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More Profit from Nitrogen

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Optimising nutrient management for improved productivity and fruit quality in cherries

Final Report

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Project team details

Provide details of all personnel involved in the project.

Name	Position	Organisation	Role	Duration of involvement
Nigel Swarts	Senior Research Fellow	TIA	Project Lead	Five years
Peter Quin	Junior Research fellow	TIA	Post doc	Five years
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Contents

Executive Summary

Nitrogen is essential for plant growth, and for producing high-yielding and high-quality crops, produce and pastures. Application of nitrogenous fertiliser is a key profit driver for agricultural industries in particular tree crops such as sweet cherry. Cherries are a significant deciduous tree crop grown in cool temperate climates of Australia with an annual value $140M.

Given the potential risk of reduced yield from insufficient nitrogen, producers generally err on the side of over-applying nitrogen as an 'insurance' policy to take advantage of good seasonal conditions, or unexpected losses. Almost no data is available for Australian cherry growing regions on the relative importance of the soil N processes and total N losses from current management. This project aimed to enhance nitrogen use efficiency and improve profitability through better understanding these multiple interactions via three main objectives:

1. Determine N demand and cycling through the soil-plant-atmosphere system and develop strategies for increasing both the quantity and quality of cherry yields. The interaction with irrigation management was a particular focus to ensure recommended practices are synergistic for both irrigation and nitrogen management (fertigation).
2. Determine the contribution of mineralisation and litter recycling to a cherry orchard's nitrogen budget, improving the ability to accurately predict the contribution of mineralisation, and reducing the reliance on over-application of nitrogen in the face of uncertainty.
3. Investigate alternative more sustainable forms of N nutrition better able to match a crop's specific nitrogen requirements, and developing a better understanding of how they perform under different circumstances (especially soil type, temperature and rain fall), supporting producers choose the best alternative fertiliser in the prevailing circumstances

This project used an integrated approach to quantify plant and nitrogen recycling between seasons. Stable isotopes were used to quantify plant N demand, soil supply and current practice nitrogen use efficiency (NUE) in combination with a comparison between conventional and biological fertilisers to develop best management practices for optimising nitrogen fertiliser use, maximising productivity and reducing environmental impacts for the Australian cherry industry.

Six trials were established in two cherry orchards in southern Tasmania, known for producing premium quality crops targeted for the export market. These included

- Application of ^{15}N labelled fertiliser and recovery through whole tree excavation
- Conventional Nitrogen fertiliser applications with varied rates in a drip irrigated mature cherry orchard
- Alternative (sustainable) fertiliser applications at a half rate commercial practice in a mature cherry orchard
- Commercial and alternative fertiliser application trial in a young cherry orchard
- Foliar as alternative N source (^{15}N labelled proline) trial in a mature cherry orchard
- Litter trial decomposition and N recovery trial using ^{15}N labelled litter material applied to young cherry trees

We worked closely with growers to implement and manage the trials and results were communicated through a variety of forums including field days, workshops, state and national conferences, grey literature and peer reviewed journal articles.

The uptake of N fertiliser as determined by ^{15}N whole tree recovery trials was measured at approximately 35%. Lower uptake of fertiliser applied at the higher rate did suggest a lower NUE, yet the rate of N applied did not affect its relative distribution amongst tree organs. As expected, the amounts of fertiliser N allocated to tree organs were for the most part substantially higher with the higher rate of N applied.

The trials showed that pre-harvest N application can result in a wasteful amount being lost in fruit. Post-harvest application could increase N uptake efficiency, but if excessive can result in unnecessary N being removed in pruned material. Thus, applying most annual N post-harvest is recommended, but the balance of pre- and post-harvest application might vary from season to season depending on yield and regional climatic factors. To best inform N management, testing of fruitlet and fruit N concentrations, and that of N in plant tissue and soil, is recommended. Efficiency of N uptake can be further enhanced by applying N frequently in smaller doses, and without excessive water where possible, to avoid the loss of excess N through leaching and denitrification emissions. These losses can be further minimised by restricting N application if substantial rainfall is imminent in the week ahead.

Our data suggests that 76.5 g N/tree is likely to be a reasonable seasonable 'replenishment' quantity of N (from harvested fruit and pruning material) that would provide adequate N for optimum yield of quality fruit and healthy, but not excessive, vegetative development. Attempts to improve N uptake efficiency, would be a preferrable way to replenish tree N than increased N application.

Taking the above value of 76.5 g N/tree as an annual replenishment quantity of N required by mature trees, at an uptake efficiency of 40% at best, would require the application of about 190 g N/tree if no other inputs were considered and/or uptake efficiency improved. One additional input to the 'N cycle' to be considered is N suitable for uptake that might be supplied by the mineralisation of pruned material and shed leaves. The trials demonstrated the breakdown of leaves into mineralised N of between 3.5 kg N/ha to 5.6 kg N/ha over a 12-month period. The breakdown of stems sufficient to release N for potential mineralisation and recycling would be expected to occur over a considerably longer timeframe than for leaves. Some orchards leave long lengths of pruned stems within the tree rows. The breakdown of stems to release their considerable organic N content for potential mineralisation is very slow. The removal of all pruned material for composting, as already practiced in some orchards, is worthy of consideration. At the least, much more substantial pulverisation of pruned stems before they are replied to tree rows would seem advisable.

Alternative biological based fertiliser treatments at the nitrogen rate applied performed (45 kg N/ha) in general, comparably to the conventional calcium nitrate-based fertiliser applied at the same rate over the three seasons trialed. The feedlot waste was a relatively cheap and simple source of biologically based N and fruit quality and yield outcomes were satisfactory over the three year period. There is likely to be some variation in N rate between batches of feedlot waste so regular monitoring of source material is required. We recognise that there is a labour requirement to distribute the waste over the orchard and the volume required to supply the

necessary N to meet tree requirements may not always be available from the supplier. However, that the application only needs to be done once or twice over a season means that this is achievable from an overall management perspective. Certainly, this form of N could be complimentary to either conventional forms of N or the Organic N which is significantly more expensive yet comparatively easier to apply. The liquid based Organic N can be directly applied through existing fertigation infrastructure, however for growers considering this source of N as a viable alternative, some longer-term studies investigating the soil health benefits of this form on top of fruit quality outcomes would be necessary given the high input cost. Complementing the conventional N and feedlot waste forms with a nutrient uptake facilitator showed some early evidence of being beneficial, however the positive effect wasn't repeated in seasons 2 and 3. The biological based forms of N tested here clearly provide an effective alternative to conventional based fertilisers, yet based on ^{15}N recovery trials, we would recommend applying at a greater rate than the 45 kg N/ha trialed here for ongoing tree health and adequate nutrition. This additional cost would need to be offset by further evidence of improved long-term soil and orchard health to encourage industry to adopt these N management approaches.

Management of fertigated N application in small, regular doses is certainly constrained by the irrigation/fertigation infrastructure of each orchard. However, improvements in nitrogen use efficiency (NUE) to higher levels than those found should be possible. Regular soil testing would be necessary to improve NUE in cherry cropping systems. Another vital tool to improving NUE in cherry orchards, already undertaken in many, would be real-time monitoring of soil moisture, including that below the root zone, to prevent application of excessive irrigation water. Pursuing such a suite of improvements might well result in improvements in NUE to over 50%, with benefits to return on investment and the environment. To determine changes in NUE, regular monitoring of N forms in soil, and N contents of fruit, leaves and pruned material would be necessary. Such testing would also act as a safeguard for orchard managers aiming to decrease their applications of N, which understandably would need to proceed with a degree of caution.

This project "Optimising nutrient management for improved productivity and fruit quality in cherries" was supported by the Australian Government Department of Agriculture, Water and the Environment as part of its Rural R&D for Profit program, Hort Innovation, and The University of Tasmania and Tasmanian Government through The Tasmanian Institute of Agriculture.

Abbreviations and glossary

Nitrogen Use Efficiency (NUE): Has been defined in different ways, often dependent on the crop, but here we simply use the term to describe the percentage of applied N fertiliser taken up by cherry trees.

Fertigation: Regardless of the type of system used, fertigation of solutions containing N are best undertaken frequently with more dilute solutions (of N), rather than less frequently with stronger solutions. This will better match N supply with tree demand and help to optimise NUE and therefore, minimise losses of N to the environment, including weeds and inter-row plants.

Nitrogen fertilisers: Nitrogen is available to fertilise soil in two broad categories, organic (e.g., manure) or mineral, with the organic forms not immediately available for plant uptake when added to soil. Mineral forms of N are available as ammonium or nitrate, each suitable for application by fertigation and readily taken up by plants. Each has its disadvantages: ammonium has an acidifying effect on soil, the extent depending on the particular form, while nitrate forms are more readily lost from the soil, typically by leaching in water but also as nitrous oxide gas if the soil is very wet.

Remobilisation cycle: Nitrogen is stored by deciduous trees during winter dormancy, in branches, buds, trunk, and roots, for potential 'remobilisation' to promote new growth in spring. The process of N storage begins in autumn. As daylight hours and temperatures decrease, N is withdrawn from leaves and stored in buds, branches, trunk, and roots. The highest concentration of stored N is clearly in the buds, followed by the roots, with the concentration in the trunk and branches lower again and similar to each other. This fits in with what is known about the first growth in the following spring, where uptake of N from soil does not commence until about 30 days after full bloom. Thus, the production of flowers is totally dependent on the remobilisation of stored N. Likewise, stored N in the roots can be used to produce fresh growth of fine roots, for use once N uptake from soil commences. Some of the N stored in roots, trunk and branches is also remobilised to commence the new growth of leaf and stems.

Nitrous oxide: Nitrous oxide (N_2O) is a potent greenhouse gas, with about 300 times the warming potential of carbon dioxide. Emissions of N_2O from human activity are responsible for about 6% of climate warming, the third most significant after carbon dioxide (66%) and methane (16%). It is now also close to being the main cause of the destruction of ozone in the stratosphere. Emissions of N_2O from agriculture account for almost 70% of those from human activity. Of synthetic N fertilisers, the nitrate forms are particularly prone to producing N2O emissions, under conditions of very wet soil.

Leaching of nitrogen: The nitrate forms of N, unlike the ammonium forms, are very easily leached away from the root zone of trees, either deeper into the soil and/or sideways away from the main root area. Rainfall or excessive irrigation can be the cause, by wetting soil beyond its capacity to hold water. Leaching of nitrate into groundwater, streams and rivers is well-recognised as a major source of N pollution, contributing to algal blooms, de-oxygenation of coastal waters, and other harmful effects upon the environment. Consequently, there is increasingly strict monitoring worldwide of nitrate N in water draining from farms.

Under suitable conditions, leaching of applied nitrate N is more likely to occur: 1) within a week or two of fertiliser application, as are emissions of N2O, and 2) particularly if applied N cannot be utilised by the trees in that time. So frequent applications of nitrate fertilisers in smaller doses is preferable to larger and less frequent applications. Even if soil characteristics are not favourable to leaching of nitrate N below the trees' root zone, excessive water can transport it to the inter-row, or below the end of rows on sloping ground, reducing its uptake by tree roots.

Mineralisation: All synthetic fertiliser N is in a 'mineral' form, but once taken up is converted to 'organic' N forms within trees. Once shed as senescent leaves or removed as pruned material, the organic N content has to be 'mineralised' by soil microbes before it can be easily re-utilised by the trees. The same is true of fertiliser N that is taken up by weeds or inter-row herbage, both adding to the pool of organic N in orchards that has the potential to be recycled within the soil/tree production system.

Volatilisation: Volatilisation of N as ammonia gas (NH3) is common from animal manures and N fertilisers other than nitrate forms. However, it can occur during the mineralisation of leaves and stems if organic N supply exceeds the appetite of soil microbes. Another reason to maintain good soil health and maximise recycled N.

1 Project rationale

Improving our understanding of the impact of management on nitrogen use efficiency is an essential pre-requisite to giving producers the confidence that they can reduce nitrogen application rates and still maintain or even improve current yields and fruit quality. Over-fertilising with nitrogen, as well as unnecessarily increasing costs, can further reduce profitability through excessive vegetative growth, reduced yield, reduced quality and increased risk of disease. Excess application of nitrogen also significantly increases the risk of reduced air and water quality, and increased greenhouse gas emissions.

Consumers are increasingly interested in the nitrogen use efficiency and greenhouse gas emissions profile of their products, and the research undertaken through this project aimed to support the sweet cherry industry, will provide important information for growers to produce high quality fruit with the lowest environmental impact. This project also prepares these intensive users of nitrogen for a future where improved sensor technologies and communication networks allow for real-time monitoring of soil nutrient status, and therefore near real-time management of crop nutrition.

The main industry need addressed by this project was a better understanding of nitrogen nutrition of cherry trees to produce consistent high-quality fruit that is of export quality. Nitrogen is considered very much a 'black box' by an industry that is in its relative infancy and had adopted nitrogen nutrition strategies originally designed for apples. Cherry fruit are susceptible to lighter colour, delayed maturity and reduce firmness when nitrogen is oversupplied, so this research was designed to better understand matching nitrogen supply to tree demand to optimize nitrogen use efficiency without compromising fruit quality and tree vigour. In addition, this research aimed to determine the efficacy of various sources of N nutrition comparing outcomes to standard commercial practice. Finally, the project aimed to determine the relative inputs and outputs of N in a commercially managed orchard from one season to the next.

2 Method and project locations

This project used an integrated approach to quantify plant and nitrogen recycling between seasons. Stable isotopes were used to quantify plant N demand, soil supply and current practice nitrogen use efficiency (NUE) in combination with a comparison between conventional and biological fertilisers to develop best management practices for optimising nitrogen fertiliser use, maximising productivity and reducing environmental impacts for the Australian cherry industry.

Six trials were established in two cherry orchards in southern Tasmania, known for producing premium quality crops targeted for the export market. These trials can best be described in three parts

1. Dynamics of nitrogen uptake and recycling in cherry orchards over two seasons
2. Dynamics of litter material decomposition and soil mineralisation of N as a nitrogen source.
3. Influence of N source, rate and timing on fruit quality, nutrition, and yield of sweet cherry.

Dynamics of nitrogen uptake and recycling in cherry orchards over two seasons: Determining the NUE of NO_3^- fertiliser applied to cherry trees at different timings and rates, how much is stored in the tree throughout winter and to what extent mineralisation of leaves and pruned stems contributes to the annual orchard N cycle is of vital importance to orchard managers. Such detailed information is needed to determine the optimum quantity of N to apply, to maximise yield of the best quality fruit while minimising the deleterious effects of N lost to the environment. The following study was established to provide detailed information in relation to different timings and rates of N application, while Part 2 that follows examines the mineralisation of fallen leaves and pruned material.

The two-year research trial was conducted in a commercial sweet-cherry orchard in southern Tasmania in a cool temperate climate. Commencing in the spring of 2017, twenty-four five-year-old 'Lapins' cherry trees on Colt rootstock (*Prunus avium* L) were provided for the trial by the grower along with a minimum of one similar 'buffer' tree on either side of each trial tree.. Six randomly allocated N treatments of varying rate 5.5 atom-% ^{15}N-$Ca(NO_3)_2$, in four equal applications ,were applied pre-harvest, post-harvest or split 50:50 between the two. Fruit quality assessments were made at commercial harvest to determine the influence of N rate on fruit quality. Whole trees were excavated at dormancy of each season to determine the fate of applied nitrogen throughout the tree. Trees were split into their various organs and subsampled to determine N content. Data was analysed to compare nitrogen uptake efficiency of different rate applications over a two-year period.

Dynamics of litter material decomposition and soil mineralisation of N as a nitrogen source: When considering the cycling of N in a cherry production system, the mineralisation of shed leaves and pruned material into readily available forms of N is important as a potential source of recycled N that may be available for tree uptake. The aim of the trial was to determine potential rates of N mineralisation of leaves and pruned stems from the trees discussed in **Error! Reference source not found.**of this report, to estimate what contribution that process may make to the annual N supply of those trees. Such knowledge is of considerable importance in determining the annual

fertiliser N requirement of those trees, this in turn helping to maximise production and minimise N lost to the environment.

A trial was established on a north-easterly facing hillside at the Horticultural Research Centre (HRC), University of Tasmania, Hobart. Two-year-old 'Lapins' cherry trees on Colt rootstock, were planted into 45 L woven planter bags using soil from Wandin Valley Farms, Rosegarland, Tasmania. The trial was of three treatments × 6 replicates, using highly ^{15}N-enriched cherry leaves and stems in mesh applied to the soil surface of the trees in planter bags, to quantify their mineralisation over a 12-month period. All trees were excavated and tree organs separated into leaves, fine roots (< 2 mm), larger roots, buds, and branches: as 'main' branch, including trunk, and remaining branches. Samples were analysed by IRMS for ^{14}N:^{15}N ratio and %N content to determine the uptake efficiency of N from litter sources.

Influence of N source, rate and timing on fruit quality, nutrition and yield of sweet cherry:
Precision farming through fertigation can facilitate efficient utilisation of resources and improve returns per unit area and time to growers. Fertigation delivers both water and essential nutrients such as N directly to the active root zone of growing crops through micro irrigation systems, thereby minimising water and nutrient loss and improving productivity. Whilst fertigation is commonly practised by cherry growers in Australia, research and management guidelines for optimal supply of tree nutrient and water requirements are limited.

The trials described below aim to maximise NUE in the Australian cherry industry to increase productivity, profitability and good environmental management. The research will measure cherry tree demand for nitrogen and track its cycling through the soil-plant-atmosphere system. This will guide the development of management strategies for increasing the quantity and quality of cherry yields whilst effectively mitigating loss of nitrogen to the environment.

Trial 1: Effect of nitrogen fertiliser application on fruit quality and bioactive properties of sweet cherry (Prunus avium L) cv. 'Lapins'

The trial was established to assess the performance of different rates of nitrogen provided by calcium nitrate and alternative nitrogen sources. The trial was conducted over three consecutive seasons (2017-2020) in a commercial sweet cherry orchard at Rosegarland in southern Tasmania. Trial design was a completely randomised design with eight treatments and four replicates per treatment. In agreement with current practice, the application of an equivalent of 90 kg N / ha / season was defined as full rate N treatment. To test the hypothesis that a reduction of the application rate of nitrogenous fertilisers does not negatively impact fruit quality, a half rate (45 kg N / ha / season) was chosen for all other treatments. Alternative nitrogen sources included an organic liquid fertiliser ("Organic N"), feedlot waste (~ 2.5% N) and a monthly application (November – February) of the microbial inoculant "Soil & Seed" via drip fertigation. Fruit quality and yield was assessed over three seasons.

Trial 2: Young trees at Reid Fruits' Honeywood Orchard at Jericho, Tasmania

A three-year trial, using one-year-old 'Lapins' cherries growing on Colt rootstock, planted in July 2016, was established at Reid Fruits' Honeywood Orchard at Jericho in the Tasmanian Midlands in the spring of 2017. The 180-tree trial compared application of forms of organic N with an equivalent, and different rates, of the standard mineral N fertiliser, calcium nitrate [$Ca(NO_3)_2$].. Nine treatments were applied over three full seasons arranged in a randomised complete block

design of four replicates, each replicate consisting of five trees, the middle three being 'trial' trees and the outer two 'buffer' trees. The projected orchard application of 150 kg $Ca(NO_3)_2$ /ha/year was taken as a standard N rate, equivalent to 120 g N/tree/year. The soil microbial inoculant *Soil & Seed* was supplied by the manufacturer, BioAg Pty Ltd, Narrandera NSW and mixed with the fertiliser prior to application. The feedlot waste, freshly sourced for each season was applied to the surface of the raised growing beds in mid-November of each season. Lysimeters were installed to measure applied NO_3^- that was leached below the trees' root zone. Tree size and fruit quality measurements were made on an annual basis.

Trial 3. Foliar uptake and partitioning of applied Nitrogen as an alternative to ground applied N

The objective of this proof-of-principle study was to measure the uptake and translocation of foliar applied 15N-labelled L-proline into fruit compartments (stem, stone, skin, and flesh) and storage of the sweet cherry variety 'Lapins'. We aimed to determine whether post-harvest applied L-proline influences the N storage as evidenced by increased N allocation to storage organs such as buds and branches In addition, we investigated if pre-harvest foliar applied N influences leaf chlorophyll and fruit quality outcomes including anthocyanin and phenolic content. 15N-labelled foliar L-proline represents an opportunity to investigate this (proof-of-principle study). We compared pre and post-harvest foliar applied 15N-labelled foliar L-proline to investigate the efficiency of uptake/incorporation into leaves, fruit and branches as an alternative N source to soil applied N. We also investigated the influence of this N source on fruit quality and bioactive compounds after pre-harvest application.

Three treatments (pre-harvest, post-harvest, control) were randomly allocated to trees within each of the four blocks. L-proline (a total of 300 mg of 20 % enriched ^{15}N-labelled L-proline; 36.9 mg N / branch segment) was applied for three consecutive weeks (100 mg / week) commencing at either straw phase of fruit development (pre-harvest application) or two weeks after harvest (post-harvest application), respectively. Treatments were applied to tagged branches to wood older than two years. The whole treated branch was destructively harvested at winter dormancy to track the fate of applied Proline throughout the branch. The section treated with ^{15}N L-proline was dissected into buds, spurs, bark and inner wood and analysed for 15N content. Fruit quality and leaf N measurements were also taken.

Research Site Type	Name	Location	Coordinates	Active Site Period	Experimental treatments
Field trial	Wandin Valley Farm	Derwent Valley, southern Tasmania	42.7099°S, 146.9436°E	2017-2020	^{15}N treatments applied pre- and post-harvest over two seasons
Field trial	Reid Fruits Jericho orchards	Jericho	42.3819°S, 147.2804°E	2017-2020	Young trees: Conventional (calcium nitrate) rate treatments applied via fertigation system and alternative biological treatments applied either via fertigation or spread (i.e., manure)
Field trial	Wandin Valley Farm	Derwent Valley, southern Tasmania	42.7099°S, 146.9436°E	2017-2020	Mature trees: Conventional (calcium nitrate) rate treatments applied via fertigation system and alternative biological treatments applied either via fertigation or spread (i.e., manure)
Field trial	Wandin Valley Farm	Derwent Valley, southern Tasmania	42.7099°S, 146.9436°E	2017-2020	Mature trees: ^{15}N labelled proline delivered via foliar application as an alternative nutrient source.
Field trial	Horticulture Research Centre	Sandy Bay, southern Tasmania	42.9083 °S, 147.3242 °E	2017 - 2020	Litter bag treatments with labelled ^{15}N litter derived from a highly enriched ^{15}N treated cherry tree at Wandin Valley farms

3 Project Outcomes

This is a summation of the findings and recommendations detailed in the technical report (Appendix 1).

The results of each research component must be summarised with key messages/ recommendations for the industry. This may include amendments or changes to current industry guidelines, DDS tools or methodology for determining nitrogen budgets. Figure 1 below details the inputs and outputs of N over a season in a commercial cherry orchard. This research has attempted to develop recommendations to industry and service providers on how best to manage N resources to reduce the N footprint of cherry production whilst maintaining and even improving sweet cherry fruit quality to an export quality standard.

