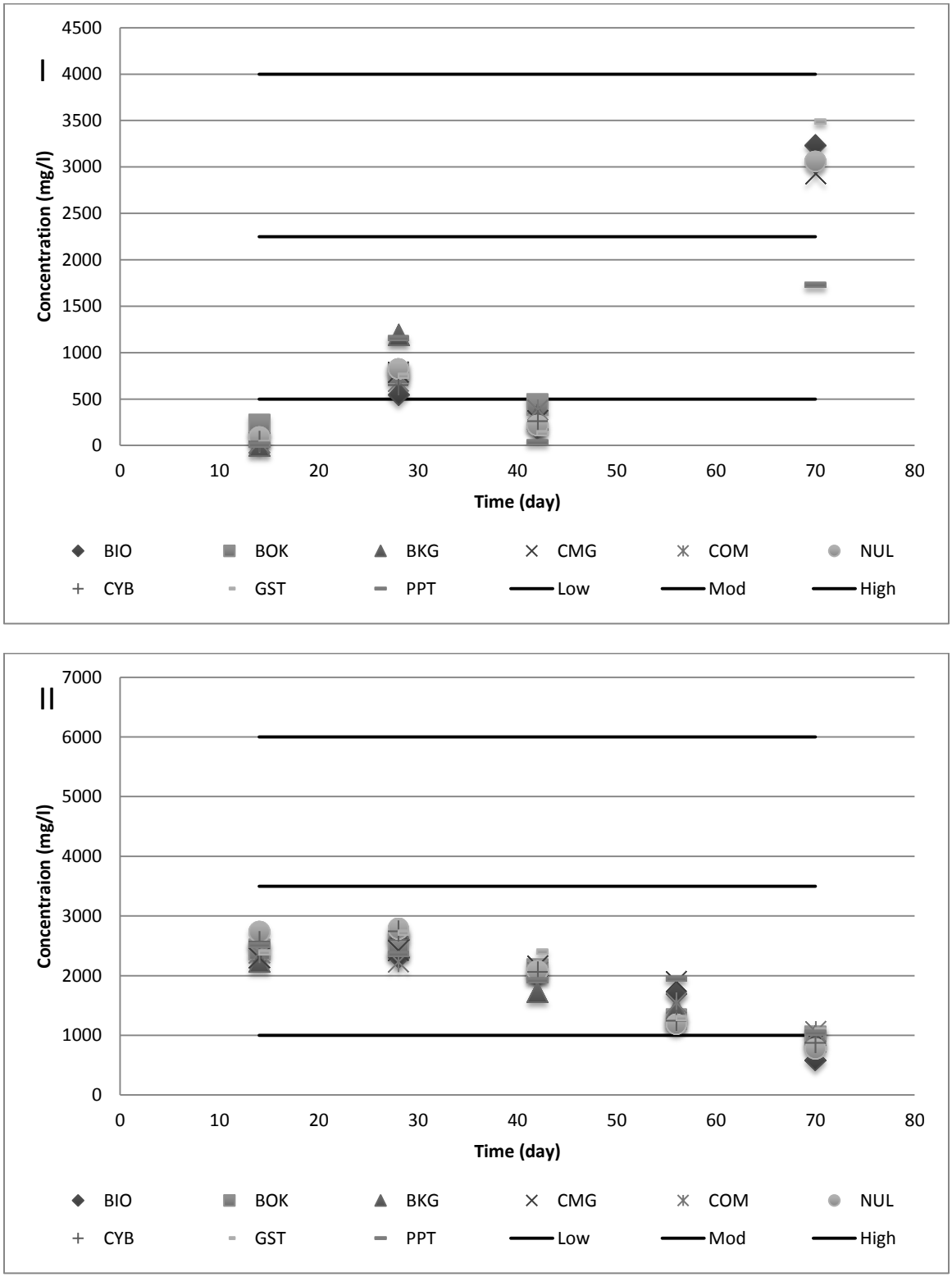


Figure 7 I – Leaf sap analysis of nitrate (NO3) levels at different periods of the experiment. II – Leaf sap analysis of leaf calcium concentrations at different periods of the experiment.

