

# Comparing nitrogen fertilisers to improve nitrogen efficiency in horticulture systems by reducing leaching loss and increasing plant uptake

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## Abstract

In this research, different nitrogen fertilisers were used to determine their effect on nitrate leaching and plant uptake in soil columns. N fertiliser was applied at a rate of 22.5 N kg ha<sup>-1</sup> with four different treatments: (i) control (no fertiliser), (ii) standard fertiliser, (iii) slow-release fertiliser and (iv) fertiliser + nitrification inhibitor (DMPP). Sandy soils were used to replicate field conditions for intensive cropping systems (horticulture). Columns were irrigated equally with 154.3mm over a period of 5 weeks. Nitrate leaching was significantly reduced with the slow-release fertiliser, but the DMPP did not alter the total N leached over 5 weeks compared to the standard fertiliser. However, use of the slow-release fertiliser led to lower plant N uptake compared with standard fertiliser and fertiliser + DMPP.

**Keywords:** nitrification inhibitors, DMPP, slow-release fertiliser, coated fertiliser, leaching, nitrate.

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## 1. Introduction

The use of fertiliser plays an important role in food security at a global scale (Lea & Morot-Gaudry 2001). In fact, up to half of the global food production depends on nitrogen (N), phosphorus and potassium fertiliser applications (Walsh & Christiaens 2016).

Nitrogen takes part in all living matter and constitutes most of the earth's atmosphere (Rayment & Lyons 2010). In agriculture, N is taken up by plants and is an essential element for crop production, as well as phosphate and potassium (Lawlor, Lemaire & Gastal 2001).

Nitrogen is quickly and efficiently absorbed by the root systems of plants when it is available in nitrate ( $\text{NO}_3^-$ ) or ammonium ( $\text{NH}_4^+$ ) forms. The amount of N that is absorbed will be determined by plant demand and also depends on the concentration of plant available N in the soil solution. However, efficiency decreases under unfavourable conditions in dry and waterlogged soils regardless of the plants having a well-developed root system. Thus, the efficiency of N is variable (Lawlor, Lemaire & Gastal 2001).

Large amounts of N fertilisers are applied to paddocks to secure and maximise yields (Walsh & Christiaens 2016). These applications of fertiliser contribute to the increase of cost of production for farmers (Mohamad Yatim et al. 2016). In actual fact, N fertiliser is the main outlay of farmers. Furthermore, there is rising concerns about nitrogen loss in agriculture (Bouchet et al. 2016).

Excessive use of nitrogen fertiliser also has a negative impact on the environment (Asing et al. 2008). Loss of nitrogen caused by excessive use of fertiliser can lead to degradation of terrestrial and aquatic systems (Jez, Soon Goo & Sherp 2016). In fact, agriculture is a major contributor to water pollution (Campisi et al. 2016). To mention some cases, levels of nitrate in both surface and ground water are frequently above the permitted level of  $10 \text{ mg L}^{-1}$  for drinking water in the United States (Bottoms et al. 2012) and  $11.3 \text{ mg L}^{-1}$  in the European Union (EU) (Leach et al. 2004). When the level of nitrate exceeds  $45 \text{ mg L}^{-1}$ , there is a hazard to human well-being and health (Portocarrero & Acreche 2014). Indiscriminate use of N fertiliser can also contribute to air pollution by increasing greenhouse gas (GHG) emissions (Bouchet et al. 2016), such as  $\text{N}_2\text{O}$ , the most damaging of all GHG (International Plant Nutrition Institute 2013b).

Inappropriate use of N fertiliser has a negative effect on produce too. It can cause toxicity in crops that are sensitive to salinity (Slamic & Jug 2016). Leafy vegetables can store high levels of nitrate in the foliage, which might be detrimental for the consumer as it can easily transform into nitrite which is toxic. Nitrite can cause respiratory problems (asphyxia) and also could result in the formation of carcinogenic nitrosamines (Steege, Stulen & Mary 2001).

Horticultural systems are characterised by intensive use of fertiliser due to establishment of crops two or three times per year (Bottoms et al. 2012). High levels of nitrogen, from either fertiliser or manures, intensive cultivation, short-cycle crops, high inputs of water make for

poor nutrient use efficiency in irrigated vegetable production systems highly susceptible to nitrate leaching (Di & Cameron 2002). Additionally, sandy soils are commonly used in these systems (Victorian Resources Online 2016). Though this type of soil is well-drained (Wachendorf, Taube & Wachendorf 2005), it lacks nutrient and water retention (Zwingmann, Mackinnon & Gilkes 2011), hence N, particularly nitrate, can be easily lost through leaching and water has to be continuously applied to support crop requirements.

Under these circumstances, growing global concern about the environment, sustainable practices (Jez, Soon Goo & Sherp 2016) and consumer health (Newbould 1989) are putting pressure to increase nitrogen use efficiency (NUE) and to reduce nitrogen losses from agriculture (Dawson, Huggins & Jones 2008). Since nitrate moves easily with water (International Plant Nutrition Institute 2013c), leaching is considered among the principal paths for nitrogen loss (Yu et al. 2015) and also it is the factor that limits improving nitrogen use efficiency the most (Ding et al. 2010).

One way to diminish nitrogen losses is via application of nitrogen fertiliser in combination with nitrification inhibitors (NI). The NIs suppress the activity of ammonia oxidizing bacteria that transforms ammonium to nitrate (nitrification). 3,4-dimethylpyrazole phosphate (DMPP) is considered to reduce loss of nitrogen through leaching and at the same time improve NUE principally in areas with excessive precipitation with coarse-textured soils (Alonso-Ayuso, Gabriel & Quemada 2016). This NI has also been recognised to reduce denitrification gasses (i.e.  $N_2O$  and  $N_2$ ) (Asing et al. 2008). Besides DMPP, there are other options to reduce losses through leaching, such as coated fertilisers. Coated fertilisers have the same proportion of nutrients as standard fertiliser but with the addition of a coated layer (Sowiński et al. 2016). This coated layer is commonly made out of a polymer which covers the granules or pellets (Ahmad, NNR, Fernando & Uzir 2015). The release of nutrients from these fertilisers is much slower and often determined by soil moisture or temperature (Compo 2015).

Ladha et al. (2005) suggests that the use of NI and slow release fertiliser should improve NUE and reduce losses of nitrogen through leaching and volatilisation. Whilst several studies have been conducted on their effect on nitrate leaching and plant uptake of both, DMPP and slow-release fertiliser, the results vary. While some authors conclude that the use of NI (Abalos et al. 2014; Di & Cameron 2004) and slow-release fertiliser (Sowiński et al. 2016) can considerably reduce the amount of nitrogen that is lost through volatilisation and/or leaching, other authors did not found any different on the effect of slow-release N fertiliser on reducing leaching losses (Chilundo et al. 2016).

## 2. Objectives

This research aims to determine whether there is an influence of NI and slow-release fertiliser on losses of nitrogen via leaching of nitrate, and whether this has a subsequent effect of increasing the level of nitrogen uptake by plants (biomass production) compared to standard fertiliser, in intensive production systems with coarse-textured soils.



### 3. Literature Review

#### 3.1. Nitrogen as a plant nutrient

Plants require many elements to develop properly (Grayston, Vaughan & Jones 1997), at least sixteen different ones (Ming & Golden 1995). Carbon, oxygen and hydrogen are considered building blocks and they are obtained from air and water. The rest of the elements, such as nitrogen, phosphorus, potassium among others are called nutrients and they are taken up from the soil (Brown 1999).

Nitrogen is considered the most important of all plant nutrients and key to achieve crop production (Berendse & Aerts 1987; Fraters et al. 2015). Its importance was determined in 1872 by G. K. Rutherford (Fageria & Baligar 2005). Nitrogen is an essential element for plant development during the vegetative and reproductive stages (Ahmad, S et al. 2016) and plays a central role in the synthesis of essential molecules, such as nucleic acids (DNA and RNA), amino acids, proteins (Mohamad Yatim et al. 2016) and adenosine triphosphate (ATP) (Wagner 2012). It also is a basic component of compounds as chlorophyll, which is indispensable for photosynthesis (Wagner 2012), and alkaloids (Fageria & Baligar 2005).

Deficiencies of N can cause premature senescence, decrease yields (McConnell et al. 1995), reduce leaf area (Zhao et al. 2005) and cause chlorosis in older leaves and even necrosis in extreme deficiency cases (Mengel et al. 2001).

#### 3.2. Nitrogen in soils

In soils, nitrogen can be found in organic and inorganic forms (nitrate and ammonium) (Mosaic 2016).

Approximately 95 to 99% of nitrogen present in soil is in organic form and comes from plant residues, stubble, animal residues, organic matter and living organisms. This form of nitrogen cannot be taken up by plants but can be transformed, through mineralisation, into available forms by microorganisms (Mosaic 2016).

Nitrogen can be found in soils in inorganic forms of nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) which are readily available for plant uptake (Sowiński et al. 2016). Both, nitrate and ammonium, are natural soil resources (Cassman, Dobermann & Walters 2002). Nitrate are highly soluble; it mostly comes from N fertiliser or the mineralisation of organic matter (Portocarrero & Acreche 2014) and its movement is affected by rainfall, evapotranspiration, irrigation practices and soil properties (International Plant Nutrition Institute 2013c).

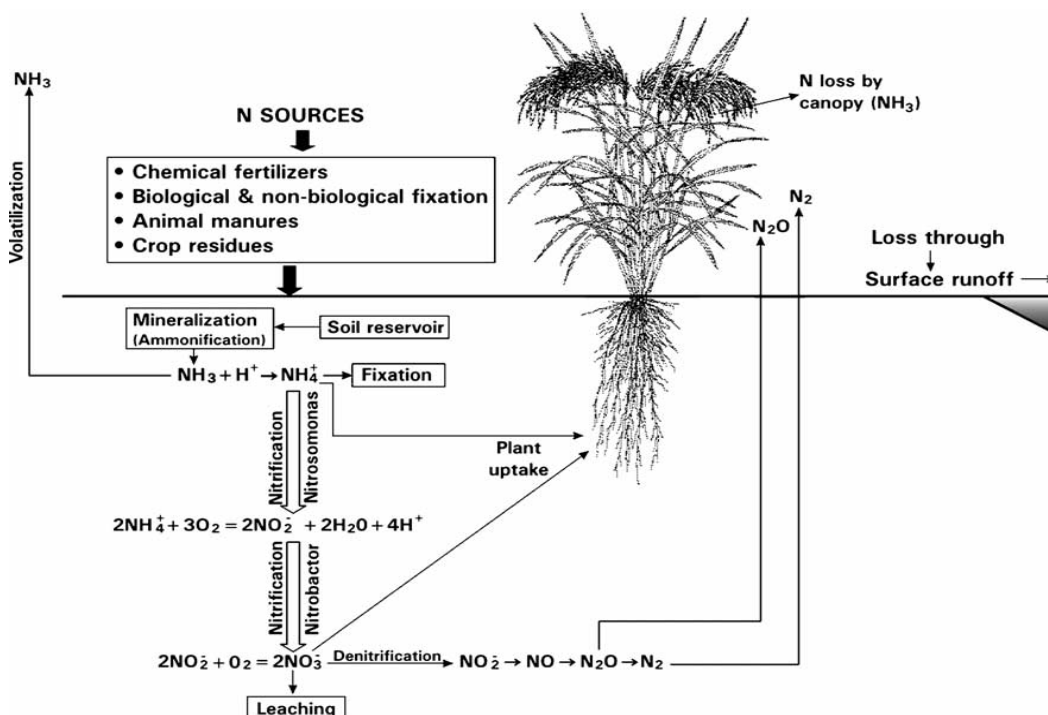
The amount of nitrate and ammonium that is readily and naturally available for plants in soils is insufficient to sustain crop production. Hence, the addition of fertilisers is required to secure produce (Hartz 2006).