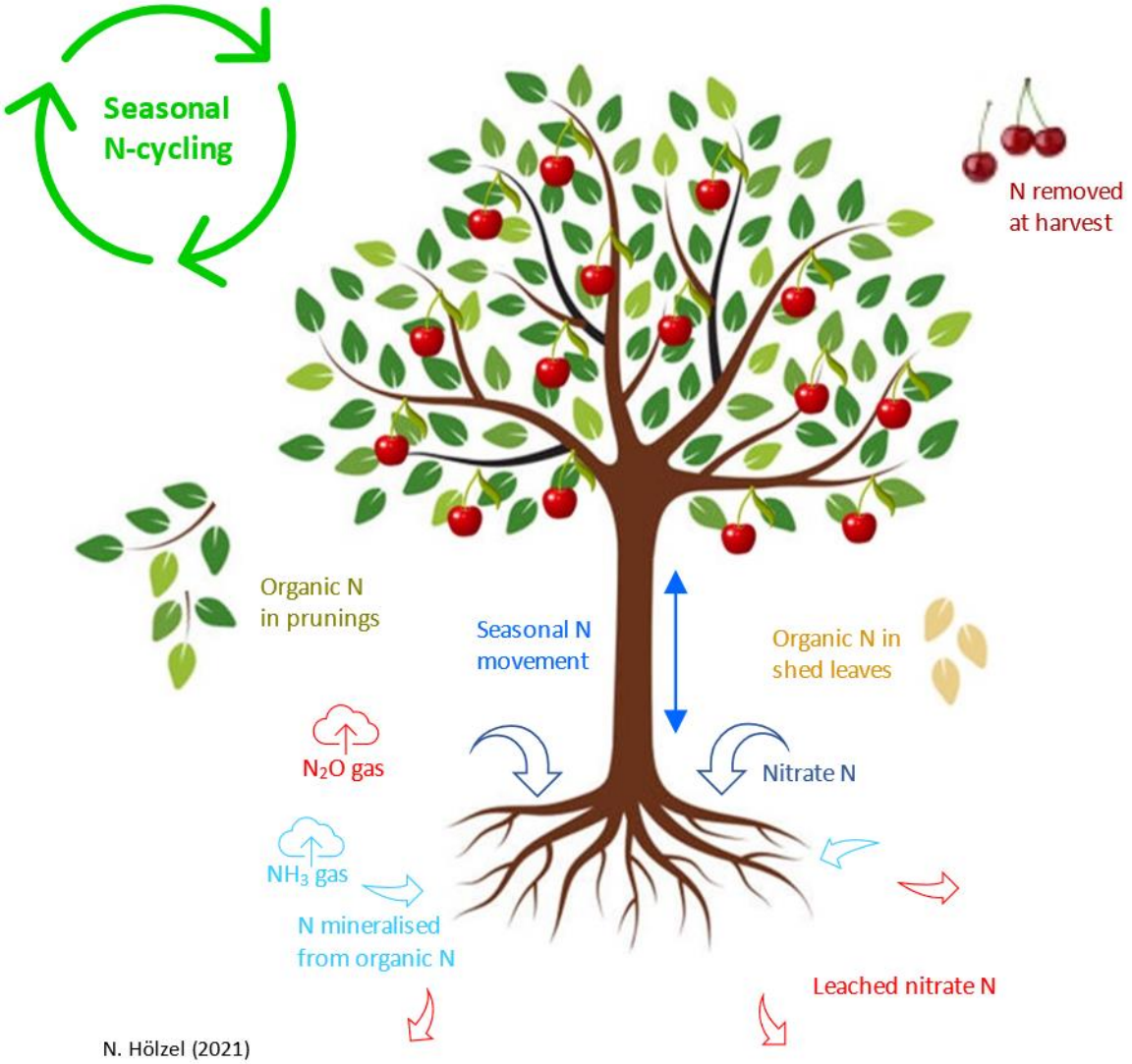

Figure 1. Diagram of inputs and outputs of nitrogen in a commercial cherry orchard

Many comparisons of applied treatments in the orchard trial examined in Part 1 of this report and to some extent, of the mineralisation trial in Part 2, have not resulted in statistically significant differences being found between treatments. In more than a few instances, this was due to large variances between treatment replicates, despite the treatment mean values being quite distinct. Consequently, some uncertainty surrounds the results of these trials, compounded by their being of relatively short duration in relation to seasonal effects. Firmer conclusions would doubtless result by trials of the applied treatments run over longer timeframes. Nonetheless, a great deal of valuable information allows the drawing of some conclusions with considerable confidence, and the making of suggestions that might improve the efficient use of N in cherry crops while maintaining good tree health with optimum yields of quality fruit. This project generated some significant outcomes for industry which have underpinned the recommendations we have made for nitrogen management in sweet cherry tree crop production systems. The outcomes are detailed following the structure detailed above for the methods.

Part 1.

It is difficult to recommend an annual quantity of $Ca(NO_3)_2$ to apply to the mature cherry trees used in the trial to sustain adequate tree growth and provide an optimum yield of high-quality fruit. Many factors can influence the trees' need for such N, from season to season, some related to climatic conditions and others to orchard management. Nonetheless, the trial results suggest that a quantity of close to the 67.5 g N applied per tree (90 kg N/ha) would be adequate, with a substantially greater quantity reducing efficiency of N uptake and thus adding to its loss to the environment. As discussed, trees that received this quantity of N in one season, applied either pre- or post-harvest, maintained sufficient N in the plant/soil system to not suffer any deficit in the total N shed in leaves or removed in pruned material in the following season, with no additional N applied. The only deficit being a lower concentration of N in the storage organs by dormancy of the second season.

As observed above, the lower concentration of N in the storage organs by dormancy of the second season could be remedied by the application of an additional 19.7 g N fertiliser (average of 19.8 and 19.6 g, above), presumably during the second season. In addition to this, account would need to be taken of N removed from the trees in fruit. The average fruit yield over the two seasons from the trees fertilised pre- or post-harvest in the first season were 11.44 and 11. 59 kg/tree, respectively. Taken as 12 kg/tree – with most orchards managers regarding an annual yield of 12-14 kg as a good result – at a sufficient N concentration of 1.667 g N/kg fresh fruit, would equate to an additional 20.0 g N/tree required to replenish N removed. At the same uptake efficiency of 40.8% used to calculate the 19.7 g N needed to maintain storage organ N concentration, 20.0 g N/tree removed per year would require the application of 49.0 g N fertiliser as replenishment. Combining the two values (19.7 + 49.0) suggests 68.7 g N/tree (91 kg N/ha) applied as $Ca(NO_3)_2$ with good management, would be close to an adequate annual replenishment to maintain the trial trees in fine health and providing optimum yields of high-quality fruit.

Part 2

The trials demonstrated the breakdown of leaves into mineralised N of between 3.5 kg N/ha to 5.6 kg N/ha over a 12-month period. The breakdown of stems sufficient to release N for

potential mineralisation and recycling would be expected to occur over a considerably longer timeframe than for leaves.

Part 3

Trial 1. Alternative biological based fertiliser treatments at the nitrogen rate applied performed (45 kg N/ha) in general, comparably to the conventional calcium nitrate-based fertiliser applied at the same rate over the three seasons trialled. The feedlot waste was a relatively cheap and simple source of biologically based N and fruit quality, and yield outcomes were satisfactory over the three-year period. There is likely to be some variation in N rate between batches of feedlot waste so regular monitoring of source material is required. We recognise that there is a labour requirement to distribute the waste over the orchard and the volume required to supply the necessary N to meet tree requirements may not always be available from the supplier. However, that the application only needs to be done once or twice over a season means that this is achievable from an overall management perspective. Certainly, this form of N could be complimentary to either conventional forms of N or the Organic N which is significantly more expensive yet comparatively easier to apply. The liquid based Organic N can be directly applied through existing fertigation infrastructure, however for growers considering this source of N as a viable alternative, some longer-term studies investigating the soil health benefits of this form on top of fruit quality outcomes would be necessary given the high input cost. Complementing the conventional N and feedlot waste forms with Soil and Seed as a nutrient uptake facilitator showed some early evidence of being beneficial, however the positive effect wasn't repeated in seasons 2 and 3. There was some evidence that Soil and Seed delayed ripening possibly due to more efficient accumulation of N as seen in the leaf N concentration charts, yet this was not conclusive either. The biological based forms of N tested here clearly provide an effective alternative to conventional based fertilisers, yet based on ^{15}N recovery trials, we would recommend applying at a greater rate than the 45 kg N /ha trialled here for ongoing tree health and adequate nutrition. This additional cost would need to be offset by further evidence of improved long-term soil and orchard health to encourage industry to adopt these N management approaches.

Trial 2. The results of this three-year trial, comparing forms of organic N applied to the soil with an equivalent, and different rates, of the mineral N fertiliser, calcium nitrate, found no significant differences in the growth of the young trees in relation to the treatments applied. This outcome was not surprising as it was known that the orchard soil was well-prepared prior to the planting of the trees some 17 months before the first application of trial treatments. All indications were of a healthy soil, with considerable mineral N available to meet the demands of young trees, and soil organic matter contents, C:N ratio and pH all suited to the purpose. with only the first real harvest of fruit as a basis for comparing the effects of the applied treatments on yield and fruit quality it was difficult to draw any conclusions. As the N already in the soil before treatment application seemed to have a dominant effect on tree growth, it is reasonable to assume that its effect on fruit yield and quality was at the least, an important factor. Thus, to make any fair judgement on the relative effects of the treatments on yield and quality of fruit, analysis of at least a further two harvests, with the same seasonal treatments applied, would seem a necessary requirement.

The leaching of nitrate N was clearly demonstrated, although precise estimation was more handicapped than in any other part of the trial by wide variation in mean values between

treatment replicates. The difficulty in installing lysimeters and replacing an 'undisturbed' soil core above them doubtless contributed considerably to this variation, as might have variation in soil drainage characteristics across the site. Nonetheless, the data did demonstrate the potential for leaching of NO_3^--N below the trees' root zone and that the likelihood that the more NO_3^--N applied, the more would be leached. The very close to linear relationship found between NO_3^--N applied and that leached certainly reinforced the relevance of the mean leached values related to each treatment, despite their associated large variances

Trial 3. Significantly elevated Atom-% 15N was found from weeks 3 and 9, i.e. after application of the pre- and post-harvest applications, respectively and thereafter remained relatively constant. Levels were significantly higher in the post- than pre-harvest application treatments. These differences in uptake may be due to higher rainfall during and following the three weeks of pre-harvest application resulting in wash-off from the leaf before absorption and incorporation into the leaf structure, or due to re-translocation of N out of leaves to other sinks for N. Due to the nature of application (foliar spray to mimic orchard practice), a mass balance is particularly difficult. Some L-proline may have been lost to the environment (due to wash-off, not quantifiable), whereas another portion may have been taken up by other parts of the branch.

The analysis of leaves from extension growth segments, which were not subjected to direct spray application, showed no evidence of increased ^{15}N levels (data not shown). This indicates that L-proline itself or N derived from L-proline was not translocated to these tree structures in detectable amounts during the growth phase of the tree, indicating that L-proline is incorporated directly where is has been applied. No significant differences were observed in total N content for fruit and branch parts between treatments. As N status is effectively controlled through orchard management practices such as soil-applied N and N reserves recycled between seasons its nit surprising that L-proline in the rates applied did not enhance total N content.

Differences in fruit quality parameters revealed a trend towards earlier ripening/maturity of fruit when branches were treated with L-proline (pre-harvest treatment). However, this trend needs to be interpreted cautiously due to small sample sizes (one treated branch per tree) and the general lack of information regarding the right timing and amounts of L-proline application for sweet cherry. To confirm this trend, studies with whole-tree or whole-block replicates, higher number of treatments (timing, doses) are necessary to evaluate L-proline application as a possible tool for short-term maturity and quality management in cherry orchards.

3.1 Project level achievements

Provide a description of project achievements against the ***final KPIs and outputs*** of the research project. As these final KPI have been worded to conclude the body of long-term investigation, please ensure the final findings are clearly articulated and linkage to impact upon current and future industry knowledge and practice is explained.

KPI no. and description	KPI Due Date	Relevant CRDC FRP Milestone Number/s.	Summary of final outcome of the research concluded by this KPI
KPI 8.6 Provide a brief and final account of the evaluation of best performing biological fertilisers in cherry crops (Output 4k) 30/9/2021	*30/6/2021*	Develop recommendations for the timing, rate and placement of biological fertilisers and blends to reduce nitrogen losses; and optimise Nitrogen Use Efficiency (NUE) both at a plot and farm scale level. (4l)	The trials showed that pre-harvest N application can result in a wasteful amount being lost in fruit. Post-harvest application could increase N uptake efficiency, but if excessive can result in unnecessary N being removed in pruned material. Thus, applying most annual N post-harvest is recommended, but the balance of pre- and post-harvest application might vary from season to season depending on yield and regional climatic factors. To best inform N management, testing of fruitlet and fruit N concentrations, and that of N in plant tissue and soil, is recommended. Efficiency of N uptake can be further enhanced by applying N frequently in smaller doses, and without excessive water where possible, to avoid the loss of excess N through leaching and denitrification emissions. These losses can be further minimised by restricting N application if substantial rainfall is imminent in the week ahead.
KPI 8.7 Provide the department with the biological fertiliser recommendations and a brief account of optimising NUE at both plot and farm-	*30/6/2021*		Alternative biological based fertiliser treatments at the nitrogen rate applied performed (45 kg N/ha) in general, comparably to the conventional calcium nitrate-based fertiliser applied at the same rate over the three seasons trialled. For growers considering these sources of N as a viable alternative, some longer-term studies investigating the soil health benefits of this form on top of fruit

KPI no. and description	KPI Due Date	Relevant CRDC FRP Milestone Number/s.	Summary of final outcome of the research concluded by this KPI
scale level (Output 4l) 30/9/2021			quality outcomes would be necessary given the high input cost. The biological based forms of N tested here clearly provide an effective alternative to conventional based fertilisers, yet based on ^{15}N recovery trials, we would recommend applying at a greater rate than the 45 kg N /ha trialed here for ongoing tree health and adequate nutrition. This additional cost would need to be offset by further evidence of improved long-term soil and orchard health to encourage industry to adopt these N management approaches.
KPI 8.9 Provide a brief and final account of calculating NUE for cherry nitrogen use (Output 5d) 30/9/2021	*30/6/2021*	Determine seasonal and inter-annual cherry plant nitrogen (N) demand, quantify N losses, uptake and calculate NUE. (5d)	Our data suggests that 76.5 g N/tree is likely to be a reasonable seasonable 'replenishment' quantity of N (from harvested fruit and pruning material) that would provide adequate N for optimum yield of quality fruit and healthy, but not excessive, vegetative development. A quantity as high as 135 g N/tree, as provided in the higher of the split 50:50 treatments appear excessive as it provided no benefits to fruit yield or quality and at dormancy the trees retained 68.5 g N/tree, compared with 59.4 g N/tree when half that rate was applied, much of the 'missing' N being lost to the environment and some removed prior to dormancy. Attempts to improve N uptake efficiency, substantially lower when the higher rate of N (135 g N/tree) was applied, would appear a preferable way to replenish tree N than increased N application. Taking the above value of 76.5 g N/tree as an annual replenishment quantity of N required by mature trees, at an uptake efficiency of 40% at best, would require the application of about 190 g N/tree if no other inputs were considered and/or uptake efficiency improved. One additional input to the 'N cycle' to be considered is N suitable

KPI no. and description	KPI Due Date	Relevant CRDC FRP Milestone Number/s.	Summary of final outcome of the research concluded by this KPI
			for uptake that might be supplied by the mineralisation of pruned material and shed leaves. The measured uptakes of N fertiliser applied over the 2017-18 season, split 50:50 between pre- and post-harvest, at the rates of 67.5 and 135 g N/tree (equivalent to 90 and 180 kg N/ha respectively) were measured as 37.9 and 29.6% respectively. While not significantly different on account of substantial variances associated with the mean values, the lower uptake of fertiliser applied at the higher rate does suggest a lower NUE. The rate of N applied apparently did not affect its relative distribution amongst tree organs. However, as might be expected, the amounts of fertiliser N allocated to tree organs were for the most part substantially higher with the higher rate of N applied.
KPI 8.11- Provide a brief and final account of the NUE benchmarks developed for the cherry industry (Output 5f) 30/9/2021	*30/6/2021*	Develop NUE benchmarks for the horticulture industry to target.	Management of fertigated N application in small, regular doses is certainly constrained by the irrigation/fertigation infrastructure of each orchard. However, improvements in nitrogen use efficiency (NUE) to higher levels than those found should be possible. Regular soil testing would be necessary to improve NUE in cherry cropping systems. Another vital tool to improving NUE in cherry orchards, already undertaken in many, would be real-time monitoring of soil moisture, including that below the root zone, to prevent application of excessive irrigation water. Some orchards leave long lengths of pruned stems within the tree rows. The breakdown of stems to release their considerable organic N content for potential mineralisation is very slow. The removal of all pruned material for composting, as already practiced in some orchards, is worthy of

KPI no. and description	KPI Due Date	Relevant CRDC FRP Milestone Number/s.	Summary of final outcome of the research concluded by this KPI
			consideration. At the least, much more substantial pulverisation of pruned stems before they are replied to tree rows would seem advisable. Pursuing such a suite of improvements might well result in improvements in NUE to over 50%, with benefits to return on investment and the environment. To determine changes in NUE, regular monitoring of N forms in soil, and N contents of fruit, leaves and pruned material would be necessary. Such testing would also act as a safeguard for orchard managers aiming to decrease their applications of N, which understandably would need to proceed with a degree of caution.

3.2 Contribution to MPfN program objectives

Provide a description of how the project has contributed to the achievement of the relevant MPfN Program Objective/s.

The objective of the MPfN Program is to enhance NUE, improving profitability and sustainable use, through better understanding the influence of contributing factors. It will:

1. *Generate a greater knowledge and understanding of the interplay of factors to optimise nitrogen (N) formulation, rate and timing across industries, farming regions and irrigated/ non-irrigated situations (Activity 5);*

This project generated significant new knowledge and understanding of how N rate and timing influenced sweet cherry fruit quality over a three -year period. Furthermore, comparison of fruit quality outcomes with conventional fertigation (Calcium nitrate) to that achieved using the same rate of N delivered via alternative more sustainable approaches such as Organic N, Soil and Seed and manure, provided substantial insight into opportunities for growers to consider when planning their nitrogen management strategies.

2. *Generate a greater knowledge and understanding of the contribution (quantifying rate and timing) of mineralisation to a crop or pasture's nitrogen budget (Activity 6); and*

Considering the different nature of the materials it was unsurprising that there was clear evidence of breakdown of leaves to release N, but not of stems. Without processing into smaller particles, rather than the approximately 50 mm sections that were used in the trial – themselves much smaller than the pruned branches left on many orchard floors – the breakdown of stems sufficient to release N for potential mineralisation and recycling would be expected to occur over a considerably longer timeframe than for leaves. About 3 g N/tree found to have been made available from the mineralisation of shed and pruned leaves. Further mineralisation of organic N from leaves remaining in the soil and, over a longer timeframe mineralised N from pruned stems, would likely contribute further N for potential uptake but could not be quantified

3. *Develop greater knowledge and understanding of how biological and conventional fertiliser formulations can better match a crop or pasture's specific N requirements (Activity 4).*

The research found a fruit N concentration of 1.7 g N/kg fresh fruit, with higher applied values deemed unnecessary. When applied to an average 12 kg/tree this equates to 20.0 g N/tree required to replenish N removed in fruit. While many factors can influence a tree's need for sufficient N to sustain adequate tree growth and provide an optimum yield of high-quality fruit—including climatic conditions and orchard management—the research found a tree N requirement of 8 g N/tree to maintain sufficient concentration of N in the storage organs.

Alternative biological based fertiliser treatments at the nitrogen rate applied performed (45 kg N/ha) in general, comparably to the conventional calcium nitrate-based fertiliser applied at the same rate over the three seasons trialled. The biological based forms of N tested here clearly provide an effective alternative to conventional based fertilisers, yet based on ^{15}N recovery trials, we would recommend applying at a greater rate than the 45 kg N /ha trialled here to meet the specific fruit and tree N requirements as described above.

3.3 Demonstrable more profit from nitrogen

Demonstrate how the research outcomes will improve the productivity and/or profitability of the industry's primary producers. Include a quantitative case study/ example where possible.

The trials showed that pre-harvest N application can result in a wasteful amount being lost in fruit or lost to the environment through leaching or as nitrous oxide emissions. In comparison, post-harvest application could increase N uptake efficiency (Figure 2).

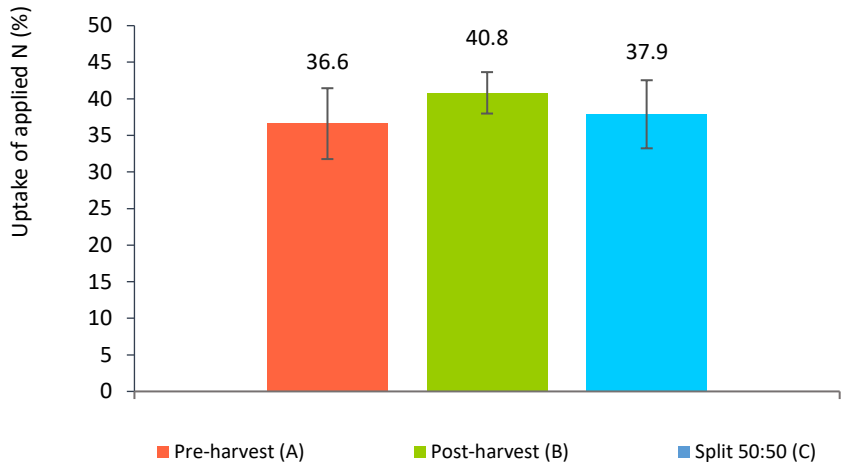

Figure 2. Fertiliser N uptake for different timings of 67.5 g N/tree application including all removed material, of trees excavated in 2018 (C) and 2019 (A and B) (error bars represent ± SE, n = 4).

Applying most annual N post-harvest is recommended, but the balance of pre- and post-harvest application might vary from season to season depending on yield and regional climatic factors. Testing of fruitlet and fruit N concentrations, and that of N in plant tissue and soil, is recommended to inform N management. Efficiency can be further enhanced by applying N frequently in smaller doses, and without excessive water where possible, to avoid the loss of excess N through leaching and nitrification emissions.

Fruit N requirements. The research found a fruit N concentration of 1.7 g N/kg fresh fruit, with higher applied values deemed unnecessary. When applied to an average 12 kg/tree this equates to an additional 20.0 g N/tree required to replenish N removed in fruit.

Tree N requirements. While factors can influence a tree's need for sufficient N to sustain adequate tree growth and provide an optimum yield of high-quality fruit—including climatic conditions and orchard management—the research found a tree N requirement of 8 g N/tree to maintain sufficient concentration of N in the storage organs.

*Analysis of farm level economic benefits***.** For an orchard density of 1330 trees per ha, and a two-year average crop of 12 t/ha, the recommended fruit and tree N requirements equate to 91

kg/ha (Table 1). Compared to a current practice of 120 kg/ha, with N applied as calcium nitrate ($Ca(NO_3)_2$) through fertigation[1], the recommendations have the potential to save $205/ha in N management, as well as reducing N losses to the environment.