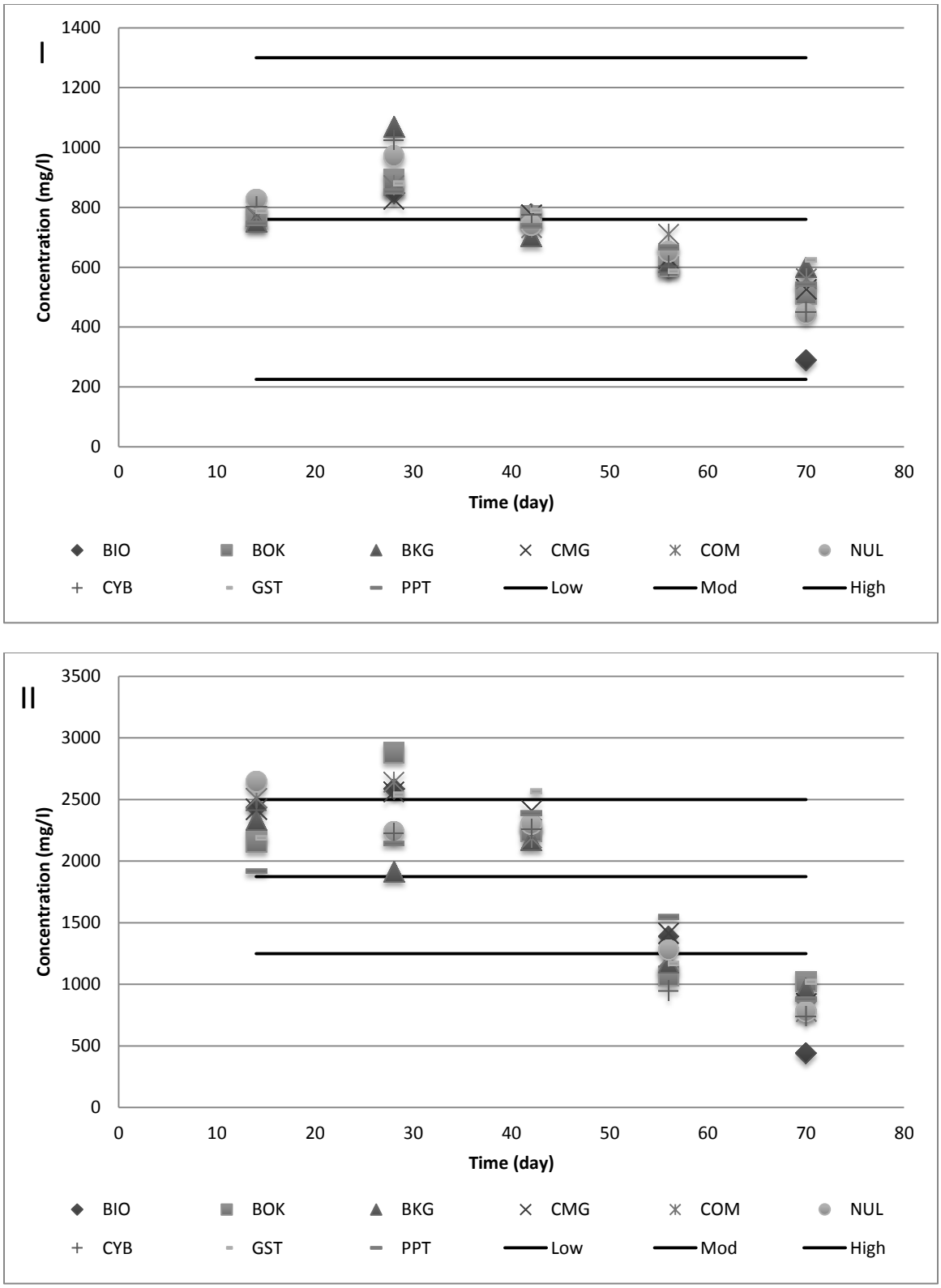


Figure 8 I – Leaf sap analysis of magnesium levels at different periods of the experiment. II – Leaf sap analysis of leaf sulfur concentrations at different periods of the experiment.