### 3.3. Nitrogen Cycle

Understanding the N cycle is critical to assess the availability and the losses of this element to plants and crops (Fageria & Baligar 2005). The cycling of N is the succession of biochemical transformations of N in which it is utilised by living organisms, changed by the decomposition of organisms, and transformed at the end to its initial oxidation state (Soil Science Society of America 2008).

The soil-plant interaction is incredibly dynamic and intricate because of the external influence of weather conditions, soil and plant factors (Figure 1). The main components of the cycle are addition, transformation, utilization and losses in the soil-plant interaction. The most important addition of N in agricultural production systems to the soil is via inorganic fertilisers. N can also be added to the soil through biological fixation, precipitation, gas absorption and organic matter (manure, stubble, etc). The major transformation processes are fixation, mineralisation (ammonification), nitrification and immobilization (Fageria & Baligar 2005).

Fixation of nitrogen can occur as a biological or as an industrial process. Biological nitrogen fixation (BNF) was discovered by Beijerinck in 1901. It is a process in which prokaryote organisms use the enzyme nitrogenase to transform the  $N_2$  (atmospheric nitrogen) to ammonia ( $NH_3$ ). The prokaryote organisms capable of performing this process include aquatic organism, as cyanobacteria, free-living soil bacteria, as *Azotobacter*, bacteria in symbiosis with plants, as *Azospirillum* and *Rhizobium*, the latter associates with legumes to fixate nitrogen in soil (Wagner 2012). Industrial fixation of nitrogen is the process in which commercial fertiliser is obtained (Johnson et al. 2005).



**Figure 1.** Nitrogen cycle (Zerulla et al. 2001).

Mineralisation is a biochemical process in which organic nitrogen is turned into inorganic form (Lin et al. 2016), this process is also known as ammonification since ammonia is the first resulting by-product (Fageria & Baligar 2005). Mineralisation is executed by microbes and occurs as a by-product of the decomposition of organic matter (Crohn 2004). As a biological process, mineralisation is affected by soil moisture, temperature and the amount of oxygen present in the soil (Johnson et al. 2005).

Nitrification is the oxidation of ammonia into nitrate. This process, like mineralisation, occurs in a short period of time (Klotz & Stein 2008). *Nitrosomonas spp.*, a bacteria naturally present in soils, take energy (C) from either CO<sub>2</sub> or organic matter, and use the N as a nutrient, turning ammonium turning it into nitrite (NO<sub>2</sub><sup>-</sup>), only for a second group of bacteria, *Nitrobacter spp.* to convert it to nitrate. This process is faster when the temperature of the soil is greater than 25°C (International Plant Nutrition Institute 2015) and substantially contributes to greenhouse emissions (Azam et al. 2001). In fact, nitrification is considered to the major source of N<sub>2</sub>O instead of denitrification (Weiske et al. 2001).

Losses in the nitrogen cycle occur through denitrification, volatilization, immobilisation and leaching (Johnson et al. 2005).

### 3.4. Nitrogen losses

Nitrogen can be lost through different ways being the most important volatilisation, denitrification, nitrate leaching (Galieni et al. 2016), immobilisation (Johnson et al. 2005) and surface run-off (Ahmad, S et al. 2016) (Figure 1). This can losses can be large, 50 to 70% of nitrogen fertiliser is lost through leaching, gas emissions of ammonia and nitrogen oxides to the atmosphere (Mohamad Yatim et al. 2016).

#### 3.4.1. Nitrate leaching

Despite ammonium representing the most common of inorganic nitrogen, nitrate is the major form of nitrogen that is leached in production systems (International Plant Nutrition Institute 2013c). This is the case due to nitrate being an anion (negatively charged) so it is not retained by either cation exchange sites or soil colloids, unlike ammonium which is positively charged (Bolan et al. 2004).

Leaching occurs when water, with nutrients dissolved in it, moves below the root zone. This takes place when field capacity of the soil is exceeded. Soils prone to leaching are located in areas where water input (rainfall or irrigation) exceeds the evapotranspiration rate. Hence, it is more common in humid than in dry climates, especially with coarse-textured soil. Sandy soils have big particles which forms macro pores, that allows water to move easily across the soil profile, and when fertiliser is applied and later dissolved, can potentially move outside the reach of the root systems (Lehmann & Schroth 2003).

However, there are techniques that can be practice to reduce nitrogen loss through leaching, such as addition of compost (Masunga et al. 2016), adopting different irrigation practices (International Plant Nutrition Institute 2013c), spreading fertilisers application through the entire season instead of doing one large application at the start (Lehmann &

Schroth 2003), adopting of new cropping technique as organic farming, site-specific nitrogen management and precision farming (Kirchmann, Johnston & Bergström 2002).

The leaching of nitrate can have different impacts on the environment. It can affect the pH of the soils by acidifying it (Li et al. 2006). In severe cases, nitrate leaching can reach ground water or surface water, which can potentially increase the permitted level of nitrate in water for human and livestock consumption (Di & Cameron 2004), it can also cause eutrophication, which affects the ecological balance of rivers and lakes (Kirchmann, Johnston & Bergström 2002).

#### 3.4.2. Ammonia volatilisation

Ammonia volatilisation is a process where ammonium is transformed to ammonia  $\text{NH}_3$  gas, which ultimately is lost (Fageria & Baligar 2005) to the atmosphere (Johnson et al. 2005). It is an entirely chemical reaction that takes place under alkaline conditions (Bolan et al. 2004).

Ammonia volatilisation can be considerably reduced by adequate management, placement and timing of nitrogen fertilisers. This process is affected by nitrogen source, fertiliser containing N in ammonium form (i.e. urea), flooded conditions, soil pH, most likely to happen when pH is greater than 8 during urea hydrolysis, soil moisture and properties, soils with higher CEC have greater ability to retain ammonium, and temperature, the higher the temperature, the higher the losses (International Plant Nutrition Institute 2013a).

Determining the amount of N that is lost through volatilisation is difficult, which can lead to perceive that plants might have greater efficiency than they actually have (Dawson, Huggins & Jones 2008). Studies show that up to 40% of N can be lost due to volatilisation, however most studies show that 10% loss is more accurate. Volatilisation is affected by many different factors, such as soil pH, temperature, soil moisture, calcium carbonate content, soil clay content, soil buffer capacity, biological activity, wind, rain, depth at which fertiliser was applied, crop canopy, stubble and fertiliser type (Schwenke et al. 2012). For example, urea is the most used nitrogen fertiliser due to its low cost per unit of N (de Campos Bernardi A et al. 2010). However, it represents near 60% of all nitrogen fertiliser consumption and contributes to nitrogen losses the most (Davis et al. 2016), especially through volatilisation (De-Xi et al. 2007).

Though applications of fertiliser can lead to volatilisation of ammonia, manure applications are considered to be the a source for nitrogen losses through this path (Søgaard et al. 2002), especially when it is applied on top of the soil instead of being incorporated into the soil (Johnson et al. 2005).

#### 3.4.3. Denitrification

The microbial reduction of nitrate into N gases in anaerobic conditions is known as denitrification (Fageria & Baligar 2005). Under water-logged conditions, there is shortage of oxygen in the soil, which forces microorganisms (bacteria) to take the oxygen from nitrate ions leading to their reduction. The process generates  $\text{NO}_2^-$ , nitric oxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ) and  $\text{N}_2$  gas (Bolan et al. 2004).

$N_2O$  is the most damaging of the gases produced by denitrification, it can stay in the air for more than 100 years and has also been connected to the lessening of stratospheric ozone. This gas is a by-product of soil nitrification and denitrification and has 298 times the global warming potential of  $CO_2$ , and a small amount can largely contribute to GHG emissions (Smeets et al. 2009).

However, losses through denitrification from chemical fertiliser account for less than 1% of total N that is applied to soils for cropping purposes but in abnormal circumstances it can account for 50% of the losses. Additionally, despite denitrification having adverse consequences in crop production, it can also have a positive impact by lowering the amount of nitrate in water (International Plant Nutrition Institute 2013b).

#### 3.4.4. Immobilisation

It is the exact opposite of mineralisation. Nitrogen is vital for all living organism so they compete for N with crops. It is the process in which available forms of nitrogen for crops are absorbed by soil-living organisms, turned into organic form, hence reducing the amount of nitrogen useful for plants (inorganic forms). Immobilisation occurs when materials with elevated carbon to nitrogen ratio (C:N ratio) are incorporated to the soil, increasing biological activity, and hence generating more demand for N (Johnson et al. 2005).

#### 3.5. Nitrogen use efficiency (NUE)

There are several definitions for NUE. Early on, some authors defined it as the quantity of organic matter taken up for the plant or stored in wood, divided by the total nitrogen lost or stored as wood. While other authors defined it as the inverse relation of total nitrogen in the total biomass. Both concepts are considered now inaccurate (Berendse & Aerts 1987).

NUE is currently defined as the percentage of all N additions that are subtracted at harvest (crop biomass), present in crop residues (stubble) and added to the soil organic matter and inorganic N pools (Cassman, Dobermann & Walters 2002). In other words, it is the highest yield obtained per unit of N that is applied to the soil and that is taken up by plants (Fageria & Baligar 2005).

It has been already established that not all the nitrogen fertiliser that is applied to soils is taken up by plants, wheat recovers 41% of total nitrogen that is applied. For example, in Australia, nitrogen use efficiency ranges from 6 to 69% in different pastures and crop systems (Chen et al. 2008). The rest of the nitrogen is either immobilised, in organic forms, or moves outside the reach of root systems, with a high risk of being leached, and large applications of N fertiliser negatively impact the NUE (Cassman, Dobermann & Walters 2002)

Though many practices are considered to favour NUE, such as modification soil chemistry, and soil and plant management (Dawson, Huggins & Jones 2008), nitrification inhibitors and slow-release fertiliser are considered to be potential solutions for improving NUE and reducing leaching losses (Fageria & Baligar 2005).

### 3.6. Nitrification inhibitors

The rate of conversion of ammonium to nitrate can be decreased by the addition of chemical compounds, known as nitrification inhibitors (NI), to nitrogen fertilisers when applied to paddocks. These compounds facilitate the conservation of nitrogen in ammonium form which can be retained by soil colloids instead of rapidly transforming it to nitrate which can be leached. NI depresses the activity of *Nitrosomonas* bacteria, responsible for nitrification. There are different compounds that are commercialised as NI, such as 2-chloro-6-(trichloromethyl)-pyridine (Nitrapyrin), dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) (International Plant Nutrition Institute 2015).