N losses to the environment: Within the resulting timeframe when N uptake is most efficient, its optimisation can clearly be maximised by applying it frequently in smaller doses, rather than less frequently in larger ones, and without excessive water. The primary reason being that trees will only utilise what they need at a given time. Any N excess to their needs risks being lost from the soil, to which the nitrate form of N is particularly prone, through leaching below the root zone or lost as nitrous oxide (N_2O) gas. Both processes are encouraged by high soil water content, be that from irrigation or rainfall. Nitrate N is particularly mobile in soil and excess water draining below and away from the root zone can carry large quantities of this dissolved form of N with it. Similarly, excess fertigation water can carry it away from tree rows. As part this trial, it was found that over 2% of N applied pre-harvest was lost as N_2O, a very significant greenhouse gas responsible for 6% of anthropogenic global warming and a catalyst for depletion of stratospheric ozone. Although this loss was primarily the consequence of a heavy rainfall event, excessive watering could produce substantial, undetected losses of applied nitrate N as N_2O. Consequently, real-time monitoring of soil water content, including that below the root zone, would be advisable to prevent application of excessive irrigation water. Avoiding excess drainage of water away from (to the inter-row) and below the root zone can help minimise leaching of soil nitrate. It is difficult to over-emphasise the importance of this, as the loss of nitrate through leaching could quite possibly account for a large portion of unutilised applied N in the trial. A slightly more conservative approach might aid in increasing nitrogen uptake efficiency to well above the values of around 40% found in this trial. To do so would help ensure that the application of 90 kg N/ha per annum, or even less, would prove to be adequate on an ongoing basis, a substantial reduction from the current annual application by management of typically, in “the low 100’s” (kg N/ha).

4 Collaboration

Many individuals contributed to the trials that provided the basis of this report, not the least of whom were many staff members of the Tasmanian Institute of Agriculture, University of Tasmania. Valuable input was also provided by many members of the More Profit from Nitrogen program, including its Coordinator, Marguerite White.

Of particular benefit was the unstinting and reliable assistance provided by our collaborators. James Clements, manager of the Rosegarland orchard of Wandin Valley Farms, and Andrew Hall, manager of Reid Fruits' Honeywood orchard at Jericho are two individuals who went out of their way to assist us, even in the busiest of times. Without their assistance and their company's agreements to host our trials, these studies would not have been possible. Of great help also were staff from BioAg Pty Ltd, in providing the soil microbial inoculant *Soil & Seed* and continuing interest in our work. This collaboration led to the establishment of another trial, involving the use of the same microbial inoculant applied to wine grapes, jointly funded by BioAg and Innovation Connections. Vital collaboration was also provided by Johannes Friedl and David Rowlings of the Institute for Future Environments, Queensland University of Technology in analysis of gas samples taken at the Rosegarland orchard – this leading to the joint publishing of a research article.

5 Extension and adoption activities

5.1 Extension of the research to the end-user

Extension activities conducted during the life of the project included:

Presentations at industry conferences: These presentations were essential for creating initial awareness of the project and for regular updates on project findings and outcomes. Presentations were made at Tasmanian Fruit Growers Annual Conferences each year of the project. These conferences attract fruit (cherry, apple and berry) growers from all over the country with strongest representation from Tasmanian and eastern states growers. The first presentation made for this project was an overview and a request for grower involvement through the hosting of research trials. We had two leading growers approach us which led to the two main study sites used for the project. These same two growers were also instrumental in the design and implantation of the trials and the communication and interpretation of results to other growers.

Presentations at national and international conferences: Presentations were made to the National Fertiliser Conference, Soil Science Australia and Annual Hort Connections conference. These presentations were helpful to ground truth the science for the project and assisted in the interpretation and communication of the results to the wider scientific community.

Field days: Results were presented at a combined Cherry Growers Australia and Fruit Growers Tasmania field day. Our host growers explained to the audience (their peers) why they agreed to host the trial and what they expected to get out of it. They also explained their perspectives on the data and interpretation of the findings which generated considerable conversation among the other growers. This was a very successful way of engaging end users of the research who are much more likely to try or adopt a new or modified practice if their peers have.

Fact sheets: We prepared numerous fact sheets for the various components of the research which were made available at a variety of events, websites and presentations. This was a very effective way of communication short snippets of detail for each individual trial.

Industry articles: Industry articles were prepared for Tree crops and Australian Fruit Grower Magazine which are national industry journals read extensively by growers all over the country. These articles always contained contact details and were a useful way of reaching a broader audience

YouTube videos: A summary video of the project was prepared for YouTube which was broadly shared across the cherry growing community.

Regular meetings with growers and advisors: The most effective form of communication with next users was always regular phone calls and impromptu meetings with growers. Whether that was in the field discussing trial implementation or problem solving or in the reseller's office buying fertiliser, these conversations were always fruitful and led to better communication of the project findings.

5.2 Recommendations to industry on adoption of the research outcomes.

Management of fertigated N application in small, regular doses is certainly constrained by the irrigation/fertigation infrastructure of each orchard. However, improvements in nitrogen use efficiency (NUE) to higher levels than those found should be possible. Regular soil testing would be necessary to improve NUE in cherry cropping systems. Another vital tool to improving NUE in cherry orchards, already undertaken in many, would be real-time monitoring of soil moisture, including that below the root zone, to prevent application of excessive irrigation water. Some orchards leave long lengths of pruned stems within the tree rows. The breakdown of stems to release their considerable organic N content for potential mineralisation is very slow. The removal of all pruned material for composting, as already practiced in some orchards, is worthy of consideration. At the least, much more substantial pulverisation of pruned stems before they are replied to tree rows would seem advisable. Pursuing such a suite of improvements might well result in improvements in NUE to over 50%, with benefits to return on investment and the environment. To determine changes in NUE, regular monitoring of N forms in soil, and N contents of fruit, leaves and pruned material would be necessary. Such testing would also act as a safeguard for orchard managers aiming to decrease their applications of N, which understandably would need to proceed with a degree of caution.

To ensure tree health and optimum yields of quality fruit with minimum nitrogen usage, monitoring is the name of the game. These guidelines are recommended:

- Test the nitrate and ammonium N content of the soil – preferably every year and at the same time, around when buds begin to swell. This will allow a year-to-year comparison of available soil prior seasonal to N fertiliser application.
- Test fruitlets N to assist in assessing pre-harvest N requirement.
- Apply nitrate fertilisers in numerous small doses to best match tree demand and minimise wastage. Remember that excess N pre-harvest can be wasted in fruit and post-harvest in pruned material.
- Test fruit N to determine how much is going ‘out the gate’ and
- Monitor soil pH and the ratio of total carbon/total nitrogen, aiming for about 6.5 and 24:1 respectively for good soil health. These conditions will be of greatest benefit to soil microbes, ensuring good tree health and optimum recycling of organic N from shed leaves and pruned material.
- Monitor other soil nutrients and micronutrients, in consultation with agronomist.
- Avoid overwatering to minimise leaching of nitrate and emissions of N_2O gas. Preferably, install soil moisture probes to assist in irrigation control. At least three depths, one being below the root zone, is ideal and at multiple locations depending on orchard size, topography, and soil variability.

Further adoption of these recommendations can be achieved by on farm demonstration with key growers and ‘early adopters’. Having growers management strategies and their rationale communicated by growers to other growers will see continued uptake of these recommendations.

6 Lessons Learnt

6.1 Research level

This research was undertaken in two main trial locations on commercial orchard blocks. The commercial growers partnering with us on the project are two of the top four or five growers in Tasmania which as a region is well known for producing premium quality fruit. There were several advantages of working with such high quality and industry respected growers. These included:

- Excellent advice and industry relevant suggestions for ensuring trial design was aligned with industry priorities
- Strong support with trial implementation including management of commercially relevant trees and high-quality management and investment in the cherry orchards over the life of the project
- Strong representation of the project by respected industry partners

There were however some significant lessons learnt from some of the disadvantages of working in the commercial blocks in which we undertook the trials. These included:

- Commercially managed trees were already managed as best as they possibly could limiting the positive or negative influence of trial treatments
- As we were excavating whole trees, and undertaking large fertigation trials, trial design had to be arranged in ways that were generally convenient to growers which meant that some compromises had to be made. The major one here was the soil type and location of the trees to be excavated which weren't as ideal as we would have liked.
- One of the sites was heavily manured prior to planting and the carryover effect limited the effects of N treatments in that orchard.
-

6.2 Industry level

At an extension event at which growers from all throughout the state of Tasmania were present, the research team presented data from the ^{15}N trials:

- N uptake efficiency could be improved if less N was applied; and
- Export grade fruit quality was not necessarily improved by adding surplus N (> 90 kg N /ha).

These two findings, although complimentary in their message, were not as well received as expected. Given that N application rates are already relatively low, and that N is relatively cheap compared to other orchard management requirements (i.e., labour), adding N at a rate that is likely to be more than necessary, is undertaken as an insurance strategy. Industry was generally surprised at the low rate if NUE that we demonstrated, and it is likely that our recommendation for less N, more often is a strategy that will be adopted if grower's have the fertigation infrastructure/technology to manage it.

6.3 Service Provider/ Primary Producer Level

Nitrogen application is considered by service providers to be a bit of a black box in tree crop orcharding, particularly for cherries. Generally, this is because there has been limited research undertaken specifically for cherries, and that the advice given to cherry growers is usually based on what is known for apples. Apples have had an extensive history of N physiological research, yet the understanding on N management for these trees generally comes from research in orchards with much lower tree densities and larger trees than reflect modern orchard production systems.

Today, with much higher yields demanded from many, more pedestrian style trees, the N recommendations have just been increased to suit the additional yields. However, cherry trees mature significantly earlier in the season than apple trees and subsequently have a lengthier post-harvest period where extension growth and N uptake can occur. This was evidence in our trial by no significant differences in NUE between pre- and post-harvest N application timings.

Our recommendations of pre- and post-harvest N supply in smaller, more frequent, doses is substantially different to current management practice and is likely to be an important strategy for future N management recommendations by service providers.

7 Appendix 1 - additional project information

7.1 Project material and intellectual property

7.1.1 Journal Papers published

Quin, P and Swarts, N and Oliver, G and Paterson, S and Friedl, J* and Rowlings, D*, "Nitrous oxide emissions from applied nitrate fertiliser in commercial cherry orchards", *Soil Research*, **59** (1) pp. 60-67. doi:10.1071/SR19333 ISSN 1838-675X (2021)

7.1.2 Journal Papers in preparation and review

Quin P and Swarts N, Nitrogen Use efficiency in commercial cherry orcharding as determined by 15N whole tree recovery (in prep)

Holzel N, Swarts N, Quin P, Close D, Bound S, Comparison of conventional and alternative sources of N in commercial cherry orcharding (in prep).

7.1.3 Conference Papers

Holzel, N and Nichols, DS and Swarts, ND and Kent, K, "Less is More: The Influence of Nitrogen Fertiliser Application on Anthocyanins in Sweet Cherries", Proceedings of the 43rd Annual Scientific Meeting of the Nutrition Society of Australia, 2-5 December 2019, Newcastle, New South Wales (2019)

Quin, P and Swarts, N and Holzel, N and Close, D, "More profit from nitrogen: nitrogen use in commercial cherry orchards", Fruit Growers Tasmania Cherry Workshop, 19 September 2019, Somercotes Cherries, Tasmania (2019) [Plenary Presentation]

Quin, P and Holzel, N and Swarts, N, "More profit from nitrogen: nitrogen use in commercial cherry orchards", More Profit from Nitrogen Partner Forum, 4-7 September 2019, Gold Coast, Queensland (2019) [Plenary Presentation]

Quin, P and Holzel, N and Swarts, N, "More profit from nitrogen: nitrogen use in commercial cherry orchards", Australian Fertiliser Industry Conference, 5-6 September 2019, Gold Coast, Queensland (2019) [Plenary Presentation]

Quin, PR and Swarts, ND and Oliver, GS and Paterson, SC and Friendl, J* and Rowlings, D* and Clements, J*, "Nitrogen use in commercial cherry orchards", Soil Science Australia, National Soils Conference, 18-23rd November 2018, Canberra (2019) [Plenary Presentation]

Swarts, N and Quin, P and Holzel, N and Close, D, "Optimising nutrient management in cherries", July 2018, Darwin, Australia, pp. 1-23. (2018) [Plenary Presentation]

Swarts, N and Quin, P and Holzel, N and Close, D, "Optimising nutrient management in cherries", August 2017, Coolangatta, pp. 1-14. (2017) [Plenary Presentation]

7.1.4 Intellectual property

Not Applicable

7.2 Equipment and assets

Not Applicable

7.3 Media and communications material

Holzel, N and Quin, P and Swarts, N, "Research Project Updates: Summer days give way to laboratory daze", Nitrogen Natters, CRDC, 9 April 2019 (2019) [Internal Newsletter]

Holzel, N and Quin, P and Swarts, ND, "Sweet results of research", The Mercury, News Corp Australia, Hobart, Australia, 5 April 2019, pp. 28-28. (2019) [Newspaper Article]

Holzel, N and Quin, PR and Swarts, ND, "Would you like your cherries peeled?", Nitrogen Matters, 8, January (2019) [Internal Newsletter]

Swarts, N and Holzel, N and Quin, P, "Nitrogen Natters", Tasmanian Institute of Agriculture, Hobart, Tasmania, 10, pp. 1-2. (2019) [Internal Newsletter]

Holzel, N and Kent, K and Swarts, ND, "Optimising fruit quality and nutritive properties of sweet cherries", Australian Tree Crop, Ag Communication Solutions Pty Ltd, Australia, August/September 2018, pp. 16-17. (2018) [Magazine Article]

Holzel, N and Quin, PR and Swarts, ND, "Plant litter, foliar nutrients and a whole lot of diggin'!", Nitrogen Natters, October (2018) [Magazine Article]

Swarts, N, "Cherries ripe for research", Tasmanian Country, News Corp Australia, Hobart, Australia, 5 January 2018, p. 10. (2018) [Media Interview]

Swarts, N, "Sacrificing cherry trees for greater good", Launceston Examiner, News Corp Australia, Tasmania, Australia, 14 June 2018, p. 32. (2018) [Media Interview]

7.4 Resource outputs for industry

Quin, P.R., Buntain, M., Swarts, N. and Holzel, N., "Nitrogen Use Guidelines for the Tasmanian Cherry Industry" (In prep).

Appendix 2 – Project technical report

MPfN Program:

Optimising nutrient management for improved productivity and fruit quality in cherries project

Final technical report

1 August 2021

Report prepared by: Dr. Peter Quin, Dr. Nigel Swarts and Nadine Holzel

University of Tasmania- Tasmanian Institute of Agriculture

Australian Government
Department of Agriculture, Water and the Environment

Hort Innovation
Strategic levy investment

CHERRY FUND

Table of contents

1. Overview

Nitrogen (N) is essential for plant growth, and for producing high-yielding and high-quality crops, produce and pastures. Application of N fertiliser is a key profit driver for agricultural industries in particular tree crops such as sweet cherry. Cherries are a significant deciduous tree crop grown in cool temperate climates of Australia with an annual value $140M.

Given the potential risk of reduced yield from insufficient N, producers generally err on the side of over-applying N as an 'insurance' policy to take advantage of good seasonal conditions, or unexpected losses. Almost no data is available for Australian cherry growing regions on the relative importance of the soil N processes and total N losses from current management. Furthermore, the potential to mitigate losses is currently limited due to a basic lack of understanding of plant N requirements and the effectiveness of various mitigation technologies and management options. This project will use an integrated approach to quantify plant N demand and cycling through the soil-plant-atmosphere system and develop strategies for increasing both the quantity and quality of cherry yields. Stable isotopes will be used to quantify plant N demand, soil supply and current practice N use efficiency (NUE) and combined biological fertilisers to develop best management practices for optimising N fertiliser use, maximising productivity and reducing environmental impacts for the Australian cherry industry.

The complexity of the N cycle makes calculating the optimum amount of N to apply difficult, especially given the uncertainties associated with the contribution of mineralisation, interactions with other management practices and the impact of weather conditions. N availability is affected by many factors including soil type and condition, field history, irrigation management practices, form, timing and placement of fertilisers, and prevailing weather.

This project aims to enhance NUE and improve profitability through better understanding these multiple interactions via three main outcomes:

1. Generating more knowledge and a better understanding of the interplay of the above factors - which combinations of management practices provide the most efficient use of N, under different soil and weather conditions. The interaction with irrigation management will be a particular focus to ensure recommended practices are synergistic for both irrigation and N management (fertigation).
2. Generating more knowledge and better understanding of the contribution of mineralisation and litter recycling to a cherry orchard's N budget, improving the ability to accurately predict the contribution of mineralisation, and reducing the reliance on over-application of N in the face of uncertainty.
3. Investigating alternative more sustainable forms of N nutrition better able to match a crop's specific N requirements, and developing a better understanding of how they perform under different circumstances (especially soil type, temperature and rain fall), supporting producers choose the best alternative fertiliser in the prevailing circumstances

Improving our understanding of the impact of management tactics on NUE is an essential pre-requisite to giving producers the confidence that they can reduce the N application rates and still maintain or even improve current yields. Over-fertilising with N, as well as unnecessarily increasing costs, can further reduce profitability through excessive vegetative growth, reduced yield, reduced quality and increased risk of disease. Excess application of N also significantly increases the risk of

reduced air and water quality, and increased greenhouse gas emissions.

Consumers are increasingly interested in the NUE and greenhouse gas emissions profile of their products, and project outcomes will support the sweet cherry industry demonstrate what they are doing to produce high quality fruit with the lowest environmental impact. This project also prepares these intensive users of N for a future where improved sensor technologies and communication networks allow for real-time monitoring of a crop or pasture's nutrition status, and therefore near real-time management of crop nutrition.

General background of NUE understanding in cherries

Nitrogen is essential for plant growth and is the most limiting nutrient for crop production in many of the world's agricultural systems (Fageria & Baligar, 2005; Goulding, Jarvis, & Whitmore, 2008). It is primarily the use of artificial fertiliser N, produced by the Haber-Bosch process, that has supported a huge increase in global food production (Fowler et al., 2013). It has been estimated that at the beginning of this century almost half of the human population was dependent on food produced by the use of such fertiliser (Erisman, Sutton, Galloway, Klimont, & Winiwarter, 2008). World N fertiliser consumption in 2017 was 107.6×10^6 tonnes, having increased from 81.2×10^6, 49.7×10^6, and 23.7×10^6 tonnes in 1997, 1977 and 1967 respectively (International Fertilizer Association, 2018). Research has shown that often less than 50% of N applied worldwide is taken up by the main cereal crops (Glass, 2003; Good & Beatty, 2011) with the remainder lost to the environment as various pollutants (Cameron, Di, & Moir, 2013; Galloway et al., 2003) – these include the nitrate (NO_3^-) form of N, widely recognised as being easily leached from soil (Di & Cameron, 2002; Environmental Protection Agency, 2014), and the potent greenhouse gas nitrous oxide (N_2O) (IPCC, 2013). Improving N use efficiency (NUE) in crops is of extreme importance to the economic production of food whilst minimising N pollution of the environment. Research has led to some instances of marked improvements in NUE. The production of maize (corn) in the United States saw the application of N fertiliser (per hectare) remain virtually unchanged from 1980 to 2010, while grain yield increased from about 104 kg to over 170 kg per kg of N applied. However, in many other instances improvements in yields have resulted from over-application of N fertiliser (Cassman, Dobermann, & Walters, 2002; International Fertilizer Association, 2014).

Perennial fruit crops, while occupying only 1% of global agricultural land, are of considerable economic importance. The N use efficiency of such crops is generally lower than 55% and mineral fertiliser N is by far the dominant source of N nutrition (Carranca, Brunetto, & Tagliavini, 2018). In many, if not the great majority of instances, the N fertiliser used is calcium nitrate ($Ca(NO_3)_2$), a convenient and economic source of both N and calcium. There are few published studies related to the application of NO_3^- fertilisers to perennial fruit trees. More relate to the application of ammonium nitrate, but its availability is now highly restricted. In the particular case of sweet cherry (*Prunus avium*) production, some studies examined the uptake efficiency of N fertilisers, but not the fate of 'lost' N (e.g. Azarenko, Chozinski, & Brutcher, 2008; Rivera, Bañados, & Ayala, 2016) and one the leaching of applied NO_3^-, to which it is highly prone (San Martino, San Martino, & Lavado, 2014). A separate study measured N_2O emissions in a Tasmanian cherry orchard fertilised with $Ca(NO_3)_2$ (N. Swarts et al., 2016), this potent greenhouse gas and ozone-depleting substance (IPCC, 2013; Ravishankara, Daniel, & Portmann, 2009) being a product resulting from the denitrification of NO_3^- fertilisers. Neither study compared the uptake or associated losses of NO_3^- fertilisers applied at different times of the season. Nor are there published studies examining the uptake of pure NO_3^- fertilisers (i.e., not ammonium nitrate) and its allocation to separate tree organs within mature sweet cherry trees.

In sweet cherry production, advice to growers often advocates applying a considerable portion of annual N post-harvest (e.g. Cherry Growers of Australia Inc, 2011), with the belief that it will substantially bolster winter-stored N and that excessive pre-harvest N may be detrimental to fruit quality. Published studies suggest that, from prior to after fruit harvest, the later N is applied the lower its uptake (Azarenko et al., 2008; Rivera et al., 2016). One of these studies (Azarenko et al., 2008) found that the NUE of ammonium sulphate fertiliser applied to 7-year-old trees, in a single dose at the rate of 45 kg N ha^{-1}, declined from 22% for that applied in early spring, to 14% pre-harvest, under 5% in mid-summer and to 2% for that applied prior to leaf abscission. When another dose of the same rate was applied to all the trees in early-mid spring of the following season, ten weeks prior to harvest, the overall two-season NUE of 25-26% was not significantly different in relation to the timing of the prior season fertiliser applications. What was apparent, as also found with the other study (Rivera et al., 2016), was that the timing of N fertiliser application had a substantial influence on the allocation of that N within the tree. This latter study found that of fertiliser N taken up, a significantly greater proportion of that applied early post-harvest was stored in roots than of that applied in early spring. For deciduous trees such as sweet cherry the storage of N during winter dormancy is important for its potential remobilisation for the following season's growth. As such, its distribution and quantity of total storage is an important question in relation to N application timing. Another important factor related to N cycling in cherry production systems is the potential mineralisation of senescent leaves and pruned material. The mineralisation of both leaves and stems left on the orchard floor may contribute substantial quantities of N for recycling within the system, but the extent of such contributions has been little studied. While such recycling and the NUE and N distribution within the trees in relation to different timing of application of pure NO_3^- fertilisers to sweet cherry trees are open questions, so too is whether NUE is affected by different rates of application of such fertiliser.

These knowledge gaps raise important questions that form the objectives and key activities of this research project. This report is compiled into three parts each describing a trial or series of trials which address the objectives of the project:

1. Determine the dynamics of N uptake and recycling through cherry trees over two seasons
2. Determine the dynamic of litter material decomposition and soil mineralisation as a N source in cherry orcharding
3. Determine the influence of N source, rate and timing on fruit quality, yield and nutrition of sweet cherry

2. Methodology, results and discussion by investigation

Part 1: Dynamics of N uptake and recycling through cherry trees over two seasons

Background

Determining the NUE of NO_3^- fertiliser applied to cherry trees at different timings and rates, how much is stored in the tree throughout winter and to what extent mineralisation of leaves and pruned stems contributes to the annual orchard N cycle is of vital importance to orchard managers. Such detailed information is needed to determine the optimum quantity of N to apply, to maximise yield of the best quality fruit while minimising the deleterious effects of N lost to the environment. The following study was established to provide detailed information in relation to different timings and rates of N application, while Part 2 that follows examines the mineralisation of fallen leaves and pruned material.