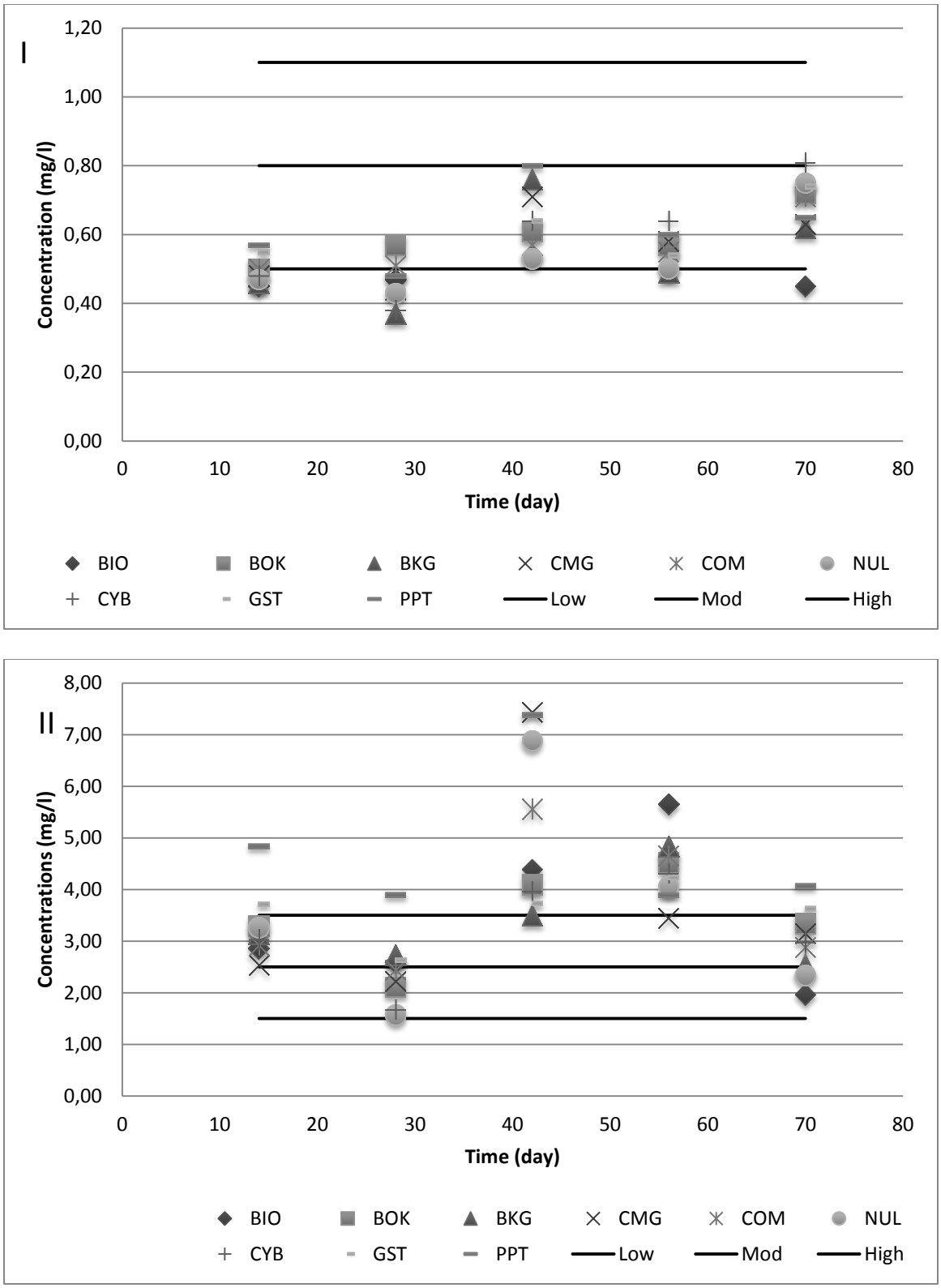
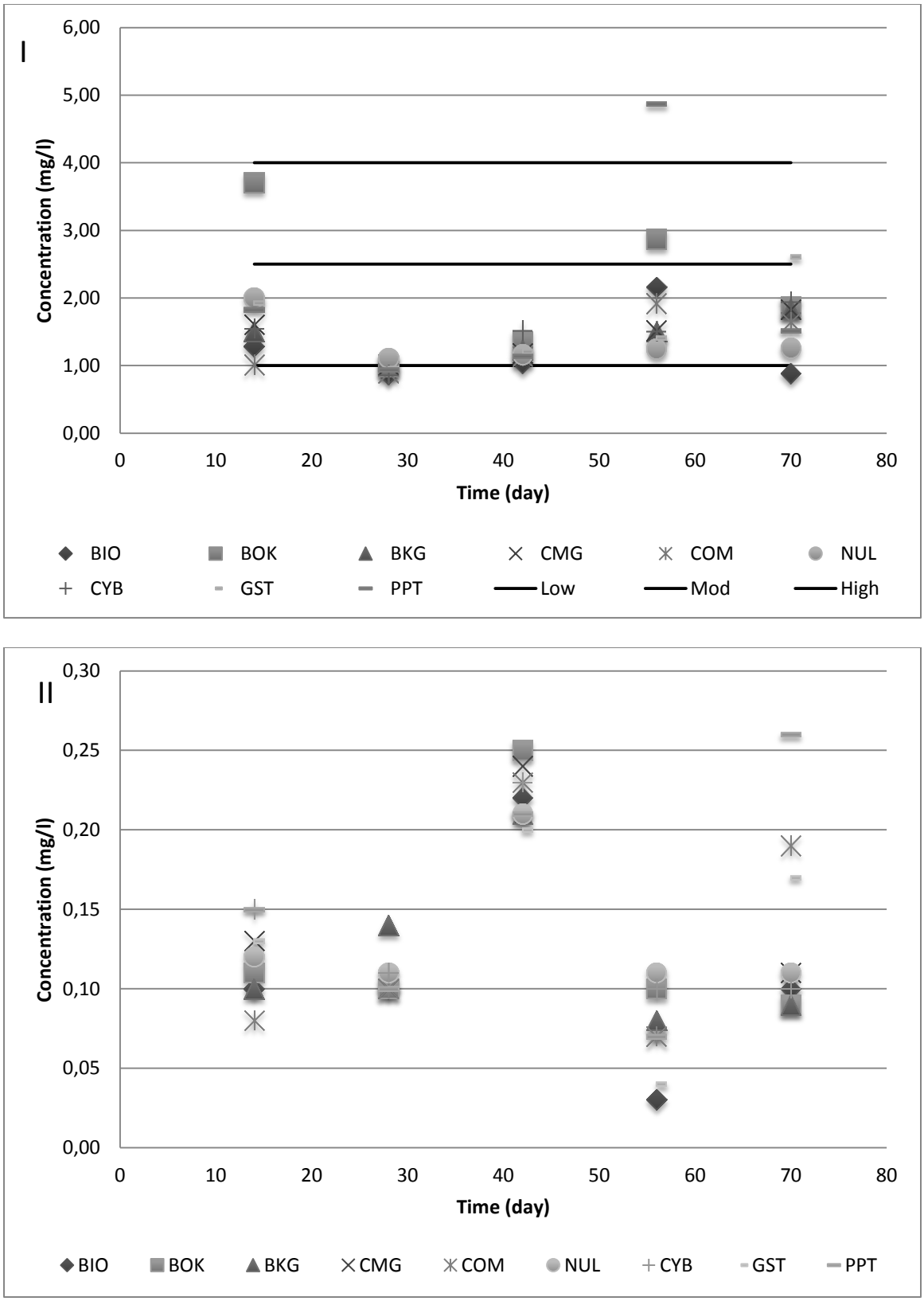


Figure 9 I – Leaf sap analysis of copper levels at different periods of the experiment. II – Leaf sap analysis of leaf iron concentrations at different periods of the experiment.