Nitrapyrin is usually considered ineffectual. Though, it can reduce N losses and boost plant nitrogen uptake, it can also be retained by soil colloids, hydrolyse and volatilise. While dicyandiamide has proved to be effective in reducing nitrification from ammoniacal fertiliser by keeping nitrogen in ammonium form for longer periods in the soil. DCD has also showed positive results in reducing nitrate leaching losses. On the other hand, DCD can be damaging to plant growth and its effects on reducing nitrate leaching is variable (Chen et al. 2008).

DMPP, on the other hand, is considered more effective and longer lasting than DCD at slowing down the nitrification process, reducing nitrous oxide emissions (Chen et al. 2008). DMPP was developed by BASF in collaboration with universities and research institutes. This NI is highly effective at very low concentrations. From 0.5 to 1.5 kg DMPP per hectare is enough to inhibit nitrification during a period of 4 to 10 weeks. The effects of DMPP vary depending on weather conditions, location qualities and the sown crop (Zerulla et al. 2001).

DMPP is commonly commercialised pre-mixed with fertiliser. It can inhibit nitrification over a period of 25 to 70 days but its effect is reduced with higher temperatures (Zerulla et al. 2001). It also keeps nitrogen in an available form ( $\text{NH}_4^+$ ) for plants (Gioacchini et al. 2006). This NI is considered to diminish nitrate leaching while improving nitrogen use efficiency especially in high rainfall areas with sandy soils (Alonso-Ayuso, Gabriel & Quemada 2016), which have a deficient water and nutrient holding capacity (Zwingmann, Mackinnon & Gilkes 2011). There are also studies that suggest DMPP reduces losses through gas emission (Asing et al. 2008).

### 3.7. Slow-release fertilisers

Decreasing nitrogen losses, providing nutrients for plant growth in a sustainable manner, reducing the application frequency and reducing the risk of toxicity due to overdose are some of the advantages of slow-release fertilisers (Xie et al. 2011).

Slow-release fertilisers require to be degraded by microorganisms present in soil before plants can absorb them. How fertiliser will be released depends on its own chemical composition (C:N ratio and N content), the physical and chemical properties of the soil (organic matter content, pH, texture) and the sizes of the granules, the smaller they are, the faster nutrients are released (Gioacchini et al. 2006).

These types of fertiliser can be classified as: inorganic materials of low solubility, chemically or biologically degradable low solubility materials, relatively soluble materials that gradually

decompose in soil and water soluble fertilisers controlled by physical barrier. The latter is the most common among all slow release fertiliser, and is referred to as coated fertiliser (Ni, Liu & Lü 2009).

Coated fertilisers are elaborated by physically coating pellets of standard fertilisers with different materials that diminish the rate at which granules dissolve (Ahmad, NNR, Fernando & Uzir 2015; Wu, L, Liu & Rui 2008) and release nutrients at a gradual rate depending on temperature and moisture (Sowiński et al. 2016), being temperature the most important of the two (Rodrigues et al. 2010).

Different materials can be used as coating such as polyethylene, polyvinyl chloride, latex (Sowiński et al. 2016), polysulfone, polystyrene (Ni, Liu & Lü 2009), sulphur, waxes, craft pine lignin (Jarosiewicz & Tomaszewska 2003).

In recent years, NIs and slow-release fertiliser have emerged as solutions to increase nitrogen use efficiency, reducing losses of nitrogen through leaching and improving plant nitrogen uptake in horticultural systems. Although a review of the literature found several studies that have looked at the impacts of Nis and slow-release fertilisers, different results have been reported since the effectiveness of these fertilisers is affected by many factors (e.g. soil type, climate crop and management). There are very few studies that have looked at these compounds in horticultural systems, as measurements of leached nitrate in the field is difficult, and no reported studies that have investigated their role in Australian vegetable soils. This project is designed to address the gap on our knowledge of how these compounds might behave in Australian horticultural soils.

## 4. Materials and Methods

### 4.1. Site and soil description

An experiment with 15 soil columns was conducted in a glasshouse, with controlled temperature (20-25°C), at Burnley Campus (The University of Melbourne) from August to October 2016.

To simulate field conditions of a horticultural cropping system, soil was sourced from Schruers vegetable farm located at Fisheries Rd, Devon Meadows VIC 3977 (38°10'29.3"S 145°19'38.2"E) (Appendix 1) that crops different vegetables, especially leeks.

Soil samples were taken from a non-cultivated area at a depth of 20 cm with a spade from 5 different locations. Soil was air-dried and later sieved.

The soil was analysed at IPL Nutrient Advantage Laboratories for physical (Table 1) and chemical (Table 2) properties. The soil's texture was determined as sandy loam.

**Table 1.** Soil analysis: Physical properties

Description	Units	Value
Silt	%	9.8
Clay	%	10.4
Sand Coarse	%	54.9
Sand Fine	%	24.9
Hydrometer Texture (Calculated)		Sandy Loam

The soil has neutral pH (Rayment & Lyons 2010), which is the ideal for plant growth (Lake 2000), cation exchange capacity (CEC) and organic carbon were found in low (Reid & Dirou 2004) concentrations. EC is associated with soil texture and CEC (Grisso et al. 2005). EC is also determined as low.

The level of nitrate nitrogen is considered as low for horticultural systems (>20 mg/kg) (Reid & Dirou 2004). Phosphorus levels were high (>15 mg kg<sup>-1</sup>) (Hazelton & Murphy 2007), while potassium was marginal for sandy loam soils (80 – 150 mg kg<sup>-1</sup>) (Agriculture Victoria 2015).

**Table 2.** Soil analysis: Chemical properties

Description	Units	Value
pH (1:5 Water)		7.8
pH (1:5 CaCl <sub>2</sub> )		7.0
Electrical Conductivity	dS/m	0.1
Nitrate Nitrogen	mg/kg	9.10
Ammonium Nitrogen	mg/kg	0.84
Phosphorus - Olsen	mg/kg	72.40
Available Potassium (Amm. Acet.)	mg/kg	130.00
Cation Exchange Capacity - incl. Al (Amm. Acet.)	cmol(+)/kg	7.52
Organic Carbon	%	0.70

#### 4.2. Experiment description

Specially designed leachate collection soil columns were used. These columns had dimensions of 17 cm of internal diameter and 18 cm of height which allow a volume of 4L of soil (Appendix 2). There was a chamber at the bottom of each column for leachate to pool and be collected from. A mesh was placed below the soil to prevent it from collapsing into the chamber. Each column contained 5.25 kg of air-dried soil with a bulk density of 1.31 g cm<sup>-3</sup>.

Lettuce of the Green Cos variety was used as an indicator plant. Three to four seedlings were transplanted into each chamber on the 29 August 2016. Fertiliser was surface applied to each chamber on the following day (30<sup>th</sup> of August 2016). The amount of fertiliser applied was calculated based on the amount of N ha<sup>-1</sup> per treatment and the recommended sowing rate. Two weeks after transplant, the smallest seedlings were removed, leaving two plants per chamber.



Each chamber was irrigated with reverse osmosis water (RO). The same amount of water was added to each chamber during the 5 weeks that the experiment lasted (Appendix 5). A typical field irrigation of 5 – 10 mm (based on commercial farm irrigation practice in the region) was applied regularly.

Plants were harvested on the 4<sup>th</sup> of October 2016.

### 4.3. Fertiliser treatments

The experiment consisted of four treatments. Each treatment had 4 replicates per treatment except for treatment 1 (control group), which will only have 3 reps due to chamber availability:

1. Treatment 1: Control group, no fertiliser is applied.
2. Treatment 2: Standard fertiliser, Blaukorn Premium (15N-1.3P-17K). Rate: 2.50 g per chamber.
3. Treatment 3: Slow-release fertiliser (coated), Basacote Plus 3M (16N-3.5P-10K). Rate: 2.34 g per chamber.
4. Treatment 4: Fertiliser + DMPP, Novatec Premium (15N-1.3P-17K). Rate: 2.50 g per chamber.

All treatments are balanced to supply  $22.5 \text{ kg N ha}^{-1}$ . Fertilizer was surface applied, a common practice in horticultural systems. Differences in phosphorus (P) and potassium (K) between treatments resulting from the N:P:K ratios in the fertiliser were not compensated because the differences were small and the levels of P in the soil were high, while the levels of K were marginal, considering the contribution of K from the fertiliser and the fact that the experiment would take last 35 days, plants would not suffer from P and K deficiencies.

The experiment was conducted with a completely randomised design (CRD) (Appendix 3).

### 4.4. Sampling

The levels of total nitrogen in the foliage, dry matter (foliage and roots) and the amount of nitrogen, as nitrate, present in the soil (top and bottom), pH and electric conductivity (EC) were compared.

Six samples of leachate were taken from each chamber. The first one was taken prior to the commencement of the experiment (before the addition of fertiliser) and the other five were taken on a weekly basis (after fertiliser application). Weekly samples were made out of two subsamples collected every two days, volume was recorded each time. These subsamples were combined to make up the weekly sample.

At harvest, foliage and roots were collected and oven dried at  $60^{\circ}\text{C}$  over two days (until weight was constant) to determine dry matter.

Additionally, two soil sample were taken from each chamber, soil was divided in top and bottom halves. To obtain the samples soil was spread on a tray, where it was later divided

into top and bottom halves. Roots were carefully retrieved from the soil. Soil was evenly spread and subdivided in quarters until sample was reached (350 – 450 g). Soil samples were oven-dried at 40°C over a period of 4 days. Mineral N, pH and electric conductivity was determined.

## 4.5. Processing samples

### 4.5.1. Leachate

After leachate was collected, samples were kept at -20°C to avoid losses and changes in nitrogen form. Once weekly samples were complete, they were filtered with filter paper Whatman 42. This process was repeated for each of the chambers. From week 3 onwards, a suction pump was used to obtain leachate since water was not moving naturally through the soil profile and leachate volumes were decreasing.

Samples for analysis were prepared in 1:5 solutions (1 ml of leachate + 4 ml of RO water) to be later analysed colourimetrically for mineral N using the Skalar SAN ++ Autoanalyser.

### 4.5.2. Foliage

Once leaves were dried, they were ground in a Cyclone grinder. To avoid contamination of the samples, the grinder was properly clean after each sample was processed.

For analysis, 0.495 to 0.505 g of ground sample were weighed and placed on crucible boats to be analysed in the C and N Leco TruMac analyser.

### 4.5.3. Soil

Mineral nitrogen was determined with 2M KCl extraction. The extract was prepared according to Rayment and Lyons (2010) procedures. Five grams (5 g) of soil were mixed with 50 ml of 2M KCl extract. Samples were mechanically shaken for one hour and then filtered with filter paper (Whatman 42). 5 ml of filtered extract were used for analysis.

pH and electric conductivity was measured in a 1:5 solution (10 g of dried soil + 50 ml of milli-Q water).