Methods

The two-year research trial was conducted in a commercial sweet-cherry orchard in southern Tasmania (42.7099°S, 146.9436°E) in a cool temperate climate. Annual rainfall for the region averages 572 mm with that in summer generally less than in other seasons (Bureau of Meteorology, 2019). Commencing in the spring of 2017, twenty-four five-year-old 'Lapins' cherry trees on Colt rootstock (*Prunus avium* L), grown with a Kym Green Bush (KGB) training system (OSU Externsion Service, 2010), were provided for the trial by the grower along with a minimum of one similar 'buffer' tree on either side of each trial tree. The trees were planted at a row spacing of 4.5 m with an average 1.67 m between trees, for a total of 1,330 trees ha^{-1}. Due to the constraints of commercial orchard management, the trial was established down one row, which ran down a gentle, north-easterly facing slope. Six randomly allocated N treatments were replicated in a four-block design within the row. Soil in the upper block (Block 1) was a Vertosol (Isbell, 2002), with a relatively shallow A horizon. This continued down the hill slope, transitioning to a texture contrast soil with a deeper A horizon at the bottom of the row. Treatments were applied by drip fertigation in the first season only (2017-18), at the timings shown in Table 1. A total of 67.5 g $tree^{-1}$ of N (373 g N m^{-2}, or 90 kg N ha^{-1} on a broadacre basis) was applied as 5.5 atom-% ^{15}N-$Ca(NO_3)_2$, in four equal applications to treatments A, B and C (Table 1), while treatment D received eight such applications for a total of 135 g N $tree^{-1}$. Treatments E and F were zero-N controls. Details of the fertigation and irrigation systems and operation are as described in Quin et al. (2021), with irrigation ceasing during the period of winter dormancy. Treatments were applied pre-harvest, post-harvest or split 50:50 between the two (Table 1). No other N was applied throughout the duration of the trial, with all other nutrient application according to orchard management practice, as was pest and weed control.

One additional tree, in an adjacent row and subject to the same management practices, was treated with 66 atom-% ^{15}N-$Ca(NO_3)_2$, applied by watering can on each of the eight dates shown in Table 1. Each application was of 15.00 g of fertiliser dissolved in 4 L of water, for a total of 21.3 g N applied, for the purpose of providing highly ^{15}N-enriched leaves and stems for autumn pruning, these to be used in the mineralisation trial (Part 2).

Table 1 The N application schedule, commencing 30 days after full bloom in the 2017-18 season; all doses equal with treatments A, B and C receiving a total of 67.5 g tree–1 of 5.5 atom-% 15N-Ca(NO3)2 and treatment D double that amount.

Days from first N application (date)	**N treatment**			**Double N treatment**
	A (pre-harvest)	**B (post-harvest)**	**C (split 50:50)**	**D (split 50:50)**
0 (9/11/17)	X		X	X
7	X			X
14	X		X	X
21	X			X
62 (9/01/18, harvest)				
70		X	X	X
77		X		X
84		X	X	X
91		X		X
Trees excavated	2019	2019	2018	2018

Combined soil moisture/temperature sensors

A 30 cm multi-depth probe (*Sentek*, Drill and Drop RS232 with Solo Head Unit) was installed in Block 2, to measure soil moisture and temperature at depths of 5, 15 and 25 cm. Values were logged half-hourly. The position of the probe and block layout are detailed in Quin et al. (2021), accompanied by soil moisture and rainfall charts (Figs. 2 & 3) for most of the 2017-18 growing season.

^{15}N enrichment

The 5.5 atom% ^{15}N-Ca$(NO_3)_2$ was produced by mixing equal weights of commercial Ca$(NO_3)_2$ (Generate Plus, total N of 15.5 %: 14.4 % nitrate, 1.15% ammonium) with 10 atom-% ^{15}N-Ca$(NO_3)_2$ (Berry and Associates, Michigan). The 66 atom-% ^{15}N-Ca$(NO_3)_2$ was produced by mixing the commercial Ca$(NO_3)_2$ with 98+ atom-% ^{15}N-Ca$(NO_3)_2$ (Berry and Associates, Michigan).

Collection of tree organs, excavation, and analysis

All fruit was harvested and weighed, in both seasons of the trial, on the day before commercial harvest in that section of the orchard. Fruit was stored overnight at 2 °C, with a selection of 25 A-grade fruit from each tree used for quality analysis the following day. A further 25 A-grade fruit from each tree were stored at 2 °C in LifeSpan® bags for quality analysis 30 days after harvest. Fruit quality was assessed by the method presented in the *Assessment of quality and bioactive properties* section of Part 3/Trial 1 of this report. Another 30 fruit from each tree were weighed and pitted the day after harvest and dried at 60 °C – flesh and pits for the first season, flesh only for the second – for subsequent total N and ^{14}N:^{15}N analysis. The dried weights of these sub-samples were used to calculate the total dry weights of harvested fruit.

Throughout the period of the trial, any leaves shed prior to harvest were captured by individual netting suspended immediately below the branches of each tree. These were cleared frequently and dried in an oven at 60 °C. Following autumn pruning, the netting was extended to fully enclose the tree and capture all senescent leaves shed up to dormancy. Senescent leaves were removed from the netting at approximately fortnightly intervals. All shed leaves were saved for later analysis, as

was a representative sub-sample (approx. 10% by wet weight) of material from the annual pruning, in this year taking place on 5th March 2018. All leaves and stems were oven-dried at 60 °C. Dried weights were recorded, with those of sub-samples used to calculate the total dry weights of pruned material. It should be noted that due to equipment malfunction these trees were not pruned in the previous season, so that the mass of pruned material was higher than it might otherwise have been.

Trial trees were excavated at dormancy (early June) in each season: those from treatments C, D, E and F in 2018 and A and B in 2019. Prior to excavation, all branches were removed with a 'major' branch on either side of the tree row being specifically tagged as 'E' or 'W'. Each remaining stump was then wrapped with a broad strap and secured to an excavator. The stump was rocked gently back-and-forth while forks were used to loosen the soil as prominence of the main roots was revealed. The stump was then lifted slightly (5–10 cm), along with further loosening of the soil. This process was repeated until roots within approximately 70 cm of the stump, down to a depth of about 40 cm, had been freed from external soil, at which point the stump, roots and attached soil was lifted clear of the ground. The soil remaining within the row was then further turned over with forks to collect any associated roots that remained behind. Each set of stump and roots were taken to another site within the orchard, where most of the remaining soil was washed off prior to transport with the branches back to the laboratory site. Stump, root, and branch material was stored overnight at ≤ 20 °C, prior to dissection the following day.

Buds were removed from all branches and weighed in three categories: those from E and W branches, and the remainder. Branches were weighed in bulk, then E and W branches individually. Each E and W branch was measured for total length and four sections, each of approximately 100 mm in length, cut from each branch: two sections began at cuts 100 and 700 mm from the but end of the branch, the third and fourth sections cut to include what was judged to be three and two-year-old wood, respectively. Each section was weighed, its location within the branch recorded along with its length and mean thickness. Fine (< 2 mm thickness) and medium (2–10 mm) roots were removed from the trunk, separated, and weighed. Any remaining soil was cleaned from the trunk and large roots (> 10 mm) excised from its main body and weighed, as was the remaining trunk. Cross-sections of approximately 50 mm thickness were cut from above and below the graft of the trunks of the 2018 excavated trees, and from below the graft only of the 2019 excavated trees as no significant differences in total N or N isotope ratios of the two sections had been found from the previous season. Cross-sections were weighed and measured for mean breadth, thickness of bark and one-year-old wood (four measurements each) and sub-samples of each removed and weighed. A series of four 10 mm diameter × 25 mm deep holes were drilled into the face of each cross-section to take sub-samples of 'outer', 'middle' and 'centre' wood, each of the three categories being homogenised and weighed. The holes were positioned by reference to growth rings, to best represent wood that was progressively older than the one-year-old wood. The mean distance from the centre of the trunk of each of the three series of four holes was recorded to enable, with the inclusion of the mean thicknesses of bark and one-year-old wood, trunk profiles of total N and N isotope ratios to be derived. All buds, sub-sections of E and W branches, (drilled) cross-sections of trunks and all trunk sub-samples were dried at 60 °C, for the time taken for each to show no further weight loss in consecutive weightings.

Soil sampling

Soil samples were taken within the tree row prior to the commencement of the trial, when it was yet to be established which trees within the row would be used for the trial. These samples were taken from depths of 0-10, 10-20, 30-40, and 50-60 cm. Further samples were taken one week prior to the excavation of trees in 2018, from a depth of 0-10 cm from four positions around each trial tree, at distances of approximately 30 cm from the trunk. Each set of four samples were homogenised, air-dried, and sieved to < 2mm.

Total N and ^{15}N analysis

All dried plant samples were homogenised, and portions prepared for analysis by Isotope Ratio Mass Spectrometry (IRMS) by grinding into a fine powder using a ball mill (Retsch, Haan, Germany), as were sub-samples of dried and sieved soil. This analysis, to determine total N and ^{14}N:^{15}N ratio, was carried out by Central Science Laboratories (CSL), University of Tasmania, Hobart.

N stable isotopes were analysed using flash combustion isotope ratio mass spectrometry (varioPYRO cube coupled to Isoprime100 mass spectrometer). Stable isotope abundances were reported in delta (∂) values as the deviations from conventional standards in parts per mil (‰) from the following equation:

$$\partial\ ^{15}N\ (‰) = [(^{15}N/^{14}N\ \text{sample}) / (^{15}N/^{14}N\ \text{standard}) - 1] \times 1000$$

$\delta^{15}N$ values were reported relative to atmospheric air. Certified Reference Materials (USGS40, USGS41, IAEA-N1 and IAEA N2) were used to correct for instrumental drift and quality assurance purposes. As recommended by IUPAC (Coplen, Krouse, & Böhlke, 1992), the value of 272 was employed for $^{14}N/^{15}N$ of N_2 in air for the calculation of atom fraction ^{15}N from measured $\delta^{15}N$ values; the applied formula (Hauck, 1983) is valid for low enrichments (<5 atom-%).

Enriched laboratory standards were prepared from mixtures of enriched and natural abundance fertilizer and calibrated against international reference standard IAEA311. The analytical performance of the instrumentation, drift correction and linearity performance were calculated from the repetitive analysis of these standards. Precision of the elemental data was 0.2 %. For isotopic measurements precision was < 0.06 ‰ up to the highest enrichment level.

^{15}N atom percentage ($^{15}N_{apc}$) was calculated from the measured $\delta^{15}N$ values and the calibration curve produced from the enriched laboratory standards. Natural abundance (NA) of ^{15}N used in the calculation was the $^{15}N_{apc}$ measured from leaf samples taken prior to the application of ^{15}N-enriched $Ca(NO_3)_2$.

Fertiliser N uptake and allocation

The proportion of N within a plant organ that was derived from fertiliser N (N_{fert}) is represented by NDF_{organ}, and:

$$NDF_{organ}\ (\%) = (^{15}N_{apc}\ \text{of organ} - NA) / (N_{fert}\ \text{atom percentage} - NA).$$

Thus, the sum of N derived from fertiliser (NDF) in all tree organs (NDF_{tree}) was calculated as:

$$NDF_{tree}\ (\text{g N tree}^{-1}) = \text{Sum of } (NDF_{organ} \times \text{dry weight of organ}) \text{ for all organs,}$$

including (except when stated) that of fruit, pruned material and shed leaves.

The NUE of each tree was calculated as:

$$NUE\ (\%) = NDF_{tree} / N_{fert} \times 100.$$

Statistical analysis

Means of calculated values were compared by ANOVA in SAS® 9.4, with least significant differences calculated at a 95% confidence interval. ANOVA was used to evaluate any block effects, with the block mean square being tested, using an F-test, against the error mean square.

Results

In the data analysis for both the ***Error! Reference source not found.*** and the *Timing of N application* section which follows there were found to be no block effects.

N rate comparison

Soil

The soil samples taken within the trial row, prior to the trial trees being determined, were only analysed for total N and organic C. The results, described relative to the position of the later-established tree blocks, are shown in Table 2. The values of total N can be compared with those in a more detailed set of soil analyses of samples taken one week prior to excavation of some of the trees in winter 2018 (Table 4).

Table 2 Organic carbon and total N in the trial row just prior to the trial's establishment in November 2017.

	Block 1		Block 2		Block 4	
Depth (cm)	Org. C (%)	Total N (%)	Org. C (%)	Total N (%)	Org. C (%)	Total N (%)
0-10	2.78	0.29	2.10	0.21	1.93	0.21
10-20	2.77	0.29	2.05	0.23	-	-
30-40	1.91	0.22	0.77	0.07	0.59	0.05
50-60	-	-	0.67	0.09	0.65	0.07

Nitrogen Use Efficiency

A comparison of the total fertiliser N uptake by trees with 67.5 g N applied and those with 135 g N applied (Figure 1) found no significant difference between the two, although the mean uptake by those with the higher rate of N applied did appear to be lower. The same was true when fertiliser N allocated to fruit was excluded from the calculations, those amounts being 20.1 and 18.1% respectively of the fertiliser N taken up from the lower and higher rates of N applied.

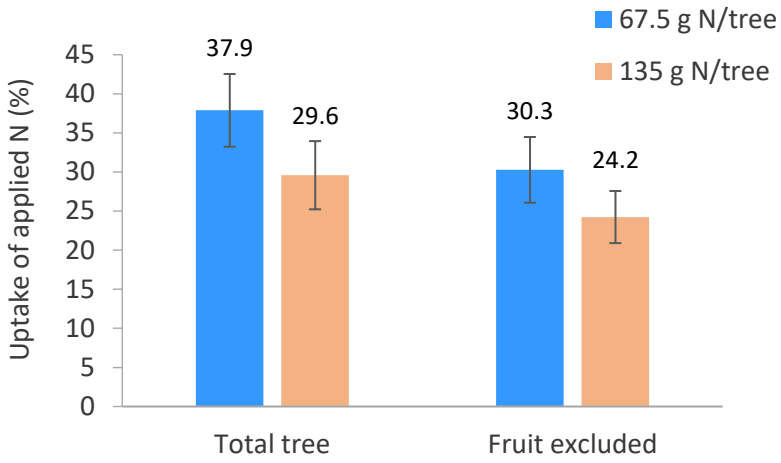

Figure 1 Efficiency of fertiliser N uptake for two rates of application, for the total tree and with fruit excluded (error bars represent ± standard error of the mean [SE], n = 4).

Fertiliser N and total N apportionment

The distribution between tree organs of fertiliser N taken up by the trees is shown in Figure 2, these values being related to the fertiliser N content of the organs at winter excavation except for fruit (at summer harvest), leaves (over the phase of senescence), and pruned material (autumn). As previously noted, the trees had not been pruned in the previous season. Nonetheless, the relatively high fertiliser content of pruned material is an indication that a substantial portion of the fertiliser N taken up was used for new canopy growth. Relative apportionment of fertiliser N taken up did not vary significantly for any organ between treatments of 67.5 g N or 135 g N applied per tree. The absolute amounts of fertiliser N taken up by the trees is shown in Figure 3. Measured on this basis the 2.21 g of fertiliser N stored at dormancy in the 2-10 mm roots of trees with 135 g N applied was significantly greater (p = 0.0165) than in the 0.90 g of fertiliser N in those of trees with 67.5 g N applied. On the same basis, the trunk was the only other tree organ that showed a significant difference in absolute fertiliser N content in relation to the amount applied, with that receiving the higher amount of N being just significantly greater (p = 0.0486) than for the lower rate of N. For the treatments of 67.5 and 135 g N applied as fertiliser the respective uptakes of 25.6 and 40.0 g N (standard errors of 3.1 and 5.9 g N, n = 4) were not significantly different.

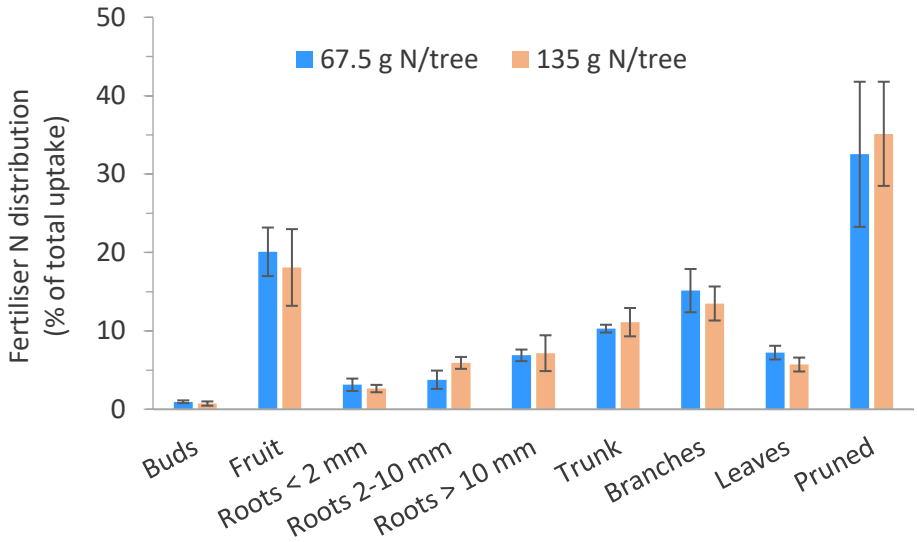

Figure 2 Relative distribution between tree organs of fertiliser N taken up by the trees (error bars represent ± SE, n = 4).

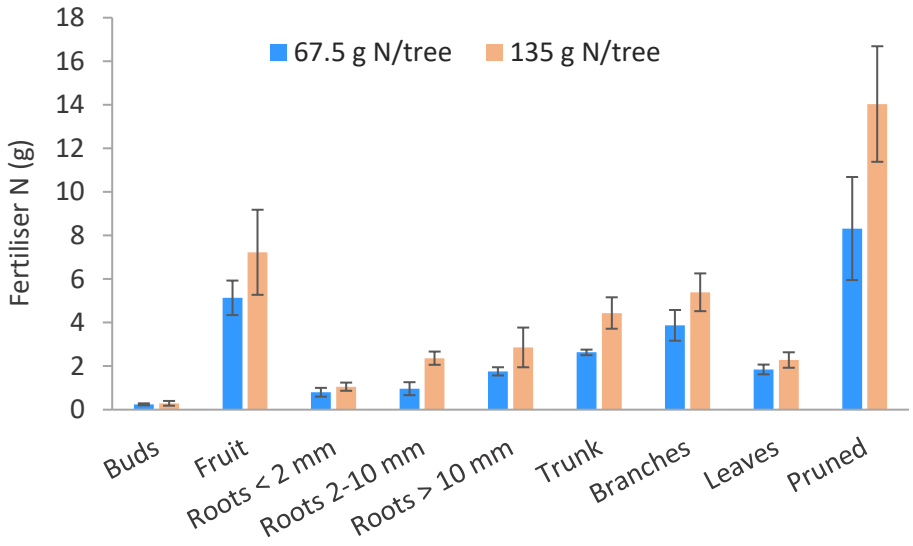

Figure 3 Fertiliser N distribution between tree organs (error bars represent ± SE, n = 4).

The concentration of fertiliser N in tree organs (mg fertiliser N/g dried tree organ) did show significant differences in some organs in relation to the amount of N applied, although there were no significant differences in the dried weights for all tree organs and for the complete trees (including fruit, shed leaves and pruned material), where those weights were 26.0 and 29.2 kg respectively for the 67.5 and 135 g N treatments. The concentrations where a significant difference was found are shown in Table 3 (plus large roots and branch, for an overview), with the level of significance (*p* value) for each.

Table 3 Concentration of fertiliser N in tree organs where a significant difference was found in relation to the quantity of fertiliser N applied, plus those of large roots and branches (n = 4).

	Roots				Trunk	Branch	Pruned	Tree
	< 2 mm	2-10 mm	> 10 mm	All roots				
N applied	**Fertiliser N (mg)/dried material (g)**							
67.5 g	2.614	1.260	0.856	1.112	0.420	0.489	4.063	1.085
135 g	3.629	3.139	1.117	1.770	0.608	0.583	5.694	1.475
p	0.0160	0.0002	(0.139)	0.0033	0.0049	(0.083)	0.0164	0.0132

Figure 4 shows the relative distribution of total N between tree organs. Relative apportionment of total N did not vary significantly for any organ between treatments of 0 g, 67.5 g or 135 g N applied per tree. Nor did the total N contents of any organ differ in relation to fertiliser N applied. For the treatments of 67.5 g N and 135 g N applied per tree the proportion of total N allocated to fruit, 22.7 and 22.3% respectively, was slightly higher than the proportions of fertiliser N (20.1 and 18.1%), although the differences were not significant. For the 0 N (control) treatment 25.3% of total N was allocated to fruit, a not significantly greater proportion than for either of the applied N treatments. In greater contrast, although again not significantly different, were the proportions of total N in pruned material of 25.4 and 25.0% for the 67.5 g N and 135 g N treatments compared to the respective 32.5 and 35.1% of fertiliser N.

The total N content of each organ is shown in Figure 5. Trees with 135 g fertiliser N applied per tree had a significantly greater total N content than the 0 N trees in branches (p = 0.0353), pruned material (p = 0.0286) and 2-10 mm roots (p = 0.0079), with no other significant differences between treatments in total N content for any organ. The concentration of N (g N/g tissue) in 2-10 mm roots was also significantly greater in those from the 135 g N treatment than in either the 67.5 g N treatment (p = 0.0169) or 0 N treatment (p = 0.0106). There were no other significant differences in N concentration for individual organs. However, although the average weights of all tree organs combined did not differ significantly between treatments, the N concentration was significantly greater in those treated with 135 g N (p = 0.0265), and 67.5 g N (p = 0.0294), than in the 0 N controls.

The total N content of the 0 N trees, including fruit, shed leaves and pruned material, was 110.0 g/tree, compared with 135.7 and 153.6 g/tree respectively for those with 67.5 and 135 g/tree of fertiliser N applied, with that in the 135 g N trees being marginally significantly greater (p = 0.0379) than in the 0 N trees. The total removed from trees of these three treatments as fruit, shed leaves and pruned material (Figure 6) was just significantly greater (p = 0.0476) in those treated with 135 g N than from the 0 N trees. For the trees with N applied, the proportions of N and fertiliser N removed as each of fruit, shed leaves and pruned material is shown in Figure 7, with there being no significant differences between the two treatments within each category.

At dormancy, trees that received 0, 67.5 and 135 g N retained a respective 51.4, 59.2 and 68.3 g N in their roots, trunks, branches, and buds, with the only significant difference being between the 0 and 135 g N treatments (p = 0.0413). The fertiliser N contents of the 67.5 and 135 g N treated trees were 10.3 and 16.4 g N and significantly different (p = 0.0395).