I – Leaf sap analysis of zinc levels at different periods of the experiment. II – Leaf sap analysis of leaf aluminum concentrations at different periods of the experiment.

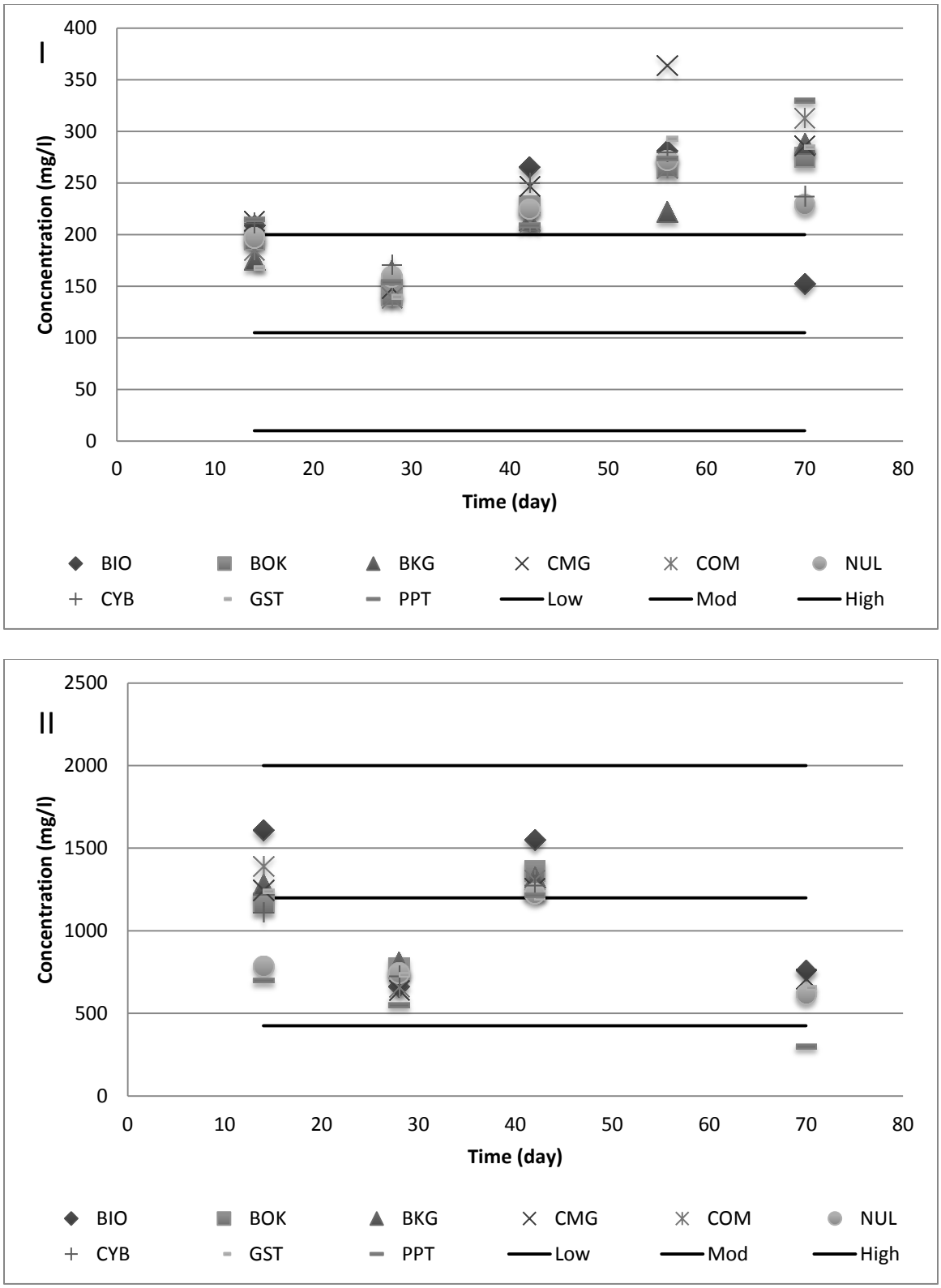


Figure 10 I – Leaf sap analysis of sodium levels at different periods of the experiment. II – Leaf sap analysis of leaf chlorine concentrations at different periods of the experiment.