## 4.6. Data analysis

Analysis of variance was conducted (ANOVA) at a 95% of confidence level to determine any differences between treatments. Additionally, the Fisher's least significant difference (LSD) at 95% level of confidence was used to determine which treatments differ from one another. Data that did not meet the assumptions for an analysis of variance (equal variances, normal distribution, independence) was transformed using Log base 10 as suggested by Rees (1989). All data was analysed using Minitab (Minitab Inc., Pennsylvania). Microsoft Excel 2016 was used for the preparation of tables and charts.

## 5. Results

### 5.1. Nitrate losses per chamber

Leachate was analysed to detect any losses of nitrogen in nitrate form over a period of 5 weeks. The amount of nitrate losses per chamber was determined using the volume of leachate collected from each column (Appendix 5) and multiplying it by the amount of nitrate present in leachate (Appendix 4). Total N leached was determined by summing up the weekly amount leached over 5 weeks.

The assumptions of equal variances within treatments was not met for ANOVA to be conducted. So the data was transformed ( $\log_{10}$ ) so statistical analysis could be valid. Results for total and weekly losses of nitrate per chamber are displayed in Table 3. Over 5 weeks the average total loss of nitrate from control was 2319  $\mu\text{g N}$ , from the standard fertiliser was 30450  $\mu\text{g N}$ , from slow-release fertiliser was 6148  $\mu\text{g N}$  and fertiliser with DMPP was 46806  $\mu\text{g N}$ .

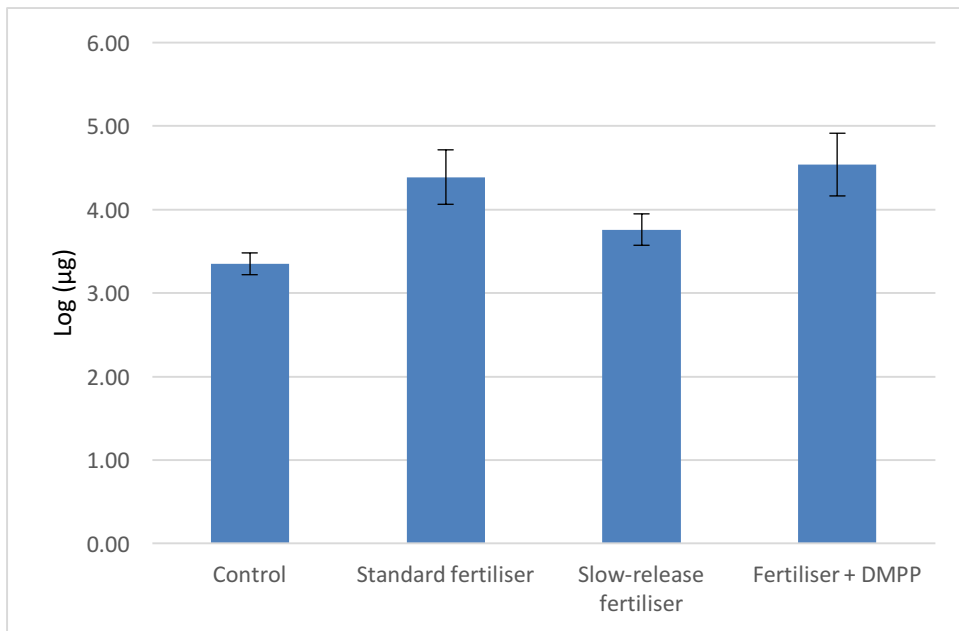
ANOVA for total nitrate losses per chamber indicated that there was a significant difference between treatments ( $p < 0.05$ ) (Appendix 18).

At the end of the experiment the treatment with slow-release fertiliser reduced the amount of total nitrate leached through the soil columns when compared to standard fertiliser. The fertiliser + DMPP treatment showed no significant difference when compared to standard fertiliser.

**Table 3.** Summary of results for nitrate losses per chamber - Log ( $\mu\text{g}$ )

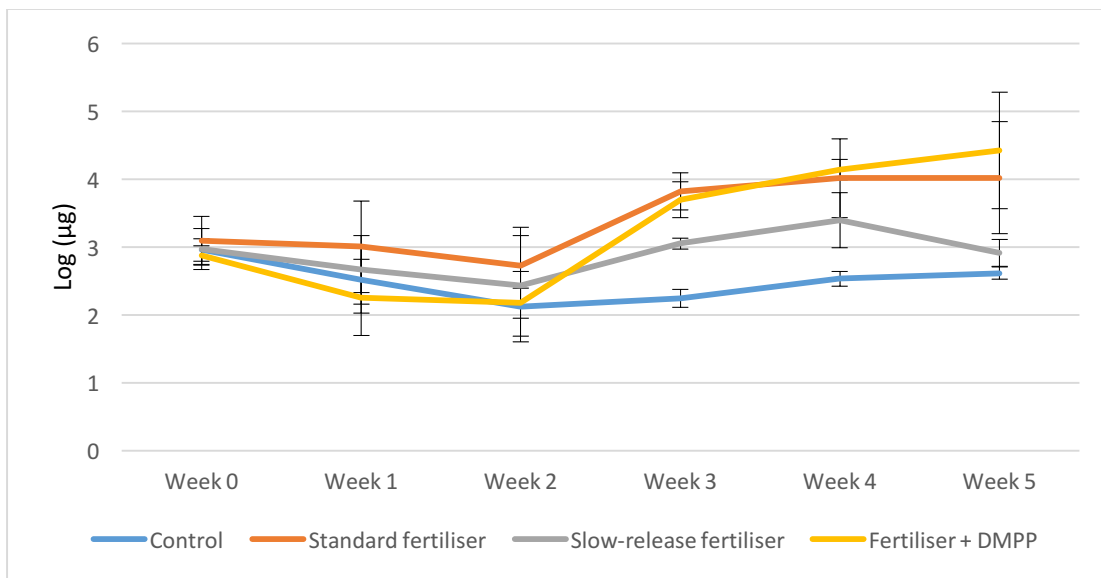
Treatments	Total		Week 0		Week 1		Week 2		Week 3		Week 4		Week 5	
	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
Control	3.352	0.130 <sup>b</sup>	2.942	0.165 <sup>a</sup>	2.335	0.493 <sup>a</sup>	1.941	0.519 <sup>a</sup>	2.238	0.130 <sup>c</sup>	2.528	0.105 <sup>c</sup>	2.613	0.087 <sup>b</sup>
Standard fertiliser	4.386	0.325 <sup>a</sup>	2.989	0.354 <sup>a</sup>	2.597	0.677 <sup>a</sup>	2.412	0.570 <sup>a</sup>	3.771	0.273 <sup>a</sup>	3.762	0.580 <sup>ab</sup>	3.438	0.826 <sup>ab</sup>
Slow-release fertiliser	3.759	0.190 <sup>b</sup>	2.893	0.299 <sup>a</sup>	2.484	0.503 <sup>a</sup>	2.065	0.738 <sup>a</sup>	3.050	0.083 <sup>b</sup>	3.255	0.403 <sup>b</sup>	2.882	0.200 <sup>b</sup>
Fertiliser + DMPP	4.538	0.378 <sup>a</sup>	2.861	0.142 <sup>a</sup>	2.049	0.559 <sup>a</sup>	2.134	0.222 <sup>a</sup>	3.641	0.264 <sup>a</sup>	4.124	0.149 <sup>a</sup>	3.941	0.855 <sup>a</sup>

In Figure 2, differences within the treatments with fertiliser can be observed. Slow-release fertiliser reduced the amount of nitrate lost through leaching per chamber when compared to the other two treatments with fertiliser (fertiliser + DMPP and standard fertiliser). Though the losses in slow-release fertiliser are 12.1% higher than the control treatment, no significant difference was found.



**Figure 2.** Total nitrate losses per chamber

At week 1 and 2 there was less nitrate leached in the fertiliser + DMPP treatment compared to standard fertiliser treatment, although these differences were not significant. However, at week 3, slow-release fertiliser reduced nitrate losses, unlike the other treatments with fertiliser, this was observed until the conclusion of the experiment. However, at week 4 and 5, nitrate losses kept increasing in treatments with standard fertiliser and fertiliser + DMPP (Figure 3).



**Figure 3.** Weekly nitrate losses per chamber.

## 5.2. Nitrogen in soil

Levels of nitrate and ammonium in the soils at the end of the 5-week experiment were below the range of detection (< 0.5 ppm).

### 5.3. Dry matter production

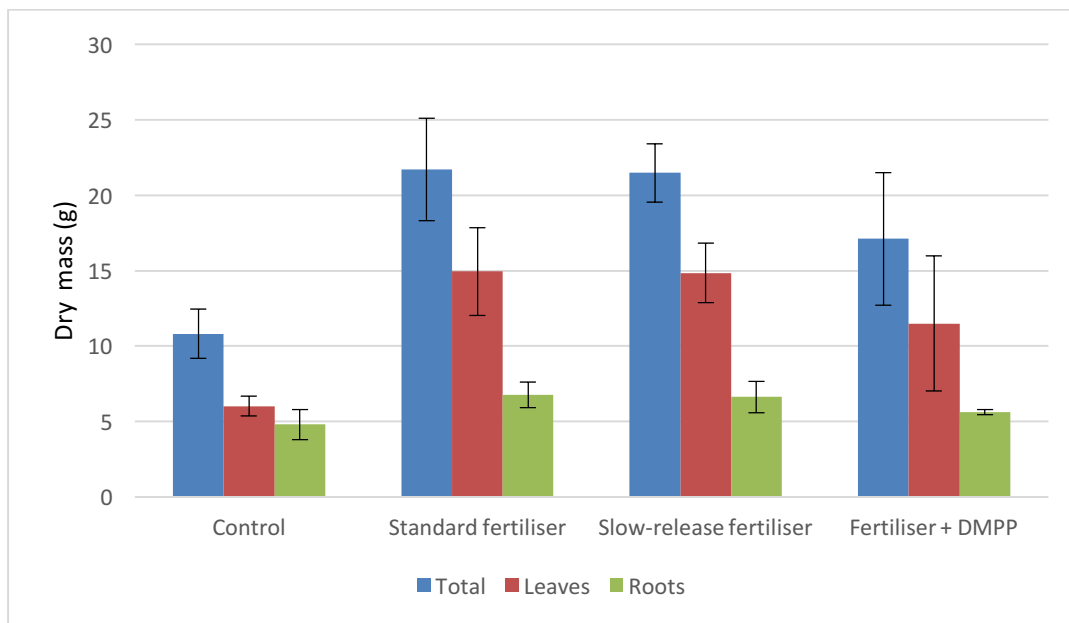
This parameter met the assumption for an analysis of variances. Therefore, data was not transformed.

ANOVA indicated differences between treatments for total, leaves and roots dry matter production (Appendix 25). Further analysis showed (Table 4) that in terms of total dry matter and leaves dry matter, there was not a significant difference between treatments that contained fertiliser. Although, there is no significant difference between treatments with fertilisers for total biomass production, the treatment with DMPP showed no significant difference when compared to the control treatment.