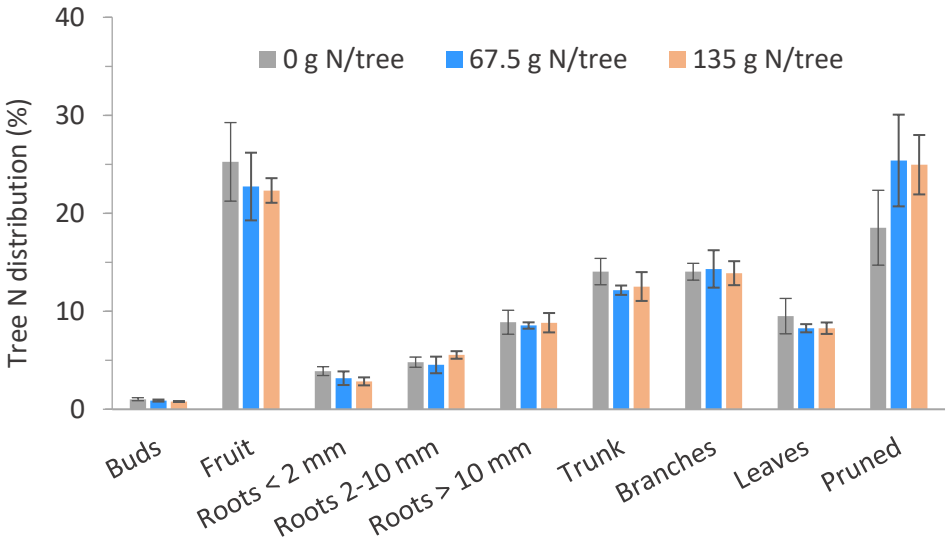

Figure 4 Relative distribution between tree organs of total tree N contents (error bars represent ± SE, n = 4).

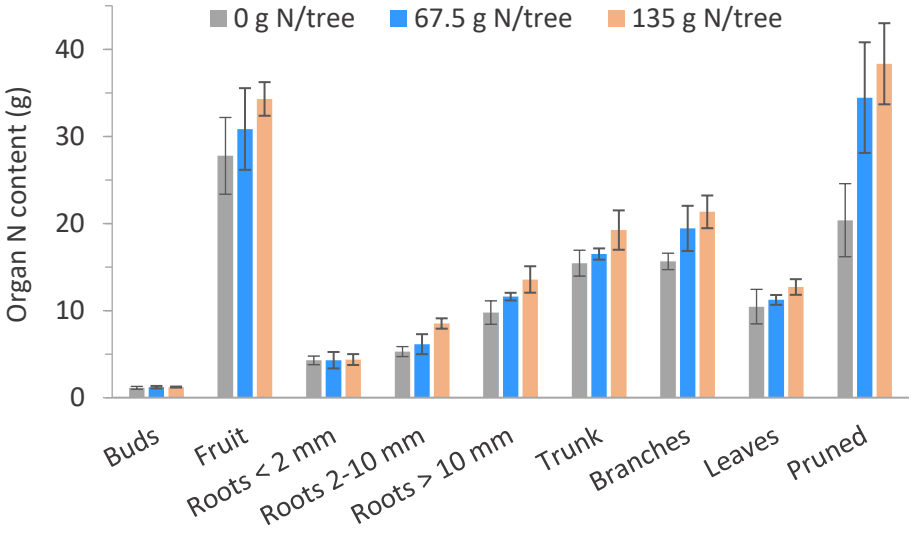

Figure 5 Total N contents of tree organs (error bars represent ± SE, n = 4).

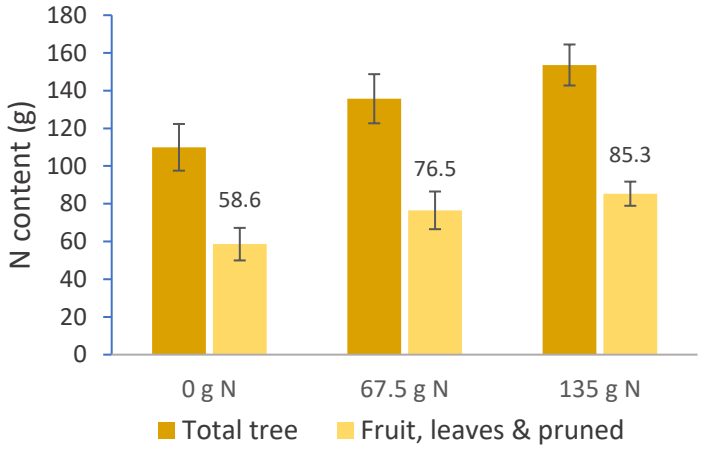

Figure 6 Total N content of all tree organs for the 2017-18 season, to which 0, 67.5 or 135 g N/tree was applied, and the corresponding quantities of N removed in fruit, leaves and pruned material (error bars represent ± SE, n = 4).

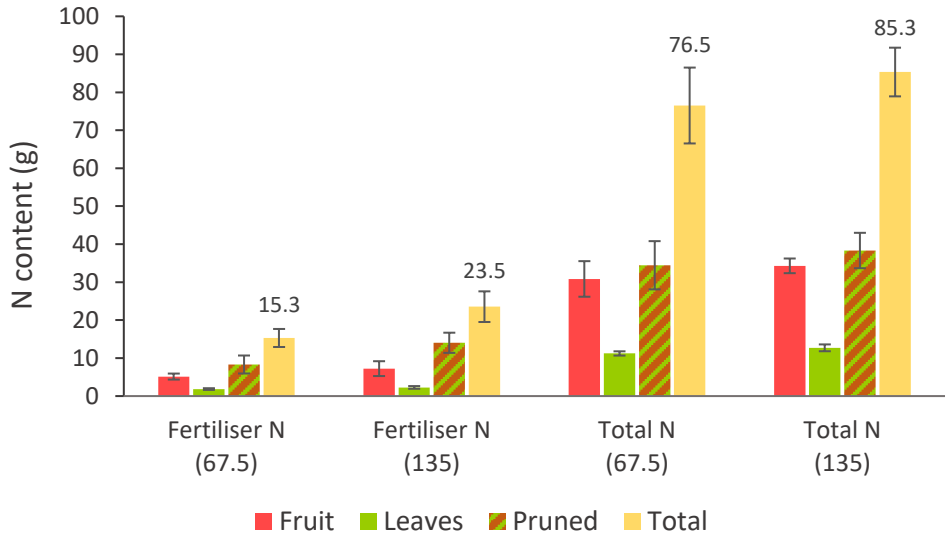

Figure 7 Fertiliser N and total N removed in fruit, senescent leaves and pruned material, and totals of the three, from trees with 67.5 and 135 g N applied (error bars represent ± SE, n = 4).

Fruit yield and quality

The mean yield of fresh fruit from trees with 67.5 g N applied was 18.24 kg/tree (SE = 1.87 kg, n =4), not significantly less than the 21.19 kg/tree (SE = 1.53 kg, n =4) harvested from trees with 135 g N applied. Nor were there significant differences between the two treatments in the concentrations of fertiliser N or total N in the fruit.

There were no significant differences found in fruit quality between the 67.5 g and 135 g N treatments, measured following harvest or after 30 days storage, within any of the measured parameters, except for their titratable acid content. In that case the fruit from trees that were treated with 0, 67.5 and 135 g N had titratable acid contents of 4.589, 4.575 and 4.319 g malic acid/L respectively when measured post-harvest, and 3.340, 3.328 and 3.167 g malic acid/L respectively when measured after 30 days of storage, in each case all being significantly different ($p < 0.0001$).

Timing of N application

From the time of N fertiliser application in the spring/summer of 2017/18 (Table 1) the total N uptake of all trees to which 67.5 g N was applied is shown in Figure 8. It should be noted that unlike the trees of treatment C which were excavated in 2018, those of treatments A and B were excavated in 2019 (Table 1). Thus, their total fertiliser uptake may reflect the additional fertiliser N in fruit, leaves and pruned material of the second season (Figure 9), be that sourced from remobilised N stored in other tree organs, further uptake of fertiliser N from soil during the 2018-19 season, or a combination of these factors. Samples taken from around all trial trees one week prior to the 2018 excavations (0 N and split 50:50 trees, Table 1) show elevated levels of ^{15}N remained in the soil (Table 4), an indication of remaining fertiliser N. In one instance, from samples taken adjacent to a 2019-excavated tree (Tree 81), this was associated with a very high nitrate concentration. Thus, given that the samples were of a limited number and only from 0-10 cm, it is possible that there was further uptake of fertiliser N by the 2019-excavated trees following winter 2018 dormancy.

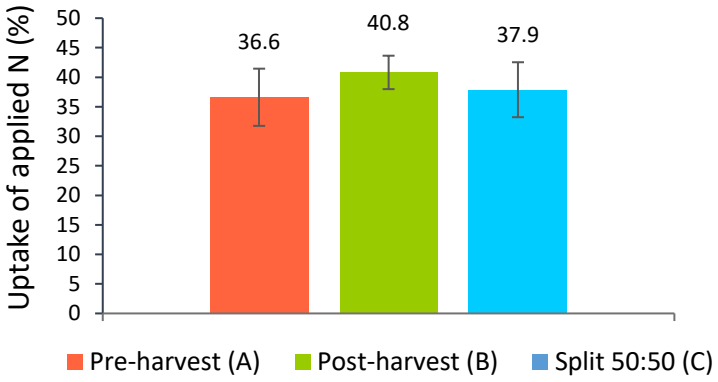

Figure 8 Percentage of fertiliser N uptake for different timings of 67.5 g N/tree application (Table 1 legend codes in parentheses), for the total tree including all removed material, of trees excavated in 2018 (C) and 2019 (A and B) (error bars represent ± SE, n = 4).

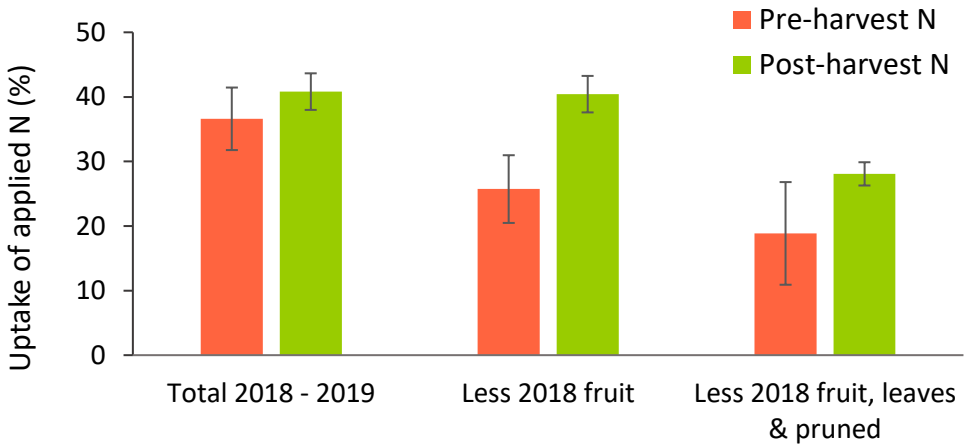

Figure 9 Uptake and allocation of fertiliser N applied pre- or post-harvest 2017/18 and excavated in 2019 (treatments A and B in Table 1), for total of all tree organs up to excavation, less 2018 fruit, and less fruit, senescent leaves and pruned material from the 2017–2018 season (error bars represent ± SE, n = 4).

Table 4 Properties of soil samples taken around trees at 0-10 cm one week prior to excavation of trees from control and both split 50:50 treatments (Table 1) on 4-06-2018. Values of $\delta^{15}N_{air}$ and atom-% ^{15}N in bold font are adjudged to be above natural abundance.

Tree	Treatment	Applied N (g)	Corrected $\delta^{15}N_{air}$	Atom-% ^{15}N	%N	%C	NH_4^+-N (μg N/g soil)	NO_3^--N (μg N/g soil)
38	Control	0	11.71	0.3706	0.24	2.97	3.24	2.06
54	Control	0	16.24	0.3722	0.21	2.12	2.73	4.79
62	Control	0	6.79	0.3688	0.21	1.89	1.71	7.48
76	Control	0	7.29	0.3690	0.21	1.89	1.38	1.83
28	Split 50:50	67.5	**103.20**	**0.4040**	0.29	3.08	7.24	4.70
48	Split 50:50	67.5	**85.38**	**0.3975**	0.21	2.13	2.00	4.20
58	Split 50:50	67.5	**117.67**	**0.4092**	0.20	1.82	1.57	10.63
85	Split 50:50	67.5	**53.34**	**0.3858**	0.22	1.97	1.73	8.47
36	Split 50:50	135	**179.56**	**0.4318**	0.28	2.94	4.39	10.68
44	Split 50:50	135	**169.72**	**0.4282**	0.23	2.49	2.26	6.88
66	Split 50:50	135	19.84	0.3735	0.18	1.80	1.66	3.20
74	Split 50:50	135	**106.45**	**0.4051**	0.21	2.03	3.74	8.68
34	Pre-harvest	67.5	**136.55**	**0.4161**	0.31	3.25	5.67	7.19
52	Pre-harvest	67.5	37.27	0.3799	0.20	2.04	1.81	2.21
68	Pre-harvest	67.5	25.03	0.3754	0.20	1.77	1.31	4.54
83	Pre-harvest	67.5	37.97	0.3802	0.20	2.06	1.74	4.19
30	Post-harvest	67.5	**48.87**	**0.3841**	0.32	3.37	6.31	7.06
46	Post-harvest	67.5	25.55	0.3756	0.20	2.14	2.26	4.52
64	Post-harvest	67.5	17.48	0.3727	0.21	1.84	2.08	2.60
81	Post-harvest	67.5	**542.06**	**0.5637**	0.23	1.94	1.92	49.91

The excess atom-% ^{15}N in leaves, from prior to the commencement of fertigation until after its cessation (Table 1), is shown for all fertigated treatments in Figure 10. This clearly demonstrates the uptake of fertiliser N following the commencement of each fertigation period (pre-harvest on 9-11-17, post-harvest on 17-01-18, and the split treatments from each of those dates). Clearly, the split 50:50 treatments continue to take up further fertiliser N from the commencement of the post-harvest period, and while not statistically significant on account of substantial variance in the mean, the peak value of the 135 g N treatment on 19-02-18 clearly demonstrates that it has the greatest uptake. The stabilisation of leaf ^{15}N from that time, and the corresponding decline in leaf % N (Figure 11) suggest that as the trees enter a period leading to dormancy the uptake of N from soil is likely declining and withdrawal of N from leaves for storage in other tree organs is underway. A similar trend for leaf % N is seen in the following season (Figure 12). However, in that case leaf N has declined for both pre- and post-harvest treatments to about 1.0% on 21-02-19, in contrast to about 2.2% on 19-02-18 in the previous season. This suggests that the trees may have lacked sufficient N, with no fertiliser applied in that season. In the previous season at this time the N content of leaves from both pre- and post- harvest treatments consisted of 20.0% fertiliser N, whereas in the second season it was 11.9 and 22.0 % respectively (Figure 12) and, although not significantly different, the leaf fertiliser N content of the pre-harvest trees was significantly less ($p < 0.05$) than those of pre-harvest trees on both 26-11-18 and 25-01-19. For the same respective treatments, all shed leaves in the second season contained 13.3% and a significantly greater 19.8 % ($p = 0.0110$) fertiliser N. Similarly, for all tree organs in the 2018-19 season, the total N content of the pre-harvest treated

trees of 101.7 g N was significantly less (p = 0.0116) than the 120.3 g N of the post-harvest treated trees. Likewise, there was a significant difference (p = 0.0279) between their respective fertiliser N contents, of 8.7 and 15.8% of total N. While there was little difference between the two treatments in N removed in fruit, leaves and pruned material in the previous season (Figure 13), these preceding differences suggest that the post-harvest treated trees had more N and fertiliser N available for the second season's growth than those treated pre-harvest, despite the fertiliser N uptake of the post-harvest trees being only marginally greater (Figure 8). The combined total N contents of fruit, leaves and pruned material of the pre- and post-harvest fertilised trees in the second season was 37.3 and 51.2 g N/tree, respectively, and represented 36.7 and 42.6% of the trees' total N content for the season. For that season, each of these organs that clearly represent new growth had a higher total N content, even if not significantly, in the post-harvest fertilised trees than the pre-harvest ones (Figure 14). The contrast was more marked with the % of N that came from fertiliser in these three organs (Figure 14) where it was significantly greater in the fruit (p = 0.0121), leaves (p = 0.0110), and pruned material (p = 0.0181), and for all the combined tree organs (p = 0.0279) of the post-harvest fertilised trees than in those of the pre-harvest ones in the second season.

Further indications of greater vigour in the 2018-19 season in the post-harvest fertilised trees than in those fertilised pre-harvest came from measures of extension growth – the mean/branch of new growth measured on five branches – and the weight of pruned material. Extension growth was measured five days before February 2019 pruning. For the post-harvest trees, it was 422 cm, although not significantly greater than the 363 cm of the pre-harvest trees, it was suggestive of greater vigour that could result from an increased N supply. Similarly, the mean dry weight of pruned material from post-harvest fertilised trees was 2.66 kg, not significantly so but nonetheless greater than the 2.06 kg from the pre-harvest fertilised trees.

Total N in material pruned in the autumn of 2018 was 24.1 and 32.8 g N/tree respectively for those fertilised with N pre- or post-harvest (**Error! Reference source not found.**). Fertiliser N comprised a respective 27.7 and 22.6% of these totals. Of leaves shed by these trees approaching dormancy in 2018, total N content was a respective 11.4 and 10.1 g N/tree, fertiliser N being 15.9 and 9.2% of these totals. Thus, the total N removed in fruit, pruned material, and shed leaves in the 2017-18 season from the trees fertilised pre- or post-harvest was 70.1 and 67.1 g N, respectively. Of these totals of material removed prior to winter 2018 dormancy, 12.0 and 8.6 g N/tree respectively was fertiliser N, representing 48.6 and 31.2% of the total fertiliser N taken up by these trees over the two seasons and well demonstrated in Figure 9. Although, due to the large variance associated with the mean values, these fertiliser losses were not significantly different, the apparently greater loss of fertiliser N from those trees fertilised pre-harvest does fit with the other factors that suggest they had lower vigour in the following season than the post-harvest fertilised trees. This was supported by the N content of tree organs at excavation at 2019 dormancy (roots, trunk, and branches – with buds), with those of the pre-harvest fertilised trees containing 64.3 g N compared with 69.0 g N in those fertilised post-harvest. While those values were not significantly different, their fertiliser N contents of 4.80 and 8.46 g N respectively, clearly were (p = 0057).

Fruit

Fruit harvested in the 2017-18 season from trees fertilised pre-harvest had a very significantly higher (p = 0.0051) N content of 34.7 g N/tree, with 21.2% from fertiliser N, than the 24.2 g N/tree of those fertilised post-harvest (**Error! Reference source not found.**). The 0.27 g of fertiliser N attributed to fruit of trees with N applied post-harvest was not significantly distinct from zero. The corresponding fresh weights of fruit from pre- and post-harvest fertilised trees were 19.78 and 17.26 kg/tree in that season, for concentrations of 1.75 and 1.40 g N/kg fruit (Table 6). The fruit yields of all treatments in

the first season (2017-2018, Table 6) were above those expected for an 'average' year, with no significant differences between treatments. Nor was there any notable correlation between those yields and the quantity of fertiliser N applied pre-harvest in that season (**Error! Reference source not found.**). In contrast, the second season's fruit yields from the pre- and post-harvest fertilised trees were much lower, and not significantly different from each other, at 3.1 and 5.9 kg/tree, respectively. Yields of fruit in the second season were much lower throughout the orchard than in the previous one, the result of biennial bearing, and for the trial trees apparently unrelated to lack of seasonal N application. The fruit N concentrations in the second season of 1.83 and 1.61 g N/kg for the pre- and post-harvest fertilised trees, respectively (Table 6), were not significantly different from the previous season's respective values, or each other, and remained higher in the trees fertilised pre-harvest. Fertiliser N comprised 13.1% of total N in 2018-19 fruit of the trees fertilised pre-harvest in the previous season, compared to 23.5% in those fertilised post-harvest. As in the previous season there were no significant differences in relation to fertiliser N timing in any of the fruit quality parameters, in this case including titratable acidity, either on harvest or after 30 days storage (*Methods*).

For all fruit harvested in the first season, the N concentration (**Error! Reference source not found.**) was higher in fruit from trees that received some applied N before harvest. However, the only significant difference was that it was higher ($p < 0.01$) in fruit from only one of the two treatments with 67.5 g N/tree applied pre-harvest, than in fruit from either of the treatments that received no N pre-harvest (**Error! Reference source not found.**). Over the two seasons, the average yields of fruit from the trees fertilised pre- or post-harvest in the first season were 11.44 and 11. 59 kg/tree respectively, and not significantly different. However, their associated fruit N concentrations, of 1.76 and 1.45 g N/kg fruit respectively, were significantly different ($p < 0.01$), due to the dominance of the first season's crop in yield and its higher N concentration.

Table 5 Total N and fertiliser N per tree removed in the 2017- 18 season in fruit, shed leaves and pruned material from trees fertilised with 67.5 g N in that season either pre- or post-harvest (n = 4).

	Pre-harvest fertilised				Post-harvest fertilised			
	Total N (g)		Fertiliser N (g)		Total N (g)		Fertiliser N (g)	
	Average	SE	Average	SE	Average	SE	Average	SE
Fruit	34.65	1.10	7.34	0.52	24.24	2.16	0.27	0.15
Leaves	11.37	1.92	1.80	0.36	10.06	2.00	0.93	0.22
Pruned	24.13	5.21	6.67	2.15	32.76	4.33	7.41	1.72

Table 6 Mean yields of fresh fruit and N concentration for harvests of seasons 2017-18 and 2018-19 *.

Applied N (g/tree) and timing	N applied (g) pre-harvest 2017-18	Fruit yield 2017-18 (kg fresh)	N concentration 2017-18 (g N/kg fresh)	Fruit yield 2018-19 (kg fresh)	N concentration 2018-19 (g N/kg fresh)
0 control	0	19.28	1.445 a	n/a	n/a
67.5 split 50:50	33.8	18.24	1.667	n/a	n/a
135 split 50:50	67.5	21.19	1.636	n/a	n/a
67.5 pre-harvest	67.5	19.78	1.753 a,b	3.10	1.840
67.5 post-harvest	0	17.26	1.401 b	5.92	1.626

* Values in the one column accompanied by the same letter are significantly different ($p < 0.01$).

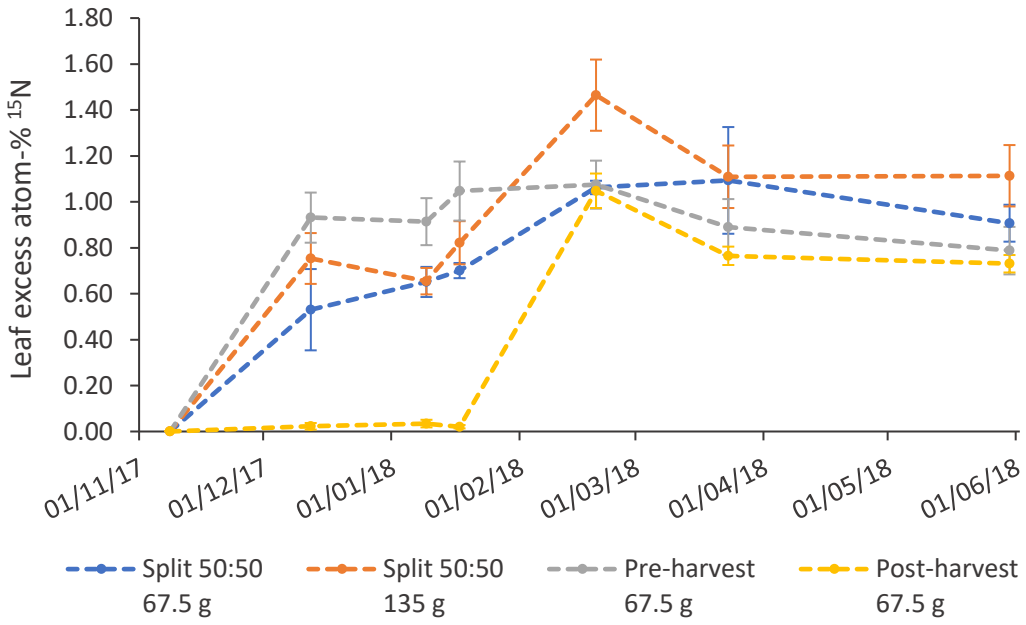

Figure 10 Excess ^{15}N-nitrogen in leaves (above natural abundance) following the application of 5.5 atom-% 15N- enriched calcium nitrate at the indicated rates (g) per tree, the pre-harvest and split treatments (Table 1) commencing for four weeks on 9-11-2017 and the post-harvest and re-commencing split treatments for four weeks on 17-01-2018 (error bars represent ± SE, n = 4).