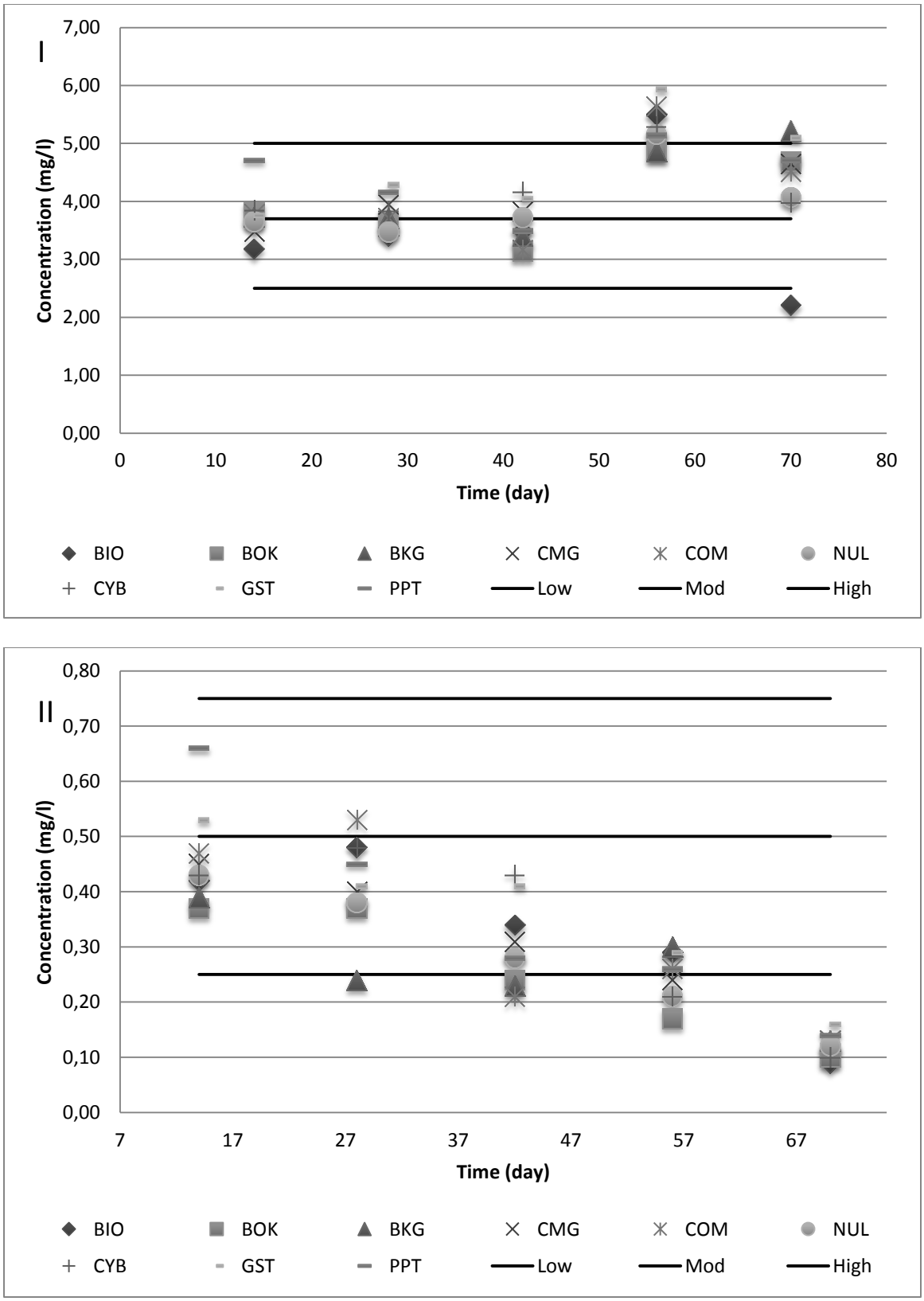


Figure 11 I – Leaf sap analysis of boron levels at different periods of the experiment. II – Leaf sap analysis of leaf molybdenum concentrations at different periods of the experiment.