**Table 4.** Summary of results for dry matter (g)

Treatments	Total		Leaves		Roots	
	Mean	StDev	Mean	StDev	Mean	StDev
<b>Control</b>	10.82	1.641	6.02	0.649	0.67	0.088
<b>Standard fertiliser</b>	21.71	3.410	14.95	2.902	0.83	0.054
<b>Slow-release fertiliser</b>	21.47	1.940	14.85	1.966	0.82	0.068
<b>Fertiliser + DMPP</b>	17.12	4.392	11.49	4.482	0.75	0.012

The standard fertiliser and slow-release fertiliser treatments had the greatest dry mass production over a period of 5 weeks, being around 27% more than in the fertiliser + DMPP treatment, although the difference was not significant (Figure 4).



**Figure 4.** Total, leaves and roots dry mass.

#### 5.4. Total nitrogen in leaves

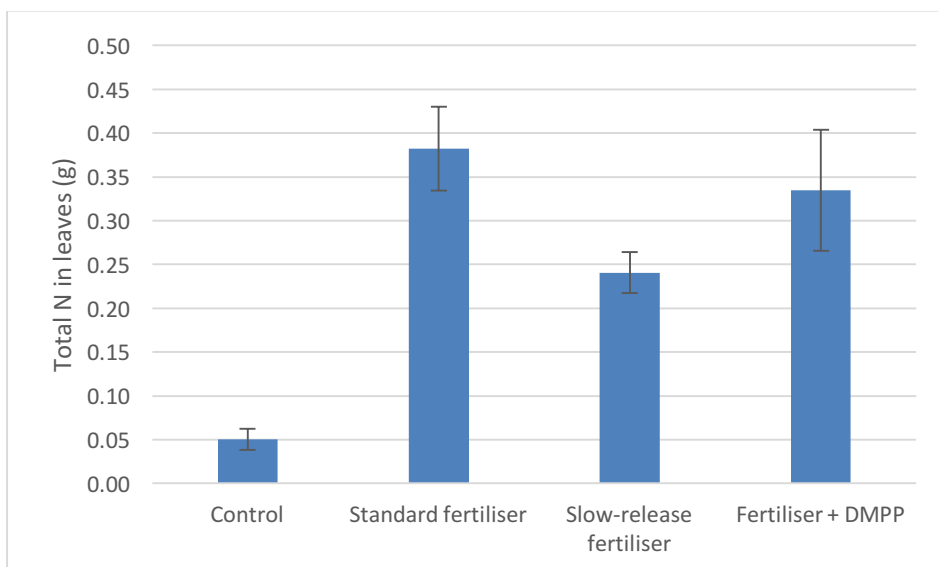
Total nitrogen in leaves was calculated using the leaves dry matter data (g) (Appendix 7) and the analysis for total nitrogen (%) in the plant (Appendix 8).

ANOVA was performed on the resulting data finding differences between treatments (Appendix 28). Further post hoc statistical analysis (Table 5) indicated that applications of fertiliser whether with or without DMPP have no significant effect on nitrogen uptake by plants after 5 weeks of the application. Plants with standard fertiliser treatment contained 14% more total N in leaves than plants with the fertiliser + DMPP treatment (Figure 5), although the difference was not significant.

**Table 5.** Summary of results for total nitrogen in leaves (g)

Treatments	Total N in leaves (g)		
	Mean	StDev	
Control	0.05	0.012	c
Standard fertiliser	0.38	0.048	a
Slow-release fertiliser	0.24	0.023	b
Fertiliser + DMPP	0.33	0.089	a

Plants with standard fertiliser treatment contained significantly more (37%) total N in leaves than plants with the slow-release fertiliser treatment (Figure 5). Control, no fertiliser application, was the treatment with the lowest total N content in leaves.



**Figure 5.** Total N in leaves (g)

## 5.5. Effect of treatments on pH and electric conductivity (EC)

Data met all the assumptions for ANOVA. Results are displayed in Table 6.

In the top soil, ANOVA showed differences in both pH and EC at the end of the experiment. Fertiliser + DMPP and standard fertiliser had no significant difference in pH. While pH level was higher in slow-release fertiliser treatment when compared to the other treatments containing fertiliser.

Regarding electric conductivity (EC), there is no significant difference between treatments that contained fertiliser. Additionally, no difference was found when comparing standard fertiliser, slow-release fertiliser and control. However, the fertiliser + DMPP treatment had significantly higher EC in the top soil.

**Table 6.** Summary of results for pH and EC ( $\mu\text{S m}^{-1}$ ).

Treatments	pH (top)		pH (bottom)		EC (top)		EC (bottom)	
	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
<b>Control</b>	7.76	0.03 <sup>a</sup>	7.83	0.04 <sup>a</sup>	62.13	3.12 <sup>b</sup>	69.80	3.82 <sup>a</sup>
<b>Standard fertiliser</b>	7.46	0.05 <sup>c</sup>	7.56	0.08 <sup>c</sup>	75.58	12.84 <sup>ab</sup>	103.73	12.68 <sup>a</sup>
<b>Slow-release fertiliser</b>	7.56	0.04 <sup>b</sup>	7.70	0.09 <sup>b</sup>	69.78	6.45 <sup>ab</sup>	111.48	65.95 <sup>a</sup>
<b>Fertiliser + DMPP</b>	7.41	0.06 <sup>c</sup>	7.62	0.07 <sup>bc</sup>	88.15	18.71 <sup>a</sup>	103.98	5.50 <sup>a</sup>

In the bottom soil part of the column, all fertiliser treatments had lower pH than the control. The pH of the standard fertiliser was significantly lower than pH of the slow-release and control treatments. There was no significant difference between the pH measured in soil treated with fertiliser + DMPP and the standard fertiliser or slow-release fertiliser. The applications of fertiliser did not affect electric conductivity in the soil at the bottom of the column. It was found that the EC of the fertiliser treatments was higher in the soil at the bottom of the column compared to that at the top.

## 6. Discussion

### 6.1. Nitrate leaching

During the first two weeks of the experiment levels of nitrate in the leachate were decreasing, suggesting that the nitrate present in the leachate samples came from nitrate naturally present in the soil which was determined to be low for horticultural systems ( $9.1 \text{ mg N Kg}^{-1}$  soil), and that N fertiliser reaches a depth of 18 cm (column length) 15 days after fertiliser application, and after 67mm (1500 ml) of water had been applied to the surface of the sandy loam soils. The observed decreases in leached nitrate was due to a combination of lower leachate volumes and reduce N concentration in the leachate. Decreases in the volume of

leachate can be explained by the amount of clay and silt, 10.4% and 9.8% respectively, present in the soil which could have moved downwards through the soil profile filling up the pores, that are common for sandy soils, slowing the movement of water through the column, and reducing the amount of leachate that was collected. From week 3 onwards, a suction pump was used to facilitate leaching as the leaching appeared to be delayed due to blocking of larger pores, which most likely explain the sudden increases in leached nitrate due to increased leachate volumes collected. Results showed that applications of slow-release (coated) fertiliser can reduce losses of nitrogen through nitrate leaching. In fact, at week 5, there was no significant difference between the slow-release and the control treatments.

Nitrate ( $\text{NO}_3^-$ ), as an anion, is not held up by colloids or cation exchange sites, which have an overall net negative charge (Bolan et al. 2004). This makes the losses of nitrogen through nitrate leaching a potential risk, especially in systems with high inputs of water and fertiliser (Di & Cameron 2002). The levels of nitrogen applied with fertiliser were high (33.33 mg N  $\text{Kg}^{-1}$  soil in nitrate form and 38.10 mg N $^{-1}$  in ammonium form), which could have increased the amount of nitrate that was leached. The DMPP delayed nitrification and appears to have slowed leaching loss in weeks 2 and 3. However, as the plant roots were shallow, the N available could not be fully utilised by the plant and was eventually leached from the column. In the DMPP treatment, there is more N available later, as it was retained as ammonium originally, and so there is increased loss of nitrate later. So in this system the DMPP seems to have only delayed, not reduced the leaching of nitrate. If lower rates of N were applied then there may have been less leaching with the DMPP treatment as the plant could utilise the retained N. However, this would have not affected slow-release fertiliser due to the presence of a coating layer, which avoided the immediate release of nutrients, thus columns treated with slow-release fertiliser showed less concentration of nitrate in leachate.

Fertiliser + DMPP treatment showed increases of nitrate per chamber later in the experiment (week 5) while treatments with standard and slow-release fertiliser were already showing decreases. This suggests that DMPP, as a nitrification inhibitor, could have delayed the transformation of ammonium into nitrate, as DMPP can restrict nitrification over several weeks (Díez López et al. 2008). Díez López et al. (2008) registered the same delaying effect on nitrification, having higher levels of nitrate at 40 days after applications in treatments containing DMPP. In contrast, Wu, SF et al. (2007) found that treatments of urea and ammonium sulphate nitrate mixed with DMPP reduced the loss of nitrate in leachate when compared to treatment without DMPP in sandy soils and the peak for nitrate losses occurred 5 days after fertiliser applications.

The effectiveness of slow-release fertiliser over DMPP on nitrate leaching showed accordance with other studies. Gioacchini et al. (2006) determined that losses of nitrate through leaching in slow-release fertiliser were lower than the losses from treatments with DMPP.

## 6.2. Soil mineral N

Nitrogen content in soil, in either nitrate or ammonium form at the end of the experiment, were below the range of detection (<0.5 ppm), which suggested that nitrogen was either leached or loss through gas emission as a consequence of denitrification.



Ammonium, being a cation, is positively charged, hence is held up by soil colloids and cation exchange sites, which is not the case for nitrate, which is negatively charged (Bolan et al. 2004). This could explain the poor levels of nitrate present in the soil at the end of the experiment which were likely lost by leaching. However, that would not be the case for ammonium, which also had low concentration in the leachate. Clay particles (negatively charged) were found in the mesh at the bottom of the column, which could have retained the ammonium, providing an explanation for the lack of ammonium in the leachate and the soil.

### 6.3. pH and electrical conductivity

Applications of N fertiliser can acidify soils and increase their electric conductivity. But, regardless of differences between treatments, the variations are minimal and for all treatments pH is still considered neutral and EC low. Higher levels of EC were found at the bottom half of the column which suggested the movement of nutrients, in this case nitrate, with water down the soil profile. Additionally, the root system of lettuce is shallow (Napier 2004), which could explain the lower EC on the top of the soil.

### 6.4. Biomass production and nitrogen uptake by plants

This research indicated that, regardless of the type of N fertiliser, there is no difference in total biomass production but there were differences in the total content of nitrogen in plants. Rodrigues et al. (2010) reported similar findings, where there was no advantage of slow release fertilisers on plant growth or yields of tall cabbage when compared to urea. Additionally, other studies conducted by Yang et al. (2016) and Abalos et al. (2014) found that DMPP had no influence on yields compared to standard fertilisers in different crop types from cereals to various vegetable crops.