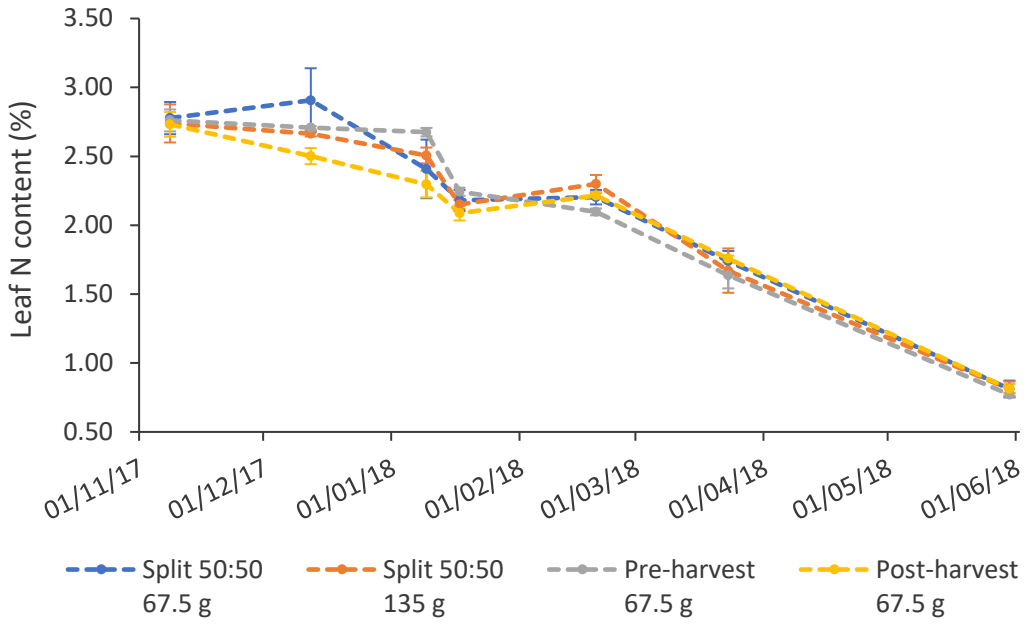

Figure 11 Nitrogen content of leaves following the application of 5.5 atom-% 15N- enriched calcium nitrate at the indicated rates (g) per tree, the pre-harvest and split treatments (Table 1) commencing for four weeks on 9-11-2017 and the post-harvest and re-commencing split treatments for four weeks on 17-01-2018 (error bars represent ± SE, n = 4).

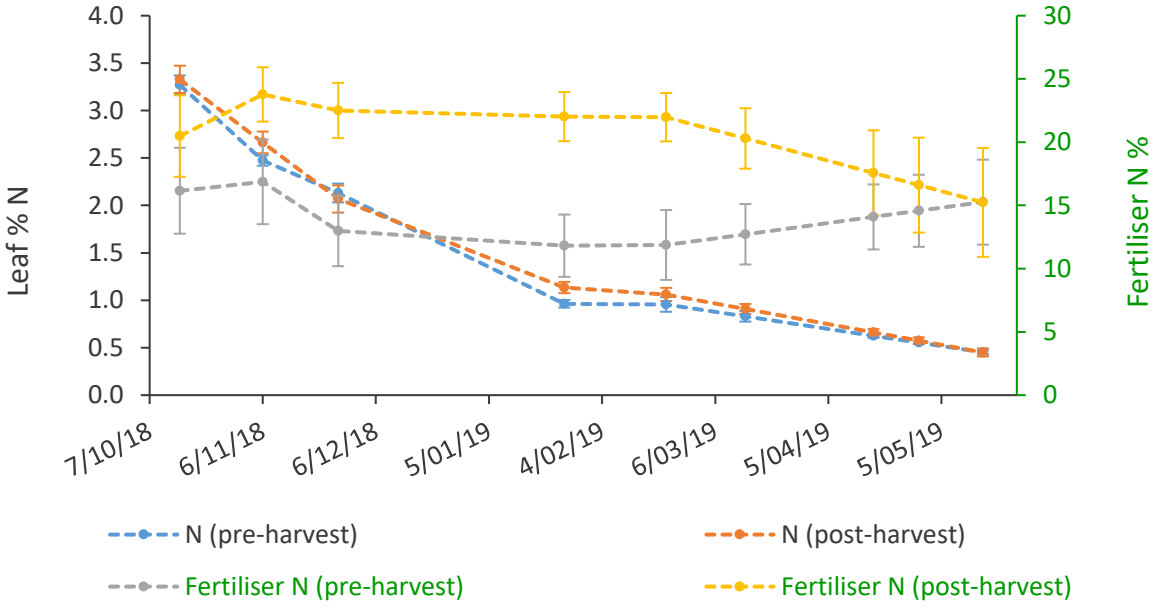

Figure 12 Percentage N and percentage N from fertiliser in leaves, from the spring of 2018 until the end of substantial leaf fall prior to winter excavation, for trees fertilised with 67.5 g N either pre- or post-harvest in the previous season (error bars represent ± SE, n = 4).

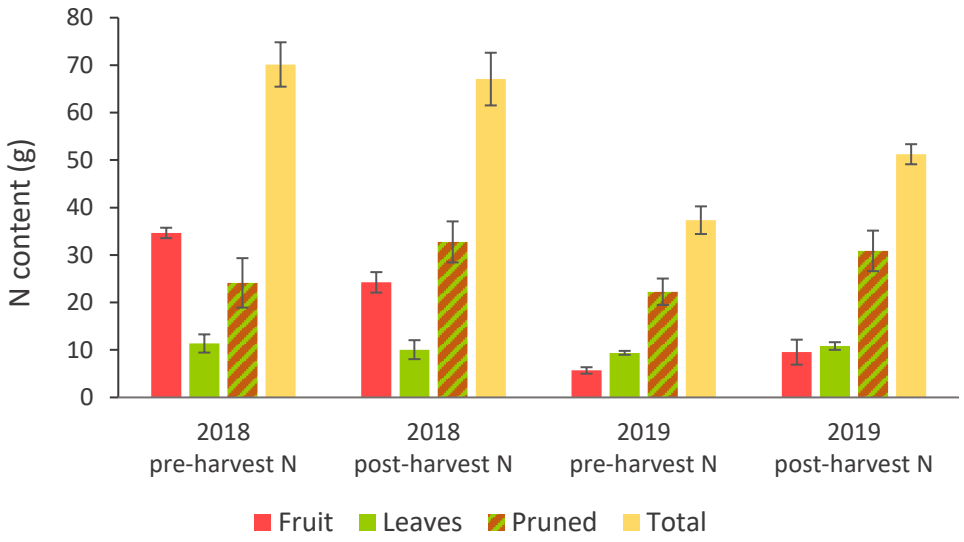

Figure 13 Nitrogen removed in fruit, leaves and pruned material, and totals of the three, in 2018 and 2019 for trees with 67.5 g N applied either pre- or post-harvest in the 2017-2018 season (error bars represent ± SE, n = 4).

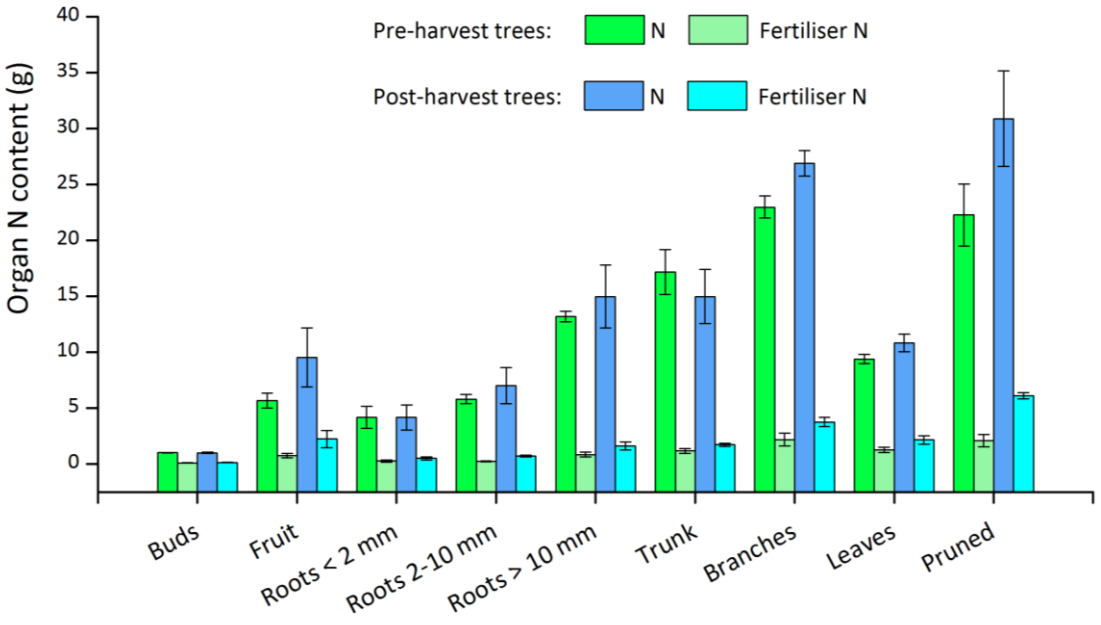

Figure 14 Total N and fertiliser N in tree organs during the 2018-2019 season (only) of trees with 67.5 g N applied either pre- or post-harvest in the previous season (error bars represent ± SE, n = 4).

Figure 15 shows the total N and the percentages of fertiliser N in each organ over the combined 2017-18 and 2018-19 seasons. Most apparent is the large quantity of N removed in pruned material, with that from the post-harvest fertilised trees being close to significantly greater (p = 0.0726) than from the pre-harvest fertilised trees. As previously discussed, a factor in this is that the pre-harvest fertilised trees lost more total N and fertiliser N in fruit than the post-harvest trees, fertiliser N very significantly (p = 0.0006) with respective combined losses of 8.09 and 2.51 g N/tree. The total N and the percentages of fertiliser N in each organ, as percentages of the tree totals, over the combined 2017-18 and 2018-19 seasons are shown in Figure 16.

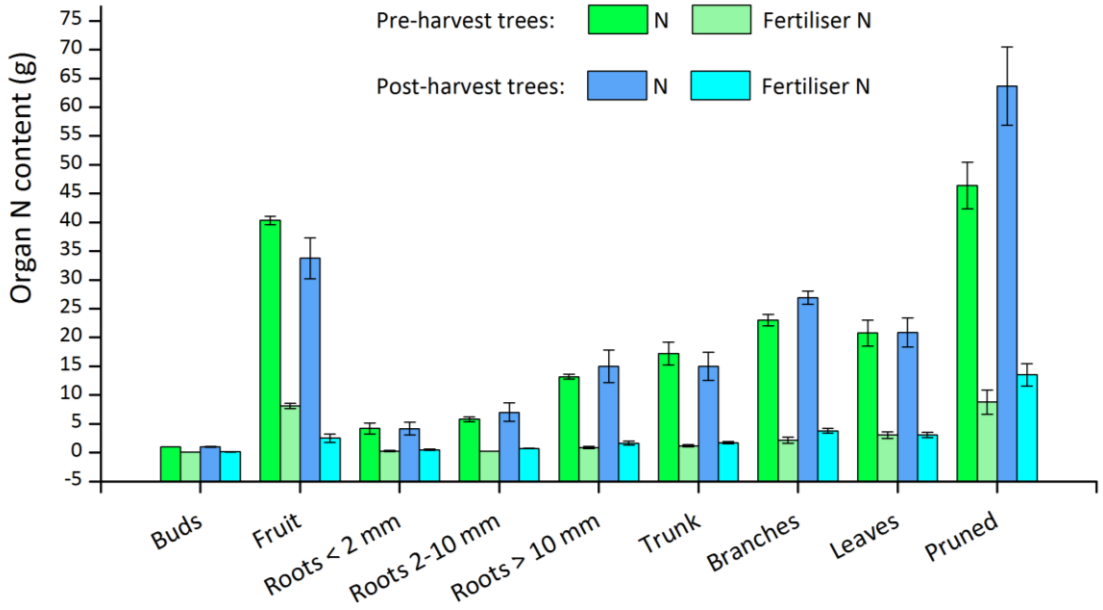

Figure 15 Total N and fertiliser N in tree organs encompassing both the 2017-18 and 2018-19 seasons of trees with 67.5 g N applied either pre- or post-harvest in the 2017-18 season (error bars represent ± SE, n = 4).

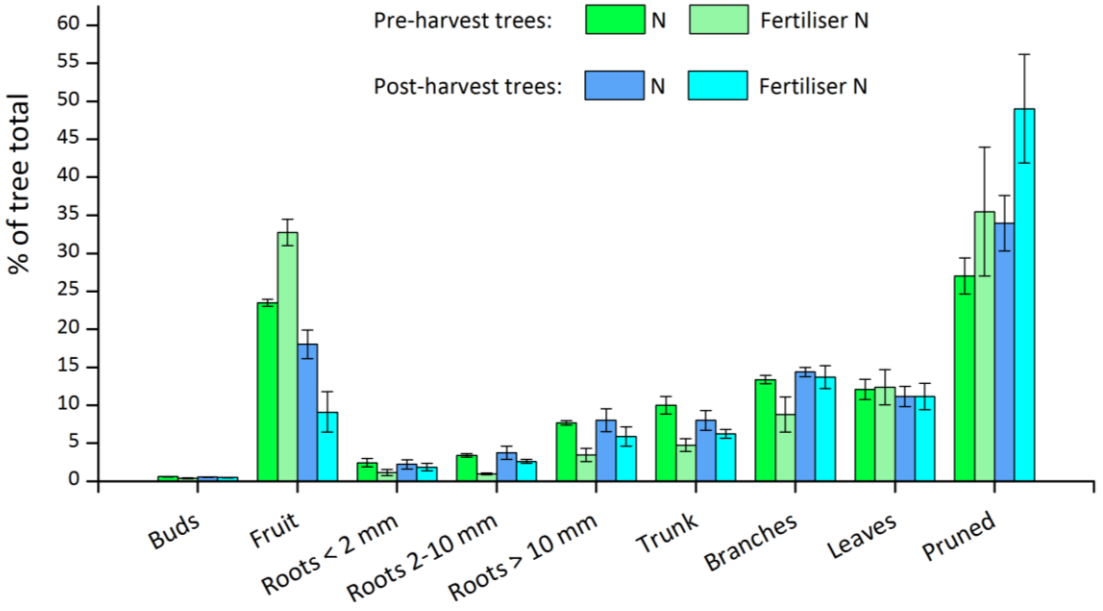

Figure 16 Percentages of contents of the tree in tree organs, of each of total N and fertiliser N, encompassing both the 2017-18 and 2018-19 seasons of trees with 67.5 g N applied either pre- or post-harvest in the 2017-18 season (error bars represent ± SE, n = 4).

Discussion

Many comparisons of applied treatments in the orchard trial examined in

Methodology, results and discussion by investigation

Part 1: Dynamics of N uptake and recycling through cherry trees over two seasonsof this report and to some extent, of the mineralisation trial in *Part 2*: Dynamics of litter material decomposition and soil mineralisation of N as a source of N, have not resulted in statistically significant differences being found between treatments. In more than a few instances, this was due to large variances between treatment replicates, despite the treatment mean values being quite distinct. Consequently, some uncertainty surrounds the results of these trials, compounded by their being of relatively short duration in relation to seasonal effects. Firmer conclusions would doubtless result by trials of the applied treatments run over longer timeframes. Nonetheless, a great deal of valuable information allows the drawing of some conclusions with considerable confidence, and the making of suggestions that might improve the efficient use of N in cherry crops while maintaining good tree health with optimum yields of quality fruit.

The measured uptakes of N fertiliser applied over the 2017-18 season, split 50:50 between pre- and post-harvest (Table 1), at the rates of 67.5 and 135 g N/tree (equivalent to 90 and 180 kg N/ha respectively) were measured as 37.9 and 29.6% respectively (Figure 1), representing 25.6 and 40.0 g N/tree. While not significantly different on account of substantial variances associated with the mean values, the lower uptake rate of fertiliser applied at the higher rate does suggest a lower NUE. The rate of N applied apparently did not affect its relative distribution amongst tree organs (Figure 2). However, as might be expected, the amounts of fertiliser N allocated to tree organs were for the most part substantially higher with the higher rate of N applied (Figure 3), with significance in differences again hampered by substantial variances. It is notable that the 5.14 g/tree of fertiliser N allocated to fruit of the 67.5 g N/tree split 50:50 treatment, with only half of that N applied pre-harvest, was not significantly less than the 7.34 g/tree of fertiliser N in fruit of that season of the trees fertilised with the full 67.5 g N/tree pre-harvest (*Timing of N application*), or the 7.23 g/tree of fertiliser N in fruit of the trees that had also received 67.5 g N to that time, as half of their 135 g N/tree split 50:50 treatment. This might suggest that if needed, pre-harvest fertiliser N is preferentially allocated to fruit production. If so, the 33.8 g of fertiliser N applied pre-harvest, did not appear to be an inadequate quantity compared with the two treatments that received 67.5 g N pre-harvest, as there were no significant differences in their fruit yields or N concentrations (Table 6), or quality parameters.

The 14.04 g/tree of fertiliser N removed in pruning from trees that received 135 g N/tree split 50:50, 35.1% of fertiliser N taken up, was clearly greater than the 8.32 g/tree of fertiliser N removed from the equivalent 67.5 g N split 50:50 trees, 32.5% of fertiliser N taken up by those trees (Figure 2 and Figure 3). The additional N did appear to drive some extra branch growth, with 660 g (dry weight) of material pruned compared to 585 g from the 67.5 g N trees, and leaf production, with 1017 g (dry weight of shed leaves) compared to 895 g, although the differences were not significant. Trees with 0 g N applied, or 67.5 or 135 g N as split 50:50 treatments, had relatively increasing quantities of N in each of their organs (Figure 5). In parallel, removed as fruit, pruned material, and shed leaves were 58.6, 76.5, and 85.3 g N/tree for the respective N treatments (Figure 6). An important effect of the higher rate of fertiliser application was its increased storage in roots and trunk where, without any substantial increase in the mass of these organs, fertiliser N was stored in a more concentrated

form, in many instances significantly so (Table 3), for potential remobilisation in the following spring. Of the total N stored at dormancy in the trees of 67.5 and 135 g of N applied as split 50:50 treatments, 10.3 g and 16.4 g respectively were fertiliser N, the difference being very significant (p < 0.0001). Nonetheless, the storage at dormancy of 68.3 g N in trees that received 135 g N as a split 50:50 treatment, compared with the 59.2 g N stored in trees that received 67.5 g N as a split 50:50 treatment (and 51.3 g N in trees that received no N fertiliser), does not appear to be a great gain for the additional 67.5 g N (90 kg N/ha) applied. It may be disadvantageous if applied at that rate long-term if the main effect on trees is to promote excessive vegetative growth – disadvantages of that being increased loss of N in shed leaves and pruned material and higher tree requirements for all nutrients and general maintenance. A very undesirable consequence of the application of the larger quantity of N fertiliser would undoubtedly be the potential loss of 126 kg N/ha of unutilised fertiliser N to the environment, compared with a lesser 56 kg N/ha from the lower rate of application.

On excavation at dormancy in 2018, trees that received 67.5 g of fertiliser N as a split 50:50 treatment in that season, with a fertiliser N uptake efficiency of 37.9%, had total N contents in storage organs (roots, trunk, and branches – with buds) of 59.2 g N. In comparison, trees that received 67.5 g N either pre- or post-harvest in the same season (Table 1) had respective totals of 64.3 and 69.0 g N upon excavation in the following season, neither being significantly different from the 2018 value, or each other. The respective fertiliser N uptake efficiencies, over two seasons, of the 2019 excavated trees were 36.6 and 40.8% respectively (Figure 8). Again, neither of these latter values were significantly different from each other or the 37.9% uptake efficiency of the 2018 excavated trees. When compared with the dried weight of storage organs, following excavation at 2018 dormancy, of trees that received 67.5 g of fertiliser N as a split 50:50 treatment, those of the trees that received 67.5 g N of fertiliser N in the same season, either as pre- or post-harvest treatments, were both found to be significantly greater (p < 0.05) following excavation in 2019. The respective dried weights being 18.6, 24.6, and 25.5 kg being indicative of tree growth over 12 months. Although the respective N contents of those organs, 59.2, 64.3, and 69.0 g N, did not vary significantly, the concentration of N (g N/g dry weight) in the storage organs of the 2019 excavated trees, were both significantly less (p < 0.05) than in the trees that received 67.5 g of fertiliser N as a split 50:50 treatment and were excavated 12 months earlier. To match the N concentration in the storage organs of those 2018 excavated trees, the storage organs of the pre- and post-harvest treated trees would have needed an extra 8.09 and 7.98 g N respectively stored at 2019 excavation. Those values would equate, at the N uptake efficiency of the 67.5 g N post-harvest treatment, 40.8%, being the best of the three treatments (Figure 8), an extra 19.8 and 19.6 g fertiliser N applied, respectively.

The leaf N content of the pre- and post-harvest treated trees on 21-02-19 (Figure 12), of 0.96 and 1.06% respectively, was in each case very significantly lower (p < 0.0001) than the respective 2.1 and 2.2% on 19-02-18 (Figure 11), suggesting that by that time in the second season the trees might have been becoming N-deficient, with not enough stored throughout dormancy to provide for a full second season's growth without additional fertiliser N. The lower leaf N of both treatments at this late stage in the second season also suggests that there could have been insufficient available N remaining in the soil to meet the trees' full demands, with some fertiliser N possibly remaining in the soil from the previous season, and other soil mineral N (Table 4), not being enough to make up for the depletion of previous soil mineral N. A contributing factor to the lower leaf N concentration was the much greater growth of leaves in the second season, with the pre- and post-harvest treated trees shedding 860 and 741 g (dry weight) of leaves respectively by dormancy in 2018, compared with 1369 and 1474 g by dormancy in 2019. Overall, the leaf N concentration in shed leaves in 2019 was very significantly lower than in 2018, for the pre-harvest fertilised trees (p = 0.0041) and the

post-harvest fertilised trees (p = 0.0004). However, the total N shed in the leaves did not differ significantly between years for either treatment. Any N deficiency did not appear to be a factor in the lower fruit yield of the second season (*Fruit*), this being an orchard-wide phenomenon of biennial bearing that included trees that had been fertilised in each season. Nor could any deficiency be termed severe, as leaf N content early in the second season (Figure 12, 6-11-2018) was comparable with that of the same trees at a similar time in the previous season (Figure 11, 8-11-2017), all being about 2.7%. It seems reasonable to conclude that in the second season the trees were in a mode of producing more vegetation and less fruit than in the previous one. The dry weight of pruned material did not vary significantly between seasons for either treatment. The N concentration of that material was only significantly less (p = 0.0234) in the second season for the trees fertilised post-harvest but, as with the shed leaves, the total N removed in pruned material did not differ significantly between years for either treatment.