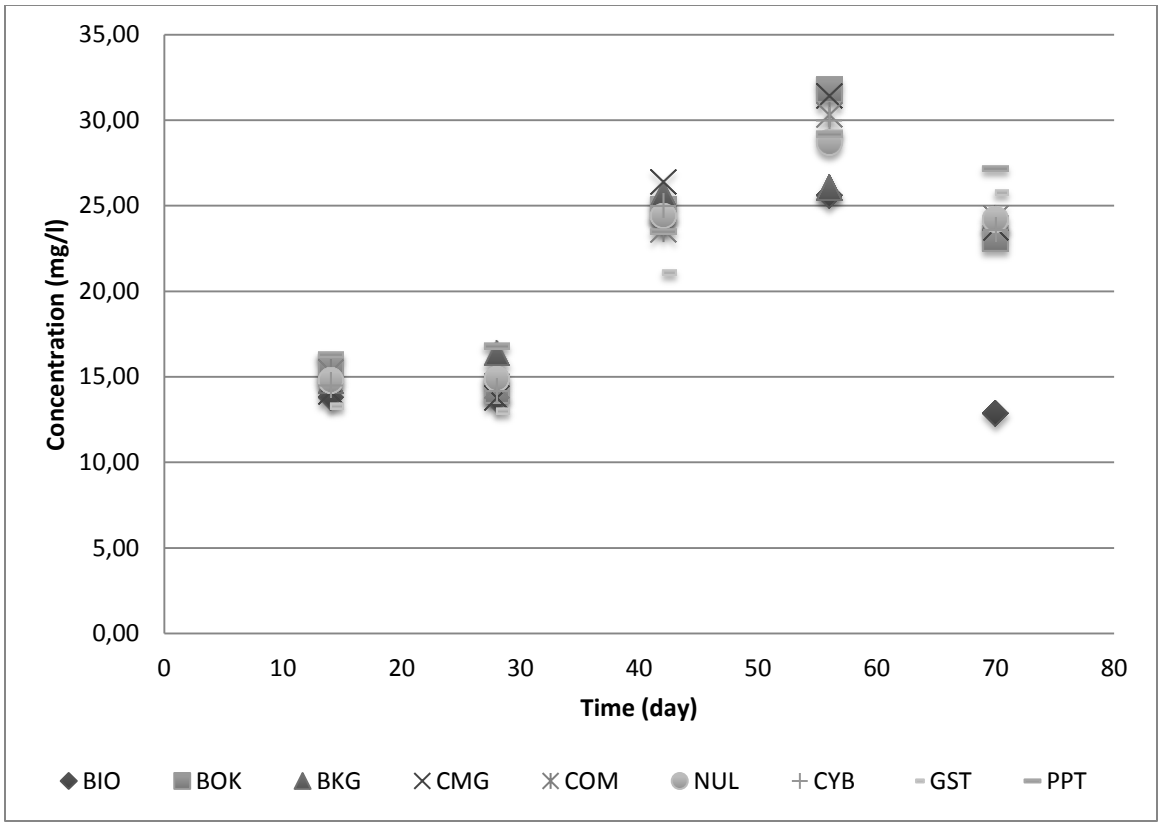


Figure 12 Leaf sap analysis of silicon levels at different periods of the experiment.