Plants treated with standard fertiliser with and without DMPP had the highest level of nitrogen in leaves after five weeks. For standard fertiliser, this could be the case because nitrogen was rapidly transformed into nitrate favouring absorption. Fertiliser + DMPP (no significant difference with standard fertiliser) was the second highest. It is important to keep in mind that lettuce, indicator plant, is cultivated for 8 to 10 weeks, and in this case it was harvested at 5 weeks when this treatment had increasing levels of nitrate in leachate. Additionally, in the presence of NI, abiotic immobilisation can occur due to competition with microorganisms for  $\text{NH}_4^+$  (Gioacchini et al. 2006).

The slow-release fertilisers are intended to make nutrients available gradually. The slow-release fertiliser used for the experiment is expected to keep releasing nutrients for a period up to 90 days depending on temperature (Compo 2015). According to manufacturer's specifications, this slow-release fertiliser has a release period of 3 to 4 months at 21°C. Temperature at the glasshouse, where the experiment was conducted, ranged from 20°C to 25°C. This leads to think that over the duration of the experiment, 35 days, slow-release fertiliser would have not completely released all the nutrients that were contained in the granules, hence obtaining a lower biomass production. The release rate of this type of fertiliser should be taken into account before applications. If the release rate of the fertiliser is not aligned with the crop production cycle, then nutrients might be released insufficiently or left in excess in soils, which would have negative impacts in yields and cost of production.

## 7. Conclusions

This research provided important information on the effect of nitrification inhibitor, DMPP, and slow-release fertiliser on nitrate leaching and plant uptake relative to a standard fertiliser used in an Australian horticultural soil. The experiment indicated that slow-release fertiliser can reduce nitrate leaching when compared to standard fertiliser in sandy soils. This can reduce the availability of nitrate for denitrification, hence reducing the environmental impacts of N in intensive vegetable production systems. DMPP, on the other hand, did not have an effect in reducing leaching losses, although the loss was delayed for 3 weeks, or increasing plant uptake when compared to standard fertiliser.

The use of N fertiliser is clearly required for production of lettuce, but the choice of N fertiliser (coated, mixed with DMPP and standard) did not affect biomass production.

Based on the results of this research, it can be stated that slow-release fertiliser can increase nitrogen used efficiency due to reducing leaching losses and improving plant uptake while maintaining biomass production.

Further research is recommended to better understand the effect of DMPP on reducing nitrate leaching losses since the results of this research were somewhat contradictory to previous studies. While this experiment has clearly shown differences in leaching between the treatments, it is recommended that future research covers extended time periods for full crop growth to fully understand the dynamics of N in the soil and the impacts on productivity. It is also recommended to do a rate study to see if better nitrogen use efficiency is achieved at lower rates, as it is possible in this study that N was supplied in excess of plant requirements in all fertiliser treatments so productivity benefits would not be clearly seen. Furthermore, this research only assessed losses through leaching leaving a gap on N gaseous losses such as from denitrification.

## 8. Acknowledgments

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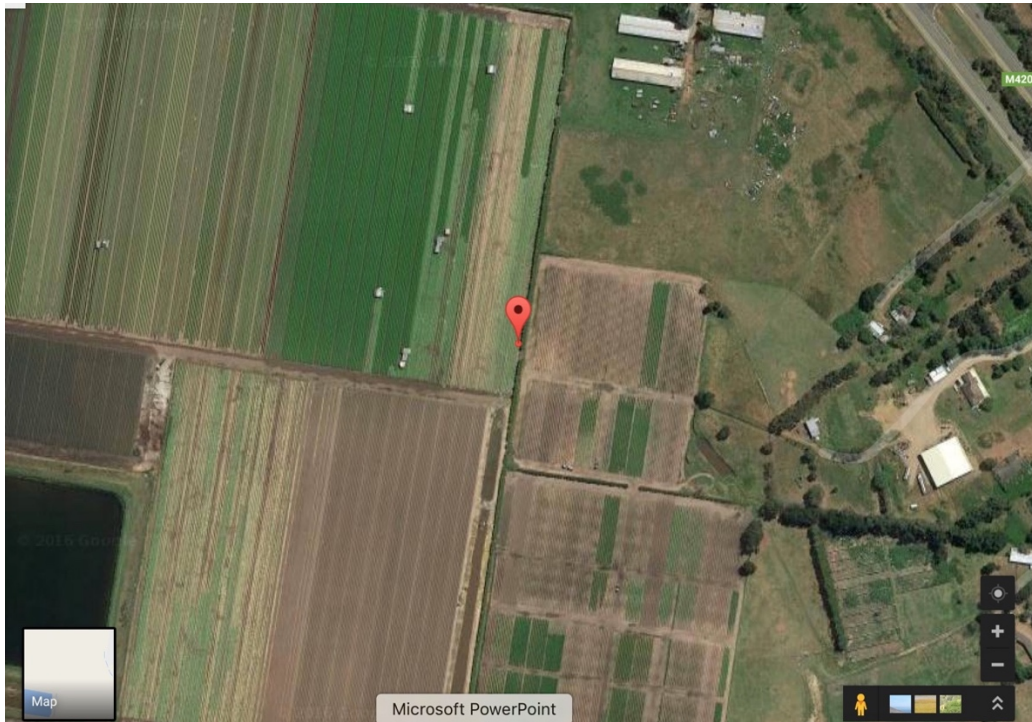
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## 10. Appendices

### Appendix 1. Satellite capture from where soil was retrieved.



Source: <https://www.google.com.au/maps/>

### Appendix 2. Chamber used as pots for the experiment.



Source: pictures taken by the author.

**Appendix 3. Experimental design.**

T2R1	T1R1	T3R1	T4R1	T3R2	T2R2	T4R2	T1R2	T3R3	T1R3	T4R3	T2R3	T1R4	T3R4	T3R4
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T = treatment

R = replication

**Appendix 4. Data for nitrate content (ppm) in leachate.**

	Raw data				Transformed data (Log10)			
	Control	Standard fertiliser	Slow-release fertiliser	Fertiliser +DMPP	Control	Standard fertiliser	Slow-release fertiliser	Fertiliser +DMPP
<b>Total</b>	51.600	261.732	189.771	215.701	1.713	2.418	2.278	2.334
	55.305	316.330	72.347	455.639	1.743	2.500	1.859	2.659
	55.337	353.185	122.007	208.445	1.743	2.548	2.086	2.319
	-	288.314	98.990	175.451	-	2.460	1.996	2.244
<b>Week 0</b>	22.150	69.650	37.900	18.650	1.345	1.843	1.579	1.271
	26.450	29.800	21.950	28.200	1.422	1.474	1.341	1.450
	26.300	27.600	37.650	22.550	1.420	1.441	1.576	1.353
	-	16.350	25.900	25.750	-	1.214	1.413	1.411
<b>Week 1</b>	13.500	42.500	28.950	12.700	1.130	1.628	1.462	1.104
	17.150	15.500	9.650	21.050	1.234	1.190	0.985	1.323
	15.900	16.950	23.400	19.350	1.201	1.229	1.369	1.287
	-	9.150	10.050	8.100	-	0.961	1.002	0.908
<b>Week 2</b>	22.150	69.650	37.900	18.650	1.345	1.843	1.579	1.271
	26.450	29.800	21.950	28.200	1.422	1.474	1.341	1.450
	26.300	27.600	37.650	22.550	1.420	1.441	1.576	1.353
	-	16.350	25.900	25.750	-	1.214	1.413	1.411
<b>Week 3</b>	2.500	63.626	34.957	24.497	0.398	1.804	1.544	1.389
	2.497	17.328	12.369	56.996	0.397	1.239	1.092	1.756
	2.887	94.692	21.649	62.782	0.460	1.976	1.335	1.798
	-	173.493	11.893	27.121	-	2.239	1.075	1.433
<b>Week 4</b>	22.150	69.650	37.900	18.650	1.345	1.843	1.579	1.271
	26.450	29.800	21.950	28.200	1.422	1.474	1.341	1.450
	26.300	27.600	37.650	22.550	1.420	1.441	1.576	1.353
	-	16.350	25.900	25.750	-	1.214	1.413	1.411
<b>Week 5</b>	2.500	3.125	13.279	76.300	0.398	0.495	1.123	1.883
	2.500	116.934	3.508	235.078	0.398	2.068	0.545	2.371
	2.500	26.874	16.772	38.429	0.398	1.429	1.225	1.585
	-	14.714	8.312	7.167	-	1.168	0.920	0.855

**Appendix 5.** Leachate volumes per week (ml) and water input (ml).

	Treatment	Saturating soil	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Chamber 1	Standard fertiliser	70	40	80	27	120	81	132
Chamber 2	Fertiliser + DMPP	195	50	15	43	131	163	265
Chamber 3	Slow-release fertiliser	125	53	35	22	42	61	42
Chamber 4	Control	165	55	55	34	49	128	142
Chamber 5	Slow-release fertiliser	160	34	20	21	82	54	138
Chamber 6	Standard fertiliser	110	41	15	37	136	188	324
Chamber 7	Control	150	36	10	21	84	175	151
Chamber 8	Fertiliser + DMPP	170	35	20	21	123	212	349
Chamber 9	Slow-release fertiliser	160	17	25	15	44	59	77
Chamber 10	Fertiliser + DMPP	185	26	5	17	120	160	104
Chamber 11	Control	170	22	5	5	70	109	206
Chamber 12	Standard fertiliser	195	21	5	10	103	72	89
Chamber 13	Fertiliser + DMPP	200	20	2.5	15	80	117	122
Chamber 14	Slow-release fertiliser	140	15	7.5	2	94	162	116
Chamber 15	Standard fertiliser	195	28	40	10	40	42	103
Water input		1000	100	200	100	250	700	1150

**Appendix 6.** Data for nitrate loss per chamber ( $\mu\text{g}$ ).

	Raw data				Transformed data (Log10)			
	Control	Standard fertiliser	Slow-release fertiliser	Fertiliser +DMPP	Control	Standard fertiliser	Slow-release fertiliser	Fertiliser +DMPP
Total	3045.55	17567.92	8184.03	37870.91	3.484	4.245	3.913	4.578
	2237.99	67017.23	3597.46	112212.49	3.350	4.826	3.556	5.050
	1673.92	25568.46	4352.10	21966.23	3.224	4.408	3.639	4.342
	-	11647.73	8461.20	15176.15	-	4.066	3.927	4.181
Week 0	1218.25	2786.00	2008.70	932.50	3.086	3.445	3.303	2.970
	952.20	1221.80	746.30	987.00	2.979	3.087	2.873	2.994
	578.60	579.60	640.05	586.30	2.762	2.763	2.806	2.768
	-	457.80	388.50	515.00	-	2.661	2.589	2.712
Week 1	742.50	3400.00	1013.25	190.50	2.871	3.531	3.006	2.280
	171.50	232.50	193.00	421.00	2.234	2.366	2.286	2.624
	79.50	84.75	585.00	96.75	1.900	1.928	2.767	1.986
	-	366.00	75.38	20.25	-	2.563	1.877	1.306
Week 2	287.30	1687.50	800.80	107.50	2.458	3.227	2.904	2.031
	88.20	96.20	116.55	273.00	1.945	1.983	2.067	2.436
	26.25	114.50	152.25	83.30	1.419	2.059	2.183	1.921
	-	239.00	12.80	141.00	-	2.378	1.107	2.149
Week 3	122.50	7635.15	1468.20	3209.16	2.088	3.883	3.167	3.506
	209.73	2356.60	1014.28	7010.55	2.322	3.372	3.006	3.846
	202.07	9753.31	952.55	7533.83	2.305	3.989	2.979	3.877
	-	6939.72	1117.98	2169.64	-	3.841	3.048	3.336
Week 4	320.00	1646.80	2335.38	13211.62	2.505	3.217	3.368	4.121
	438.86	25223.62	1043.28	21478.83	2.642	4.402	3.018	4.332
	272.50	12644.51	730.78	9669.47	2.435	4.102	2.864	3.985
	-	2129.71	5902.31	11455.88	-	3.328	3.771	4.059
Week 5	355.00	412.47	557.71	20219.63	2.550	2.615	2.746	4.306
	377.50	37886.51	484.05	82042.12	2.577	4.578	2.685	4.914
	515.00	2391.79	1291.47	3996.58	2.712	3.379	3.111	3.602
	-	1515.50	964.23	874.38	-	3.181	2.984	2.942