It is difficult to recommend an annual quantity of $Ca(NO_3)_2$ to apply to the mature cherry trees used in the trial to sustain adequate tree growth and provide an optimum yield of high-quality fruit. Many factors can influence the trees' need for such N, from season to season, some related to climatic conditions and others to orchard management. Nonetheless, the trial results suggest that a quantity of close to the 67.5 g N applied per tree (90 kg N/ha) would be adequate, with a substantially greater quantity reducing efficiency of N uptake and thus adding to its loss to the environment. As discussed, trees that received this quantity of N in one season, applied either pre- or post-harvest, maintained sufficient N in the plant/soil system to not suffer any deficit in the total N shed in leaves or removed in pruned material in the following season, with no additional N applied. The only deficit being a lower concentration of N in the storage organs by dormancy of the second season. Nor was the yield or quality of fruit in the second season affected, and its average N concentration over the two seasons was comparable to that from most of the non-zero N treatments in the first season (Table 6). A parallel trial (Part 3, *Trial 1. Effect of N fertiliser application on fruit quality and bioactive properties of sweet cherry (Prunus avium L) cv. 'Lapins'.)*, held over three years using trees of the same age and heritage in an adjacent row to this trial's trees, found that fruit yield varied from year to year, but that quality did not deteriorate over time with the annual application of 67.5 g N /tree as a split 50:50 treatment. As observed above, the lower concentration of N in the storage organs by dormancy of the second season could be remedied by the application of an additional 19.7 g N fertiliser (average of 19.8 and 19.6 g, above), presumably during the second season. In addition to this, account would need to be taken of N removed from the trees in fruit. The average fruit yield over the two seasons from the trees fertilised pre- or post-harvest in the first season were 11.44 and 11. 59 kg/tree, respectively. Taken as 12 kg/tree – with most orchards managers regarding an annual yield of 12-14 kg as a good result – at a sufficient N concentration of 1.667 g N/kg fresh fruit (Table 6, with higher values deemed unnecessary), would equate to an additional 20.0 g N/tree required to replenish N removed. At the same uptake efficiency of 40.8% used to calculate the 19.7 g N needed to maintain storage organ N concentration, 20.0 g N/tree removed per year would require the application of 49.0 g N fertiliser as replenishment. Combining the two values (19.7 + 49.0) suggests 68.7 g N/tree (91 kg N/ha) applied as $Ca(NO_3)_2$ with good management, would be close to an adequate annual replenishment to maintain the trial trees in fine health and providing optimum yields of high-quality fruit.

It is common practice in many cherry orchards to apply a major part of annual N supply post-harvest. This appears to be influenced by beliefs that, 1) N applied pre-harvest, particularly close to it, can be detrimental to fruit quality, and 2) N removed in fruit needs to be replenished post-harvest to ensure adequate N storage through winter dormancy to initiate the following spring's growth. Whether any N applied pre-harvest could be detrimental to fruit quality seems questionable, but

certainly excess N, or application close to harvest, could be (refer Part 3, *Trial 1.* Effect of N fertiliser application on fruit quality and bioactive properties of sweet cherry (*Prunus avium* L) cv. 'Lapins'.). Certainly, the trial results appear to demonstrate that application of N only pre-harvest can result in a wasteful amount being lost in fruit, and that post-harvest application could be marginally more efficient for N uptake (Figure 8). Thus, applying most annual N post-harvest appears logical, but the balance of pre- and post-harvest application might vary from season to season, depending on yield and regional climatic factors, and would need to be judged by orchard management. Such a decision would be well informed by testing of fruitlet and fruit N concentrations, and that of N in plant tissue and soil, practices that are already common throughout the industry. It is known that soil N uptake does not commence until around 30 days after full bloom, so application of N to soil much before that time would be wasteful. In Tasmania, N uptake would likely begin to diminish between late February and mid-March, as soil temperatures and daylight hours declined. Consequently, post-harvest N application beyond that time would also seem inadvisable.

Within the resulting timeframe when N uptake is most efficient, its optimisation can clearly be maximised by applying it frequently in smaller doses, rather than less frequently in larger ones, and without excessive water. The primary reason being that trees will only utilise what they need at a given time. Any N excess to their needs risks being lost from the soil, to which the nitrate form of N is particularly prone, through leaching below the root zone (refer to *Leached nitrate* in Part 3 of this report) or lost as nitrous oxide (N_2O) gas. Both of these processes are encouraged by high soil water content, be that from irrigation or rainfall. Nitrate N is particularly mobile in soil and excess water draining below and away from the root zone can carry large quantities of this dissolved form of N with it. Similarly, excess fertigation water can carry it away from tree rows. Losses through leaching are known to be large (Cameron et al., 2013; Zhang, Tian, Zhang, & Li, 1996), and might account for the majority of applied N not taken up by the trial trees. As part this trial, it was found that over 2% of N applied pre-harvest was lost as N_2O (Quin et al., 2021), a very significant greenhouse gas responsible for 6% of anthropogenic global warming and a catalyst for depletion of stratospheric ozone (Ravishankara et al., 2009). Although this loss was primarily the consequence of a heavy rainfall event, excessive watering could produce substantial, undetected losses of applied nitrate N as N_2O. Consequently, real-time monitoring of soil water content, including that below the root zone, would be advisable to prevent application of excessive irrigation water. Avoiding excess drainage of water away from (to the inter-row) and below the root zone can help minimise leaching of soil nitrate. It is difficult to over-emphasise the importance of this, as the loss of nitrate through leaching could quite possibly account for a large portion of unutilised applied N in the trial. With the orchard manager being instructed to have irrigation water "running out the bottom of the rows", a slightly more conservative approach might aid in increasing N uptake efficiency to well above the values of around 40% found in this trial. To do so would help ensure that the application of 90 kg N/ha per annum, or even less, would prove to be adequate on an ongoing basis, a substantial reduction from the current annual application by management of typically, in "the low 100's" (kg N/ha).

Management of fertigated N application in small, regular doses is certainly constrained by the irrigation/fertigation infrastructure of each orchard. However, improvements in NUE to higher levels than those found should be possible, as has been achieved in other crops. One example being the production of maize (corn) in the United States , which saw the application of N fertiliser (per hectare) remain virtually unchanged from 1980 to 2000, while the efficiency of grain yield per kg of applied N increased from about 43% to 58% (Cassman et al., 2002). Regular soil testing was a large

contributor to this improvement, as would be necessary to improve NUE in cherry cropping systems. Some orchards leave long lengths of pruned stems within the tree rows. As observed in *Part 2*: Dynamics of litter material decomposition and soil mineralisation of N as a source of Nof this report, the breakdown of leaves for potential mineralisation of contained organic N is fairly rapid, contributing about 4 kg N/ha per annum in mineral form from leaves of trees with 67.5 g N applied, but of stems to release their considerable organic N content, breakdown is very slow. The recycling of organic N contained in leaves and stems through mineralisation is a potentially very important source of N for orchard trees (refer *Part 2*), as is that contained in suppressed weeds and mown inter-row herbage. The removal of all pruned material for composting, as already practiced in some orchards, is worthy of consideration to aid this process. At the least, much more substantial pulverisation of pruned stems, before they are re-applied to tree rows, would seem advisable to greatly speed up the recycling of the substantial quantities of organic N in such material.

Pursuing a suite of management practices as suggested might well result in improvements in NUE to over 50%, with benefits to return on investment and the environment. To determine changes in NUE, regular monitoring of N forms in soil, and N contents of fruit, leaves and pruned material would be necessary. Such testing would also act as a safeguard for orchard managers aiming to decrease their applications of N, which understandably would need to proceed with a degree of caution.

Part 2: Dynamics of litter material decomposition and soil mineralisation of N as a source of N

Background

Mineralisation of plant organic matter remaining on the surface of or incorporated into soil releases the nutrients in those compounds into the soil in soluble inorganic forms . These may then be easily available to plants, although recent research has found that organic forms, which may be complex (Kelley & Stevenson, 1995) can also be utilised by plants in some circumstances (Schimel & Bennett, 2004). As a first step, "Mineralization results in the production of ammonium (NH_4^+). Under favourable conditions, ammonium is further converted by microorganisms to nitrate. Build-up of nitrates in soil followed by heavy rains can move plant available nitrate below the root zone. Nitrate is a form of N that is highly mobile and easily moves with water." (Killpack & Buchholz, 1993). Such favourable conditions include soil with a C:N ratio of less than about 24:1 – a ratio higher than this resulting in immobilisation of added N to meet the requirements of soil microorganisms, while if the ratio is lower added N is in excess of those needs and is available for mineralisation (Reuter, Gensel, Elvert, & Zak, 2020; USDA, 2011).

In considering the cycling of N in a cherry production system, the mineralisation of shed leaves and pruned material into readily available forms of N is important as a potential source of recycled N that may be available for tree uptake. The aim of the trial was to determine potential rates of N mineralisation of leaves and pruned stems from the trees discussed in

Methodology, results and discussion by investigation

Part 1: Dynamics of N uptake and recycling through cherry trees over two seasons

of this report, to estimate what contribution that process may make to the annual N supply of those trees. Such knowledge is of considerable importance in determining the annual fertiliser N requirement of those trees, this in turn helping to maximise production and minimise N lost to the environment.

Methods

A trial was established on a north-easterly facing hillside at the Horticultural Research Centre (HRC), University of Tasmania, Hobart (42.9083 °S, 147.3242 °E). The climate for the region is oceanic, with mild, occasionally cold, rainy winters and cool, quite rainy summers. The site is enclosed with rabbit-proof fencing and bird netting at a height of approximately 3 m, preventing intrusion by unwanted species. The hillside is terraced and covered in weed matting to provide level surfaces free of undesirable plant growth.

Two-year-old 'Lapins' cherry trees on Colt rootstock, supplied by Reid Fruits Pty Ltd, were planted into 45 L woven planter bags (410 mm dia. × 370 mm high), using soil from Wandin Valley Farms, Rosegarland, Tasmania. The soil had a C:N ratio of 11.5:1, thus being suitable for mineralisation of added N. Planting was carried out at the Rosegarland orchard in August 2018 and, after delays in preparation of the HRC site, were transported there in mid-April 2019, having received no fertiliser in the intervening period. Soil was sampled in early October 2018 from a randomly selected three bags and analysed for total N, NO_3^--N, and ammonium-N (NH_4^+-N) (Table 7). On arrival at the HRC site the trees in bags were supported on galvanised steel mesh (50mm squares × 4 mm) placed on bricks, above large saucers (approx. 20 L) of 430 mm diameter intended to collect leachate for recycling into the bags. Trees were spaced, as evenly as was practically possible given site encumbrances, approximately 1.6 m apart on each terrace.

The trial was of three treatments × 6 replicates (Table 8), using highly ^{15}N-enriched cherry leaves and stems applied to the soil surface of the trees in planter bags, to quantify their mineralisation over a 12-month period. The trees were spread over sections of the five terraces of the site in a randomised design – a randomised complete block design being considered impractical due to the nature of the site, including different levels within each terrace and only partial availability of some terraces. The aspect of the site and situation of adjacent trees guaranteed that no trees received unequal access to sunlight throughout the day, regardless of season. Water was supplied during the growing season by an automated watering system through 2 drippers per bag (4 L h^{-1}), situated approximately 13 cm either side of and in line with the tree trunk. Watering duration was adjusted up to twice weekly, in accordance with climatic conditions, to maintain adequate soil moisture.

The ^{15}N-enriched leaves and stems, pruned in autumn 2018 from a cherry tree treated with 66 atom-% ^{15}N-$Ca(NO_3)_2$ (*Methods*, Part 1 of this report) were used for treatments. These had been stored, in a dry environment and unexposed to sunlight, until deployment in the trial at tree dormancy in mid-June 2019. Commensurate with the average air-dried weights of 2018-pruned leaves and stems per unit of orchard area of the trees discussed in the previous section of this report, either leaves or stems were applied to the soil surface of the planter bags at , evenly spread, and held in place below a single layer of synthetic insect mesh. The stems were cut into sections of approximately 50 mm, and categorised as either 'tip', 'mid', or 'butt', depending on the section of pruned branch from

which they came. The stem sample for each tree was made up to consist of 24, 32, and 44% respectively by weight of each of these categories, as this had been found to be the mean representation of the categories from six randomly selected pruned branches with leaves removed. The mesh was pinned to the soil close to the perimeter of the bags with thin wires (0.90 mm galvanised) fashioned for the task and inserted into the soil. The leaves, stems and mesh were left undisturbed for the full duration of the trial. Both leaves and stems were analysed by IRMS prior to deployment, for ^{14}N:^{15}N ratio and %N content (Central Science Laboratories (CSL), University of Tasmania, Hobart), these details included in Table 8.

The trial continued until 23-06-2020, approximately three weeks after the trees reached winter dormancy. Weeds were of little consequence throughout the trial, with glyphosate applied on a single occasion, in February 2020, for suppression. The trees were netted before leaf senescence in 2020 to capture all shed leaves, which were regularly emptied from the netting, dried, weighed and kept in paper bags, separately in accordance with date of collection, for later IRMS analysis with the other tree organs.

After removal of the insect mesh and remaining treatment material, three soil samples of 0-150 mm depth × 20 mm diameter were taken from each planter bag on 22-06-2020, from positions of approximately 150 mm from, and close to equally spaced (i.e., 120^{o} apart) around the tree trunks. Each set of samples was homogenised, air-dried, and sieved to < 2 mm, and ground into a fine powder using a ball mill (Retsch, Haan, Germany) in preparation for analysis. All trees were excavated over the following two days, care being taken to capture all roots as the soil was washed from them. Tree organs were separated into leaves (previously collected), fine roots (< 2 mm), larger roots, buds, and branches: as 'main' branch, including trunk, and remaining branches. Prior to dissection the main branch was measure for overall length, and thickness at three points: 100 mm above the graft, about 30 mm above the junction with the first secondary branch, and at a further 400 mm above that point. Three cross-sectional slivers, of 3-4 mm thickness, were cut at each of these points and retained in separate categories. All organs and sub-categories of branches were dried at 60 ^{o}C, until no further weight loss was observed, and dry weights of each category recorded.

Once dried, a branch sub-sample was created for each tree from the three categories of cross-sectional slivers by apportioning a weight of each category, as a fraction of that of the total sub-sample, according to the ratios of their cross-sectional areas calculated from their measured thicknesses at the time of cutting. These sub-samples and sub-samples of each dried tree organ, including the separate categories of leaves, were then ground into fine powders using a ball mill. Finally, for each tree a sample of fine powder was prepared for analysis, consisting of an homogenised mixture of all the ground components, each weighed into the mixture using a five-figure balance (Shimadzu, AUW220D), proportionally according to the fraction of the total tree dried weight that each dried organ category represented. These and prepared soil samples were analysed by IRMS for ^{14}N:^{15}N ratio and %N content (CSL, Hobart) and soil also analysed for NO_3^--N and NH_4^+-N (Plant Science, University of Tasmania) by the potassium chloride extraction method.

The ^{15}N-N content of each analysed sample was multiplied by the total dried weight of the combined tree organs (g) to quantify the ^{15}N-N content (g) of each excavated tree, including controls:

$$\text{Tree } ^{15}\text{N-N content (g)} = \text{sample (atom-\% } ^{15}\text{N} \times \%\text{N} \times 10^{-4}) \times \text{total organ dried weight (g)}$$

Similarly, the soil ^{15}N-N content was determined by using the estimated average dried weight of soil in a bag, on the assumption that the soil samples were a fair representation of the soil bag contents (refer to *Discussion* section):

$$\text{Soil } {}^{15}\text{N-N content (g)} = \text{sample (atom-\% } {}^{15}\text{N} \times \%\text{N} \times 10^{-4}) \times \text{bag soil dried weight (g)}$$

For each of the calculated tree and soil ^{15}N-N contents, that of the control treatment was deducted from that of each of the other treatments (leaves or stems) to give the increase in ^{15}N-N of tree or soil resulting from application of that treatment. These net ^{15}N-N contents of tree and soil were combined for each treatment and those values divided by the respective ^{15}N-N contents of the treatments to determine the percentage of ^{15}N-N in the tree and soil combined that came from that of each applied treatment.

All statistical analysis was in accordance with that in the *Statistical analysis* section of this report.

Results

The planter bag soil NH_4^+-N, NO_3^--N, and %N contents, measured in early October 2018, are shown in Table 7. These measurements were taken well in advance of treatment application in mid-June 2019, but with no fertiliser applied in the intervening period. Table 8 shows the atom-% ^{15}N enrichment, %N content and the air-dried weight per planter bag of the applied treatments, plus the ^{15}N-N contents of the applied treatments, calculated from the other parameters in the table using an equation of the same form of those immediately above in the *Methods* section.

Table 7 Planter bag NH_4^+-N, NO_3^--N, and %N in early October 2018, prior to treatment application (n = 3).

NH_4^+-N		NO_3^--N		N	
Mean	SE	Mean	SE	Mean	SE
µg N/g soil				%	
19.33	1.20	51.67	4.98	0.22	0.01

Table 8 Air-dried weight, %N, ^{15}N-enrichment level of treatments applied to soil of mineralisation trial trees, and the ^{15}N-N content of each applied treatment (n = 4).

Treatment applied	Air-dried weight (g)	%N		Atom-% ^{15}N		^{15}N-N (g)	
		Mean	SE	Mean	SE	Mean	SE
Control	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Leaves	34.6	1.986	0.037	16.564	2.851	0.113626	0.019674
Stems	42.0	0.741	0.011	9.649	0.797	0.030024	0.002663

The results of analysis of (combined) dried tree organ samples, shown in Table 9, revealed that only those trees with ^{15}N-enriched leaves applied to the soil had atom-% ^{15}N values above natural ^{15}N abundance . The means and standard errors for each treatment of the calculated ^{15}N-N values (from equation in *Methods* section, above) and %N are shown in Table 10, along with the differences between means. There were no significant differences between the control values of ^{15}N-N in the combined tree organs and those associated with either of the treatments of ^{15}N-enriched leaves or stems (Table 10). This was despite the mean values of atom-% ^{15}N associated with the leaves treatment (Table 9) being very significantly higher ($p < 0.0001$) than those of the control treatment. However, when combined with their respective %N and dry weight values to give ^{15}N-N (as in equation above), with the means of the control treatment being higher than those of the leaves treatment in each case, although not significantly, no significant differences in ^{15}N-N resulted. This result suggests that although substantial leaf N was mineralised and taken up by the trees, factors

associated with the slightly lower %N and considerable variance in tree dry weight values associated with that treatment have nullified any significance of the measurement.

For the stems treatment, none of the associated atom-% ^{15}N values in the trees (Table 9) were above natural abundance. With the ^{15}N-N values for that treatment also not being significantly different from those of the control treatment, the uptake of any N mineralised from the stems can be considered negligible. The %N in the trees from that treatment was just significantly less (p = 0.0480) than for the control treatment but, again with the tree dry weight values not being significantly different from those of the controls, this difference was apparently of little consequence.

Table 9 Measured atom-% ^{15}N, %N and total dried weight of combined organs of excavated trees from mineralisation trial, with calculated ^{15}N-N content of each tree (values of $\delta^{15}N_{air}$ and atom-% ^{15}N in bold font were adjudged to be above natural abundance).

TREE					
Treatment	Corrected $\delta^{15}N_{air}$	Atom-% ^{15}N corrected	%N	Total dry weight (g)	^{15}N-N (g)
Control	3.28	0.3684	0.72	720.1	0.0191
Control	3.74	0.3686	0.47	1030.8	0.0179
Leaves	***554.81***	**0.5612**	0.50	627.9	0.0176
Stems	7.17	0.3698	0.50	881.8	0.0163
Control	3.47	0.3685	0.57	770.7	0.0162
Control	3.84	0.3686	0.53	777.7	0.0152
Stems	11.36	0.3712	0.55	740.4	0.0151
Stems	7.22	0.3698	0.48	856.4	0.0152
Leaves	***312.61***	**0.4767**	0.48	1073.7	0.0246
Stems	11.45	0.3713	0.41	835.6	0.0127
Stems	7.45	0.3699	0.46	718.8	0.0122
Control	3.93	0.3686	0.58	667.3	0.0143
Leaves	***670.19***	**0.6015**	0.51	441.1	0.0135
Control	6.12	0.3694	0.62	637.9	0.0146
Leaves	***303.05***	**0.4733**	0.57	669.8	0.0181
Stems	14.22	0.3722	0.54	669.9	0.0135
Leaves	***593.07***	**0.5746**	0.56	502.4	0.0162
Leaves	***421.09***	**0.5146**	0.54	948.5	0.0264

Table 10 Mean values of calculated ^{15}N-N values and %N of combined tree organs and differences between means (n = 6).

TREE				
Treatment applied	^{15}N-N (g)		%N	
	Mean	SE	Mean	SE
Control	0.016202	0.000784	0.5817	0.0346
Leaves	0.019384	0.002040	0.5267	0.0145
Stems	0.014172	0.000655	0.4900	0.0213
Net values				
Leaves - Control	0.003182	0.002186	-0.0550	0.0375
Stems - Control	-0.002030	0.001022	-0.0917	0.0406

In calculation of soil ^{15}N-N content from soil samples taken the day before the trees were excavated, using the equation in the *Methods* section above, bag soil dry weight was calculated by multiplying the typical volume of soil in a bag by the soil bulk density, where the typical soil in a bag (with little variation) was of 38 cm diameter × 27 cm deep, of bulk density was 1.05 g cm^{-3}. Thus:

$$\text{Dry soil/bag} = \pi \times (38/2 \text{ cm})^2 \times 27 \text{ cm} \times 1.05 \text{ g cm}^{-3} = 32{,}152 \text{ g}$$

This weight of dry soil was used for all calculations of soil ^{15}N-N, that are shown in Table 11. Analysis of the soil samples again showed that only those trees with ^{15}N-enriched leaves applied to the soil had atom-% ^{15}N values above natural ^{15}N abundance (Table 11). However, unlike the comparison of ^{15}N-N values for combined tree organs, for soil the mean ^{15}N-N of the leaves treatment (Table 12) was very significantly greater ($p < 0.0001$) than that of the control treatment. Those of the control and stems treatments were not significantly different, as were none of the means of %N (Table 12), and NO_3^--N, or NH_4^+-N values (Table 13), between any of the three treatments.

Table 11 Measured atom-% ^{15}N, %N, ^{15}N-N, NO_3^--N and NH_4^+-N of planter bag soil of excavated trees from mineralisation trial, with calculated ^{15}N-N content of each tree (values of $\delta^{15}N_{air}$ and atom-% ^{15}N in bold font were adjudged to be above natural abundance).