**Appendix 7.** Raw data for dry mass (g).

	Control	Standard fertiliser	Slow-release fertiliser	Fertiliser +DMPP
<b>Total</b>	12.63	24.09	20.98	15.54
	10.40	16.67	22.82	11.75
	9.43	23.41	23.15	19.48
	-	22.67	18.94	21.70
<b>Leaves</b>	6.75	16.74	14.31	9.99
	5.82	10.64	17.34	5.99
	5.50	15.80	15.14	13.73
	-	16.61	12.61	16.26
<b>Roots</b>	5.88	7.35	6.67	5.55
	4.58	6.03	5.48	5.76
	3.93	7.61	8.01	5.75
	-	6.06	6.33	5.44

**Appendix 8.** Total nitrogen and samples weight.

	Control	Standard fertiliser	Slow-release fertiliser	Fertiliser +DMPP
<b>TOTAL N (%)</b>	0.94	2.55	1.92	3.54
	0.82	2.96	1.29	3.41
	0.73	2.51	1.58	2.81
	-	2.34	1.80	2.44
<b>Sample weight (g)</b>	0.4998	0.4995	0.5004	0.5008
	0.5005	0.4991	0.5024	0.5018
	0.5008	0.5031	0.4984	0.4998
	-	0.4980	0.4985	0.5026

**Appendix 9.** Soil pH data

	Control	Standard fertiliser	Slow-release fertiliser	Fertiliser +DMPP
<b>Top half</b>	7.72	7.51	7.51	7.46
	7.78	7.46	7.56	7.38
	7.77	7.47	7.57	7.33
	-	7.72	7.60	7.46
<b>Bottom half</b>	7.81	7.53	7.73	7.70
	7.81	7.64	7.57	7.54
	7.88	7.60	7.71	7.64
	-	7.81	7.77	7.61

**Appendix 10.** Soil electric conductivity (EC) data ( $\mu\text{g m}^{-1}$ )

	Control	Standard fertiliser	Slow-release fertiliser	Fertiliser +DMPP
<b>Top half</b>	59.90	77.30	63.50	99.40
	65.70	92.30	65.30	100.30
	60.80	61.80	73.20	92.30
	-	59.90	77.10	60.60
<b>Bottom half</b>	71.80	119.90	71.30	103.10
	72.20	106.70	209.70	112.00
	65.40	90.40	75.40	100.40
	-	71.80	89.50	100.40

**Appendix 11.** Statistical analysis for Total nitrate loss - Log (ppm).

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	3.72671	1.24224	16.57	0.0002
Error	11	0.82469	0.07497		
Total	14	4.55140			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	1.73280	0.01746	(1.55971, 1.90590)
Standard fertiliser	4	2.48146	0.05564	(2.33156, 2.63137)
Slow-release fertiliser	4	2.05491	0.17568	(1.90501, 2.20482)
Fertiliser + DMPP	4	2.38890	0.18405	(2.23899, 2.53881)

*Pooled StDev = 0.136218*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	2.48146	A
Fertiliser + DMPP	4	2.38890	A
Slow-release fertiliser	4	2.05491	B
Control	3	1.73280	C

**Appendix 12.** Statistical analysis for nitrate loss at week 0 - Log (ppm).

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.041361	0.0137871	0.57	0.6491
Error	11	0.268223	0.0243839		
Total	14	0.309584			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	1.39592	0.04379	(1.19749, 1.59435)
Standard fertiliser	4	1.4929	0.2605	(1.3210, 1.6647)
Slow-release fertiliser	4	1.47728	0.11905	(1.30544, 1.64913)
Fertiliser + DMPP	4	1.37121	0.07799	(1.19937, 1.54306)

*Pooled StDev = 0.156153*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	1.4929	A
Slow-release fertiliser	4	1.47728	A
Control	3	1.39592	A
Fertiliser + DMPP	4	1.37121	A

*Means that do not share a letter are significantly different.*

**Appendix 13.** Statistical analysis for nitrate loss at week 1 - Log (ppm)

## Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.019301	0.0064338	0.13	0.9377
Error	11	0.527694	0.0479722		
Total	14	0.546996			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	1.18867	0.05312	(0.91034, 1.46699)
Standard fertiliser	4	1.2523	0.2771	(1.0113, 1.4934)
Slow-release fertiliser	4	1.2044	0.2467	(0.9634, 1.4454)
Fertiliser + DMPP	4	1.15556	0.19065	(0.91452, 1.39659)

*Pooled StDev = 0.219026*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	1.2523	A
Slow-release fertiliser	4	1.2044	A
Control	3	1.18867	A
Fertiliser + DMPP	4	1.15556	A

*Means that do not share a letter are significantly different.*

**Appendix 14.** Statistical analysis for nitrate loss at week 2 - Log (ppm)

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.41461	0.138204	0.85	0.4936
Error	11	1.78186	0.161987		
Total	14	2.19647			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	0.75676	0.15508	(0.24531, 1.26820)
Standard fertiliser	4	1.1620	0.5823	(0.7191, 1.6049)
Slow-release fertiliser	4	1.0295	0.3716	(0.5866, 1.4724)
Fertiliser + DMPP	4	0.7938	0.3173	(0.3509, 1.2367)

*Pooled StDev = 0.402477*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	1.1620	A
Slow-release fertiliser	4	1.0295	A
Fertiliser + DMPP	4	0.7938	A
Control	3	0.75676	A

*Means that do not share a letter are significantly different.*

**Appendix 15.** Statistical analysis for nitrate loss at week 3 - Log (ppm)

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	3.72671	1.24224	16.57	0.0002
Error	11	0.82469	0.07497		
Total	14	4.55140			



**Means**

Treatment	N	Mean	StDev	95% CI
Control	3	0.41893	0.03635	(0.07098, 0.76687)
Standard fertiliser	4	1.8145	0.4235	(1.5132, 2.1158)
Slow-release fertiliser	4	1.2616	0.2224	(0.9603, 1.5630)
Fertiliser + DMPP	4	1.5940	0.2125	(1.2927, 1.8954)

*Pooled StDev = 0.273810*

**Grouping information using the Fisher LSD method and 95% confidence**

Treatment	N	Mean	Grouping
Standard fertiliser	4	1.8145	A
Fertiliser + DMPP	4	1.5940	A B
Slow-release fertiliser	4	1.2616	B
Control	3	0.41893	C

*Means that do not share a letter are significantly different.*

**Appendix 16. Statistical analysis for nitrate loss at week 4 - Log (ppm)****Analysis of variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	4.85401	1.61800	23.91	<0.0001
Error	11	0.74435	0.06767		
Total	14	5.59837			

**Means**

Treatment	N	Mean	StDev	95% CI
Control	3	0.398518	0.0010010	(0.0679580, 0.7290778)
Standard fertiliser	4	1.8464	0.4271	(1.5601, 2.1326)
Slow-release fertiliser	4	1.3809	0.2347	(1.0946, 1.6672)
Fertiliser + DMPP	4	1.92163	0.10284	(1.63536, 2.20791)

*Pooled StDev = 0.260132*

**Grouping information using the Fisher LSD method and 95% confidence**

Treatment	N	Mean	Grouping
Fertiliser + DMPP	4	1.92163	A
Standard fertiliser	4	1.8464	A
Slow-release fertiliser	4	1.3809	B
Control	3	0.398518	C

*Means that do not share a letter are significantly different.*

**Appendix 17.** Statistical analysis for nitrate loss at week 5 - Log (ppm)

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	3.01584	1.00528	4.02	0.0371
Error	11	2.75042	0.25004		
Total	14	5.76626			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	0.397940	0	(-0.237478, 1.033358)
Standard fertiliser	4	1.2897	0.6514	(0.7395, 1.8400)
Slow-release fertiliser	4	0.9532	0.3000	(0.4029, 1.5034)
Fertiliser + DMPP	4	1.6735	0.6344	(1.1232, 2.2238)

*Pooled StDev = 0.500038*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Fertiliser + DMPP	4	1.6735	A
Standard fertiliser	4	1.2897	A
Slow-release fertiliser	4	0.9532	A B
Control	3	0.397940	B

*Means that do not share a letter are significantly different.*

**Appendix 18.** Statistical analysis for total nitrate loss per chamber - Log ( $\mu\text{g}$ )

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	3.20302	1.06767	13.23	0.0006
Error	11	0.88765	0.08070		
Total	14	4.09066			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	3.35242	0.12999	(2.99144, 3.71340)
Standard fertiliser	4	4.3862	0.3248	(4.0736, 4.6988)
Slow-release fertiliser	4	3.75877	0.18952	(3.44616, 4.07139)
Fertiliser + DMPP	4	4.5378	0.3784	(4.2252, 4.8504)

*Pooled StDev = 0.284069*

## Grouping information using the fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Fertiliser + DMPP	4	4.5378	A
Standard fertiliser	4	4.3862	A
Slow-release fertiliser	4	3.75877	B
Control	3	3.35242	B

*Means that do not share a letter are significantly different.*

**Appendix 19.** Statistical analysis for nitrate loss per chamber at week 0 - Log ( $\mu\text{g}$ )

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.037386	0.0124619	0.18	0.9074
Error	11	0.759217	0.0690197		
Total	14	0.796603			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	2.94229	0.16474	(2.60844, 3.27613)
Standard fertiliser	4	2.9889	0.3542	(2.6998, 3.2781)
Slow-release fertiliser	4	2.8929	0.2990	(2.6037, 3.1820)
Fertiliser + DMPP	4	2.86097	0.14197	(2.57186, 3.15009)

*Pooled StDev = 0.262716*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	2.9889	A
Control	3	2.94229	A
Slow-release fertiliser	4	2.8929	A
Fertiliser + DMPP	4	2.86097	A

*Means that do not share a letter are significantly different.*

**Appendix 20.** Statistical analysis for nitrate loss per chamber at week 1 - Log ( $\mu\text{g}$ )

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.67366	0.224553	0.69	0.5746
Error	11	3.55843	0.323494		
Total	14	4.23209			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	2.3351	0.4930	(1.6124, 3.0579)
Standard fertiliser	4	2.5974	0.6769	(1.9715, 3.2234)
Slow-release fertiliser	4	2.4840	0.5032	(1.8580, 3.1099)
Fertiliser + DMPP	4	2.0494	0.5592	(1.4235, 2.6753)

*Pooled StDev = 0.568765*

Grouping information using the Fisher LSD method and 95% confidence.

Treatment	N	Mean	Grouping
Standard fertiliser	4	2.5974	A
Slow-release fertiliser	4	2.4840	A
Control	3	2.3351	A
Fertiliser + DMPP	4	2.0494	A

*Means that do not share a letter are significantly different.*

## Appendix 21. Statistical analysis for nitrate loss per chamber at week 2 - Log ( $\mu\text{g}$ )

### Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.43494	0.144979	0.48	0.7002
Error	11	3.29545	0.299586		
Total	14	3.73039			

### Means

Treatment	N	Mean	StDev	95% CI
Control	3	1.9413	0.5192	(1.2457, 2.6368)
Standard fertiliser	4	2.4119	0.5699	(1.8096, 3.0143)
Slow-release fertiliser	4	2.0650	0.7381	(1.4627, 2.6674)
Fertiliser + DMPP	4	2.1344	0.2218	(1.5320, 2.7367)

*Pooled StDev = 0.547345*

Grouping information using the fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	2.4119	A
Fertiliser + DMPP	4	2.1344	A
Slow-release fertiliser	4	2.0650	A
Control	3	1.9413	A

*Means that do not share a letter are significantly different.*

**Appendix 22.** Statistical analysis for nitrate loss per chamber at week 3 - Log ( $\mu\text{g}$ )

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	4.92759	1.64253	37.09	<0.0001
Error	11	0.48718	0.04429		
Total	14	5.41477			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	2.23843	0.13041	(1.97101, 2.50586)
Standard fertiliser	4	3.7714	0.2733	(3.5398, 4.0030)
Slow-release fertiliser	4	3.05008	0.08290	(2.81848, 3.28167)
Fertiliser + DMPP	4	3.6414	0.2637	(3.4098, 3.8730)

*Pooled StDev = 0.210449*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	3.7714	A
Fertiliser + DMPP	4	3.6414	A
Slow-release fertiliser	4	3.05008	B
Control	3	2.23843	C

*Means that do not share a letter are significantly different.*

**Appendix 23.** Statistical analysis for nitrate loss per chamber at week 4 - Log ( $\mu\text{g}$ )

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	4.90188	1.63396	11.32	0.0011
Error	11	1.58741	0.14431		
Total	14	6.48930			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	2.52763	0.10531	(2.04490, 3.01036)
Standard fertiliser	4	3.7622	0.5803	(3.3441, 4.1802)
Slow-release fertiliser	4	3.2554	0.4034	(2.8373, 3.6735)
Fertiliser + DMPP	4	4.12435	0.14912	(3.70629, 4.54241)

*Pooled StDev = 0.379882*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Fertiliser + DMPP	4	4.12435	A
Standard fertiliser	4	3.7622	A B
Slow-release fertiliser	4	3.2554	B
Control	3	2.52763	C

Means that do not share a letter are significantly different.

**Appendix 24.** Statistical analysis for nitrate loss per chamber at week 5 - Log ( $\mu\text{g}$ )

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	3.80959	1.26986	3.19	0.0665
Error	11	4.37585	0.39780		
Total	14	8.18544			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	2.61298	0.08662	(1.81151, 3.41446)
Standard fertiliser	4	3.4383	0.8261	(2.7442, 4.1324)
Slow-release fertiliser	4	2.8816	0.2001	(2.1875, 3.5757)
Fertiliser + DMPP	4	3.9408	0.8551	(3.2467, 4.6349)

Pooled StDev = 0.630717

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Fertiliser + DMPP	4	3.9408	A
Standard fertiliser	4	3.4383	A B
Slow-release fertiliser	4	2.8816	B
Control	3	2.61298	B

Means that do not share a letter are significantly different.

**Appendix 25.** Statistical analysis for total dry mass production (g)

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	260.169	86.7229	8.72	0.0030
Error	11	109.427	9.9479		
Total	14	369.596			

**Means**

Treatment	N	Mean	StDev	95% CI
Control	3	10.8200	1.6408	(6.8120, 14.8280)
Standard fertiliser	4	21.710	3.410	(18.239, 25.181)
Slow-release fertiliser	4	21.4725	1.9396	(18.0015, 24.9435)
Fertiliser + DMPP	4	17.118	4.392	(13.647, 20.588)

*Pooled StDev = 3.15404*

**Grouping information using the Fisher LSD method and 95% confidence**

Treatment	N	Mean	Grouping
Standard fertiliser	4	21.710	A
Slow-release fertiliser	4	21.4725	A
Fertiliser + DMPP	4	17.118	A
Control	3	10.8200	B

*Means that do not share a letter are significantly different.*

**Appendix 26. Statistical analysis for dry mass production in leaves (g)****Analysis of variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	174.737	58.2458	6.54	0.0084
Error	11	97.965	8.9059		
Total	14	272.703			

**Means**

Treatment	N	Mean	StDev	95% CI
Control	3	6.0233	0.6493	(2.2311, 9.8156)
Standard fertiliser	4	14.948	2.902	(11.663, 18.232)
Slow-release fertiliser	4	14.8500	1.9658	(11.5658, 18.1342)
Fertiliser + DMPP	4	11.493	4.482	(8.208, 14.777)

*Pooled StDev = 2.98428*

**Grouping information using the Fisher LSD method and 95% confidence**

Treatment	N	Mean	Grouping
Standard fertiliser	4	14.948	A
Slow-release fertiliser	4	14.8500	A
Fertiliser + DMPP	4	11.493	A
Control	3	6.0233	B

*Means that do not share a letter are significantly different.*

**Appendix 27.** Statistical analysis for dry mass production in roots (g)

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.0506664	0.0168888	4.81	0.0224
Error	11	0.0386256	0.0035114		
Total	14	0.0892920			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	0.67488	0.08833	(0.59958, 0.75018)
Standard fertiliser	4	0.82762	0.05373	(0.76240, 0.89283)
Slow-release fertiliser	4	0.81699	0.06811	(0.75177, 0.88220)
Fertiliser + DMPP	4	0.749996	0.012139	(0.684783, 0.815208)

*Pooled StDev = 0.0592572*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	0.82762	A
Slow-release fertiliser	4	0.81699	A
Fertiliser + DMPP	4	0.749996	A B
Control	3	0.67488	B

*Means that do not share a letter are significantly different.*

**Appendix 28.** Statistical analysis for total nitrogen in leaves (g)

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.214720	0.0715733	24.17	<0.0001
Error	11	0.032571	0.0029610		
Total	14	0.247291			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	0.050533	0.011973	(-0.018614, 0.119681)
Standard fertiliser	4	0.38205	0.04785	(0.32217, 0.44193)
Slow-release fertiliser	4	0.24078	0.02334	(0.18089, 0.30066)
Fertiliser + DMPP	4	0.33498	0.08904	(0.27509, 0.39486)

*Pooled StDev = 0.0544150*



## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Standard fertiliser	4	0.38205	A
Fertiliser + DMPP	4	0.33498	A
Slow-release fertiliser	4	0.24078	B
Control	3	0.050533	C

*Means that do not share a letter are significantly different.*

**Appendix 29. Statistical analysis for pH (top)**

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.240252	0.0800839	32.10	<0.0001
Error	11	0.027442	0.0024947		
Total	14	0.267693			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	7.75667	0.03215	(7.69320, 7.82014)
Standard fertiliser	4	7.45500	0.05447	(7.40003, 7.50997)
Slow-release fertiliser	4	7.56000	0.03742	(7.50503, 7.61497)
Fertiliser + DMPP	4	7.40750	0.06397	(7.35253, 7.46247)

*Pooled StDev = 0.0499469*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Control	3	7.75667	A
Slow-release fertiliser	4	7.56000	B
Standard fertiliser	4	7.45500	C
Fertiliser + DMPP	4	7.40750	C

*Means that do not share a letter are significantly different.*

**Appendix 30. Statistical analysis for pH (bottom)**

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	0.144218	0.0480728	8.79	0.0029
Error	11	0.060142	0.0054674		
Total	14	0.204360			

**Means**

Treatment	N	Mean	StDev	95% CI
Control	3	7.83333	0.04041	(7.73937, 7.92729)
Standard fertiliser	4	7.55500	0.08347	(7.47363, 7.63637)
Slow-release fertiliser	4	7.69500	0.08699	(7.61363, 7.77637)
Fertiliser + DMPP	4	7.62250	0.06652	(7.54113, 7.70387)

*Pooled StDev = 0.0739420*

**Grouping information using the Fisher LSD method and 95% confidence**

Treatment	N	Mean	Grouping
Control	3	7.83333	A
Slow-release fertiliser	4	7.69500	B
Fertiliser + DMPP	4	7.62250	B C
Standard fertiliser	4	7.55500	C

*Means that do not share a letter are significantly different.*

**Appendix 31. Statistical analysis for electric conductivity (top) ( $\mu\text{S}\cdot\text{m}^{-1}$ )****Analysis of variance**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	1297.46	432.486	2.82	0.0885
Error	11	1688.99	153.545		
Total	14	2986.45			

**Means**

Treatment	N	Mean	StDev	95% CI
Control	3	62.133	3.121	(46.387, 77.879)
Standard fertiliser	4	75.575	12.836	(61.938, 89.212)
Slow-release fertiliser	4	69.775	6.449	(56.138, 83.412)
Fertiliser + DMPP	4	88.150	18.712	(74.513, 101.787)

*Pooled StDev = 12.3913*

**Grouping information using the Fisher LSD method and 95% confidence**

Treatment	N	Mean	Grouping
Fertiliser + DMPP	4	88.150	A
Standard fertiliser	4	75.575	A B
Slow-release fertiliser	4	69.775	A B
Control	3	62.133	B

*Means that do not share a letter are significantly different.*

**Appendix 32.** Statistical analysis for electric conductivity (bottom) ( $\mu\text{S}\cdot\text{m}^{-1}$ ).

## Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	3	3368.6	1122.88	0.90	0.4697
Error	11	13648.3	1240.75		
Total	14	17016.9			

## Means

Treatment	N	Mean	StDev	95% CI
Control	3	69.800	3.816	(25.039, 114.561)
Standard fertiliser	4	103.725	12.675	(64.961, 142.489)
Slow-release fertiliser	4	111.48	65.95	(72.71, 150.24)
Fertiliser + DMPP	4	103.975	5.499	(65.211, 142.739)

*Pooled StDev = 35.2243*

## Grouping information using the Fisher LSD method and 95% confidence

Treatment	N	Mean	Grouping
Slow-release fertiliser	4	111.48	A
Fertiliser + DMPP	4	103.975	A
Standard fertiliser	4	103.725	A
Control	3	69.800	A

*Means that do not share a letter are significantly different.*