SOIL						
Treatment	Corrected $\delta^{15}N_{air}$	Atom-% ^{15}N corrected	%N	^{15}N-N (g)	NO_3^--N (µg N/g soil)	NH_4^+-N (µg N/g soil)
Control	7.04	0.3697	0.18	0.21397	0.465	0.824
Control	7.42	0.3698	0.19	0.22594	0.541	0.911
Leaves	***399.58***	**0.5070**	0.18	0.29345	0.575	1.525
Stems	12.05	0.3715	0.17	0.20304	0.378	0.845
Control	7.70	0.3699	0.19	0.22600	0.555	1.309
Control	8.54	0.3702	0.20	0.23808	0.428	1.026
Stems	14.81	0.3724	0.17	0.20357	0.510	0.847
Stems	13.59	0.3720	0.18	0.21530	0.363	1.303
Leaves	***352.43***	**0.4906**	0.19	0.29968	0.399	1.266
Stems	12.40	0.3716	0.19	0.22700	0.469	0.886
Stems	12.35	0.3716	0.17	0.20310	0.571	0.729
Control	7.96	0.3700	0.17	0.20226	0.420	0.645
Leaves	***464.64***	**0.5298**	0.18	0.30659	0.585	1.327
Control	7.76	0.3700	0.19	0.22601	0.427	1.038
Leaves	***650.00***	**0.5944**	0.19	0.36314	0.424	1.095
Stems	13.28	0.3719	0.20	0.23915	0.262	1.023
Leaves	***369.55***	**0.4965**	0.19	0.30334	0.667	1.251
Leaves	***218.24***	**0.4437**	0.20	0.28529	0.339	0.880

Table 12 Mean values of calculated ^{15}N-N values and %N of planter bag soil and differences between means (n = 6).

SOIL				
Treatment applied	^{15}N-N (g)		%N	
	Mean	SE	Mean	SE
Control	0.222042	0.005034	0.1867	0.0042
Leaves	0.308581	0.011342	0.1883	0.0031
Stems	0.215193	0.006171	0.1800	0.0052
Net values				
Leaves - Control	0.086539	0.012409	0.0017	0.0052
Stems - Control	-0.006849	0.007964	-0.0067	0.0067

Table 13 Ammonium-N and nitrate-N contents of soil from grow bags, one day prior to tree excavation (n = 6).

Treatment applied	NH_4^+-N		NO_3^--N	
	Mean	SE	Mean	SE
	µg N/g soil			
Control	0.959	0.092	0.473	0.025
Leaves	1.224	0.089	0.498	0.053
Stems	0.939	0.082	0.426	0.046

The combined mean values of ^{15}N-N found in dried tree organs and soil (Table 10 and Table 12 respectively), resulting from treatment applications, are shown in Table 14. Compared with that in the control treatment, the combined mean ^{15}N-N in tree and soil was very significantly higher (p < 0.0001) for the leaves treatment but not significantly different for the stems treatment. The net values, with the background (control) ^{15}N-N content deducted, are also shown in Table 14 (Leaves – Control and Stems – Control). From these net values the percentage of ^{15}N-N in each applied treatment that was transferred into the combined tree organs and soil related to that treatment, was determined, i.e., net ^{15}N-N value in tree and soil combined divided by treatment ^{15}N-N content (values in Table 8).

Table 14 Combined ^{15}N-N content of tree and soil of each treatment, the net values (control deducted) related to each ^{15}N-applied treatment, and the percentage of ^{15}N-N in each applied treatment transferred into tree and soil combined (n = 6).

TREE and SOIL				
Treatment applied	^{15}N-N (g)			
	Mean	SE		
Control	0.238244	0.005095	Combined ^{15}N-N content from applied treatment (%)	
Leaves	0.327964	0.011524		
Stems	0.229365	0.006206	Mean	SE
Net values				
Leaves - Control	0.089721	0.012600	78.96	17.60
Stems - Control	-0.008879	0.008029	-29.57	26.87

From Table 14 it can be seen that the transfer of ^{15}N-N from stems applied to the soil surface, to that treatment's trees and soil, is negligible. The negative value of -29.57% is meaningless, given the size of its accompanying standard error. On the other hand, the transfer of 79% of ^{15}N-N from leaves applied to the soil surface into that treatment's trees and soil is substantial, with 96.5% of that found in the soil. To estimate how much of that 96.5% was in the form of mineral N, the NH_4^+-N values in Table 13 have been used. Even though those values of the leaves treatment were not quite significantly greater (at 95% confidence interval) than those of the control treatment (p = 0.0648), they served as the best guide available of the extent of mineralisation of the excess ^{15}N-N content (above control) in the soil of that treatment (Table 12). The net concentration of NH_4^+-N in the soil of the leaves treatment (Table 13: [1.224 - 0.959] µg N/g soil), multiplied by the dry weight of soil/planter bag (32,152 g), gave a value of 0.008535 g NH_4^+-N (SE = 0.004113, n = 6). This value is 9.86% (SE = 4.96%, n = 6) of the excess ^{15}N-N content in the soil of the leaves treatment, and an approximate guide to the extent of N mineralisation of orchard leaves over a 12-month period. Of the trees discussed in

Methodology, results and discussion by investigation

Part 1: Dynamics of N uptake and recycling through cherry trees over two seasons

of this report – that received fertiliser N: treatments A, B, C, and D in Table 1 – and the control trees that received none, the N content of leaves shed and pruned in the 2017-18 season, when fertiliser N was applied, and the leaf N which might be annually mineralised, is shown in Table 15.

Table 15 For each treatment: total N in pruned and shed leaves, % of total N in pruned leaves, mineralised leaf N per tree and per hectare.

Treatment and application timing	Fertiliser N (g N/tree)	Total N in all leaves (g)	N from pruned leaves (%)	Mineralised leaf N per tree (g)	Mineralised leaf N per hectare (kg)
Control	0	23.5	55.5	2.32	3.0
A. Pre-harvest	67.5	26.8	57.7	2.65	3.5
B. Post-harvest	67.5	32.9	69.4	3.24	4.3
C. Split 50:50	67.5	34.9	67.8	3.44	4.6
D. Split 50:50	135	39.0	67.4	3.85	5.1

Discussion

Mineralisation

Considering the different nature of the materials it was unsurprising that there was clear evidence of breakdown of leaves to release N, but not of stems. Without processing into smaller particles, rather than the approximately 50 mm sections that were used in the trial – themselves much smaller than the pruned branches left on many orchard floors – the breakdown of stems sufficient to release N for potential mineralisation and recycling would be expected to occur over a considerably longer timeframe than for leaves.

It is acknowledged that the calculation of N mineralised from leaves was based on a not quite significant measure of excess NH_4^+-N in the soil of that treatment. Nonetheless, the resulting calculated data are considered reasonable estimations of mineralised leaf N from the trees examined over a 12-month period (Table 15), particularly in light of additional factors:

- The uptake of mineralised N into the trees of the leaves treatment, though of minor importance, was not included in the calculations of N mineralised.
- When establishing the trial, the intention was to empty water drained from the planter bags into the saucers beneath them (*Methods*) back into the planter bags, so as to not lose any N that might have come from the applied treatments. Unfortunately, this proved to be impractical as, commencing with 100 mm of rain in August 2019, elevated soil water content regularly made addition of drained water impossible, with saucers overflowing as a result. Consequently, with NO_3^--N particularly prone to leaching, some treatment N that had been nitrified from NH_4^+-N might have lost from the system. Other soil NO_3^--N, not from the applied treatments, might also have been lost by the same mechanism. The low NO_3^--N concentrations in all trial soils at the time of tree excavation (Table 13) were perhaps

indicative of leaching having occurred, although the much lower concentrations of both NO_3^--N and NH_4^+-N at this time than shortly after planting (Table 7) are indicative of the trees' demands for mineral N during the intervening period.

Consideration of all factors suggest that the calculated mineralised leaf N values are likely reasonable and could even be underestimates. Certainly, as only about 10% of the excess ^{15}N-N from the applied leaves that was found in the soil was determined to have been mineralised, the remainder must be assumed to remain as forms of organic N. This is of considerable importance, as much of this remainder might be mineralised over a longer timeframe under favourable conditions. There are tests available for 'potentially mineralisable N' (PMN) in soil, but "this potential would need to be scaled back using seasonal soil water and temperature conditions in a modelling framework to allow calibration of PMN" (Moody, 2018). This was beyond the scope of this trial. Under some circumstances, NH_4^+-N can be lost to the environment through volatilisation during the process of mineralisation, but only if amino acid–N supply (due to protein depolymerization) exceeds microbial N demand (Reuter et al., 2020). Quantification of any such losses that might have occurred during the mineralisation of leaves was also beyond the scope of this trial.

Part 3: Influence of N source, rate and timing on fruit quality, nutrition and yield of sweet cherry

Trial 1. Effect of N fertiliser application on fruit quality and bioactive properties of sweet cherry (*Prunus avium* L) cv. 'Lapins'.

Background

Deciduous fruit trees accumulate and store nutrients at the end of the growing season for remobilisation in the following spring (Loescher, Mccamant, & Keller, 1990). This resource remobilisation is critical for growth of flowers, fruit, leaves and shoots, yet little is known about seasonal nutrient budgets and the storage and remobilisation of nutrients (Frak, Le Roux, Millard, Guillaume, & Wendler, 2006). This is particularly true in a region such as southern Australia where limited research into the seasonal, soil and cultivar implications on nutrient requirements of sweet cherry has been completed. However, principles gleaned from research in other regions can be used to guide nutrient management and identify region specific research questions.

In highly studied macro-nutrients such as N, it is well recognised that increasing the rate of N application can increase vegetative growth and yield but adversely affect fruit quality by decreasing fruit colour and firmness (Fallahi, Righetti, & Proebsting, 1993; GH Neilsen, Neilsen, Ferree, & Warrington, 2003; Oberly & Boynton, 1966). James (2011) suggested that applying too much before harvest can cause uneven ripping, delay ripening and reduce fruit shelf-life. In addition, studies have shown that the efficacy of N application in orchards is related to irrigation practice as excess water can leach N below the root zone (D. Neilsen & Neilsen, 2002) while soil-water stress and may reduce the tree's capacity for nutrient uptake. Therefore, the regulation of N and water is a crucial management consideration for commercial orchard production. The effectiveness of matching nutrient supply with tree demand requires a sophisticated understanding of seasonal cherry tree N recycling to maximize the advantages inherent in being able to apply N and water simultaneously. Fertigation and foliar nutrient application are important tools in the management of cherry nutrition and provide a more precise solution to meeting tree nutrient demand.

Precision farming through fertigation can facilitate efficient utilization of resources and improve returns per unit area and time to growers. Fertigation delivers both water and essential nutrients such as N directly to the active root zone of growing crops through micro irrigation systems, thereby minimising water and nutrient loss and improving productivity (Klein, Levin, Bar-Yosef, Assaf, & Berkovitz, 1989). Whilst fertigation is commonly practised by cherry growers in Australia, research and management guidelines for optimal supply of tree nutrient and water requirements are limited.

The trial described below aims to compare conventional N fertilisers with readily available biological alternatives on the fruit quality outcomes in a commercial sweet cherry orchard. This will guide the development of management strategies for increasing the quantity and quality of cherry yields whilst effectively mitigating loss of N to the environment.

Methods

Study site.

A trial was conducted over three consecutive seasons (2017-2020) in a commercial sweet cherry orchard at Rosegarland in southern Tasmania (42.71°S, 146.94°E, 130 m above sea level). Trees were cv. 'Lapins' on Colt rootstock, planted in 2012 and trained to a Kym Green Bush system (Green,

2005) with intra-row spacing of 1.7 m and inter-row spacing of 4.5 m (1300 trees/ha) and a weed-free strip along the tree line. The trial was established along two gently sloping rows with south-west to north-east orientation. Climate and soil characteristics are described in detail in (Quin et al., 2021). Whilst commercial management continued throughout the duration of the trial, N was excluded from the management program and applied via a modified drip fertigation system.

Experimental design.

Trees were selected during late winter in August 2017 based on uniformity of appearance. Trial design was a completely randomised design with eight treatments and four replicates per treatment. Individual plots consisted of three trees and the centre tree of each plot was used for sampling (sample tree), with the trees on either side functioning as buffer trees. Treatments were allocated at random to each plot.

Treatments and fertiliser application

The trial was established to assess the performance of different rates of N provided by calcium nitrate, alternative N sources. Details of each treatment are provided in **Error! Reference source not found.**6. In agreement with current practice, the application of an equivalent of 90 kg N / ha / season was defined as full rate N treatment. To test the hypothesis that a reduction of the application rate of nitrogenous fertilisers does not negatively impact fruit quality, a half rate (45 kg N / ha / season) was chosen for all other treatments. Alternative N sources included an organic liquid fertiliser ("Organic N"), feedlot waste (~ 2.5% N) and a monthly application (November – February) of the microbial inoculant "Soil & Seed" via drip fertigation. Before trial commencement, separate drip fertigation lines were set up for each treatment. Pre-harvest fertigation commenced in November, approximately four weeks after full bloom and consisted of five weekly applications (fertigated treatments) or an annual one-off surface application (feedlot waste treatments). Post-harvest application commenced one week after commercial harvest and consisted of five weekly applications (fertigated treatments). A zero N control (no N application) and a grower's control (usual management practice, no alteration of N regime) functioned as comparison.

Table 16 Fertiliser and orchard floor management strategies.

Treatments	Application method	Annual N (kg/ha)	Pre-harvest N (kg/ha)	Post-harvest N (kg/ha)	Soil&Seed®
1 Zero N control	fertigation	0	0	0	-
2 Calcium nitrate 90	fertigation	90	45	45	-
3 Calcium nitrate 45	fertigation	45	22.5	22.5	-
4 Calcium nitrate 45+ Soil&Seed®	fertigation	45	22.5	22.5	monthly
5 "Organic N®" 45	fertigation	45	22.5	22.5	-
6 Feedlot waste 45	surface application	45	45	-	-
7 Feedlot waste 45 + Soil&Seed®	surface application	45	45	-	monthly
8 Grower's control	fertigation and foliar	180*	100*	80*	-

* values for grower's control are approximate due to seasonal adjustments by the orchard management.

Sampling and assessments

Soil

Soil samples were collected four times in each season: immediately prior to the first pre-harvest fertiliser application (November), one week after the last pre-harvest application (December), at harvest (January), and one week after the last post-harvest fertiliser application (February). Three cores were taken around each sample tree with a push corer to a depth of 10 cm, combined, air-dried, homogenised, sieved to < 2 mm, and ground with a Retsch MM 200 ball mill prior to total N analysis.

Leaves

Leaf samples (10 mature leaves per timing) were taken at regular intervals of two to four weeks from November to April each season. The chlorophyll content was estimated in the quarter of each leaf closest to the petiole using a SPAD-502 Plus Chlorophyll Meter (Konica Minolta, Japan), and the results expressed as the mean of the measurements of ten leaves. Leaves were then prepared for total N analysis for the same key dates as above. Leaves were placed into paper bags and weighed immediately prior to drying at 60 °C and again after 48 h to determine the dry weight. Dried samples were ground to a fine powder with a Retsch MM 200 ball mill prior to total N analysis.

Trunk girth, limb girth, extension growth

Trunk girth (cm) was measured 10 cm above the graft union on the centre tree in each plot in August of each season. Growth is represented as % increase from the initial measurement prior to trial commencement. Two representative limbs on opposing sites of each tree were selected and tagged prior to the second season. Girth (cm) was measured at the base of each limb and limb growth is represented as % growth from the initial measurement. The length (cm) of each extension growth segment was measured in February of each season, immediately before extension growth was routinely pruned with cutter bars.

Fruit set

In season 2 and 3 all flowers were counted on the two selected limbs at full bloom (mid-October) and all fruit was counted one week before commercial harvest (late December). Fruit set was calculated using the following formula:

$$Fruit\ set\ (\%) = 100 * \frac{number\ fruit}{number\ flowers}$$

Harvest and sub-sampling

Fruit from each sample tree was harvested by hand in line with commercial maturity (9 January 2018, 31 December 2018, 30 December 2019) and standard commercial practice. Harvest took place in the morning and fruit was stored in the shade before transport to the laboratory. The cherries were kept refrigerated until grading within 24 h of harvest. Fruit was weighed and graded into two categories: blemish-free cherries with stems attached of > 22 mm diameter (“A-grade”) and “other”. The weight of each category was recorded to calculate pack out (% of A-grade fruit). Sub-samples of 25 representative A-grade fruit were randomly selected for quality assessment at harvest. A second set of 25 fruit was stored in modified atmosphere (LifeSpan) bags at 1-2°C for 30 days and subjected to fruit quality assessment to determine the changes in quality parameters during storage. Weight loss (%) was determined by weighing the sample before and after storage and calculating the difference between initial and final weights. Additional samples of 25 fruit were dried to determine fruit dry matter content and prepared for nutrient analysis to obtain total N content.

Assessment of quality and bioactive properties

All sub-samples were handled identically. Fruit was removed from the fridge and laid out in trays to minimise damage caused by handling of the fruit. When the fruit had reached ambient laboratory temperature, quality assessments were conducted with quality parameters always measured in the same order. Skin colour was determined using a colour chart (Australian Cherry Colour Guide, Cherry Growers Australia) with ratings from 1 (pale red) to 6 (dark red) and a Chroma meter CR-400 (Konica Minolta, Japan) measuring *L** (lightness), *a** (redness) and *b** (yellowness). The measurement was taken in the middle of the fruit opposite the suture side, output in the CIE L*a*b* color space (CIE, 1976) and the values of L*, a* and b* then converted from Cartesian to cylindrical co-ordinates (Arfken, Weber, & Harris, 2013), to yield parameters in the CIE L*C*h* colour space of hue (*h*°) and chroma (*C**), using the formulae:

$$h^{\circ} = \arctan(a^{*}b^{*-1})$$

$$C^{*} = \sqrt{a^{*2} + b^{*2}}$$

This colour space is used as "its system correlates well with how the human eye perceives color" (Konica Minolta, 2020).

The weight (g) of individual fruit with stem attached was determined with an electronic balance (Mettler Toledo, Switzerland). Fruit size (mm) was determined by measuring the diameter at the widest axis of each fruit using digital Vernier callipers (DigiMax, Wiha-41101, Wiha Switzerland). Fruit compression firmness (g/mm) was determined with a FirmTech 2 fruit firmness tester (BioWorks Inc., USA). A fruit texture analyser (Güss, model S-20, South Africa) was employed to record flesh firmness and skin puncture force with a 2 mm diameter probe. The skin of one cheek of the fruit was removed immediately prior to flesh firmness measurement. Skin puncture force was measured on the opposite cheek of the same cherry. Stem retention force (g) was measured using a stand mounted Mark-10 Series 5 force gauge (Mark-10, USA). Titratable acidity (TA) of the juice was determined with a Mettler Toledo G20 Compact Titrator equipped with an automatic 'Rondolino' titration stand. Samples of 10 mL juice were diluted with 50 mL purified water and titrated against 0.1M NaOH. The results were expressed as malic acid content in g/L.

Total N analysis

According to sample matrix and expected N content, between 1.0 and 10 mg of sample were weighed in tin capsules and analysis for total N was performed using a Thermo Finnigan EA 1112 Series Flash Elemental Analyser, with results expressed as % dry weight.

Statistical analysis

Results were analysed with IBM SPSS Statistics Version 26. A Shapiro-Wilk test was used to assess normality and all variables were normally distributed. Descriptive and one-way analysis of variance (ANOVA) with Duncan's/Tukey's post hoc multiple comparisons was used to determine significant differences between treatments. The significance level for all analyses was set at $p \leq 0.05$.

Results and Discussion

Harvest of season 1 (2017-2018) took place one week later compared to the following seasons, which is indicated by overall darker and softer fruit and reduced stem removal force. No significant treatment effects were detected in season 1 which is likely due to the deciduous nature of cherry trees and carryover effects from orchard management practices before trial commencement. This leads to the conclusion that, dependent on orchard and management history, the establishment of a new N fertiliser regime may not result in immediate effects on fruit quality.

Season 2 (2019-2020) was characterised by reduced yields. This phenomenon affected the block as a whole and was likely due to a biennial bearing effect from overcropping in season 1. This low crop load of high-quality fruit (pack-out of 68 ± 9 %) was considered an advantage for the detection of treatment effects.

To allow comparability and detect seasonal effects, harvest in season 3 (2019-2020) took place the same day as in season 2, in alignment with the grower's decision. Overall quality was lower in season 3 as indicated by a low pack-out (38 ± 10 %), mainly due to blemishes and cracks, which may have been caused by a combination of specific weather conditions. The assessment of "A-grade" cherries revealed that season 3 fruit was smaller, lighter in colour and sweeter than season 2 fruit but showed comparable firmness and higher stem retention.

The following sections will provide a detailed description of the effect of different N management practices on fruit N contents and selected fruit quality outcomes.

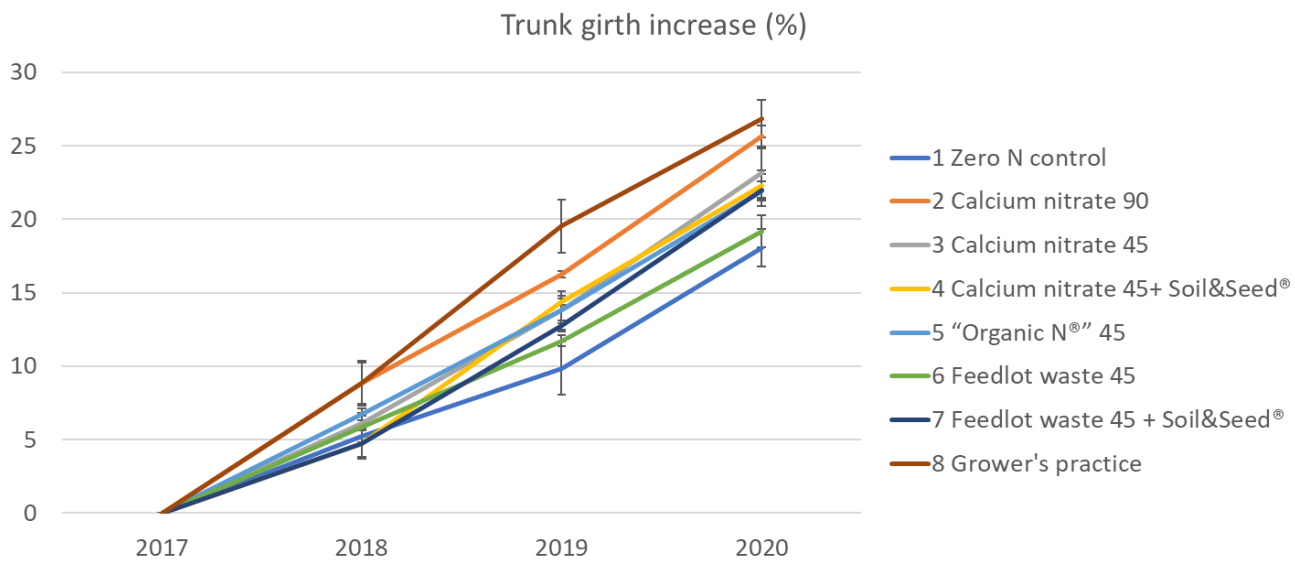

Figure 17 Trunk girth increase as a percentage from the commencement of the trial in 2017 through to the conclusion in 2020 under eight nitrogen treatments

Increase in trunk girth from 2017 through to 2020 reflected the amount of N applied Figure 17. The highest rate applied from the grower's practice and Calcium nitrate at 90 kg N ha having significantly higher trunk girth increase than the other treatments.

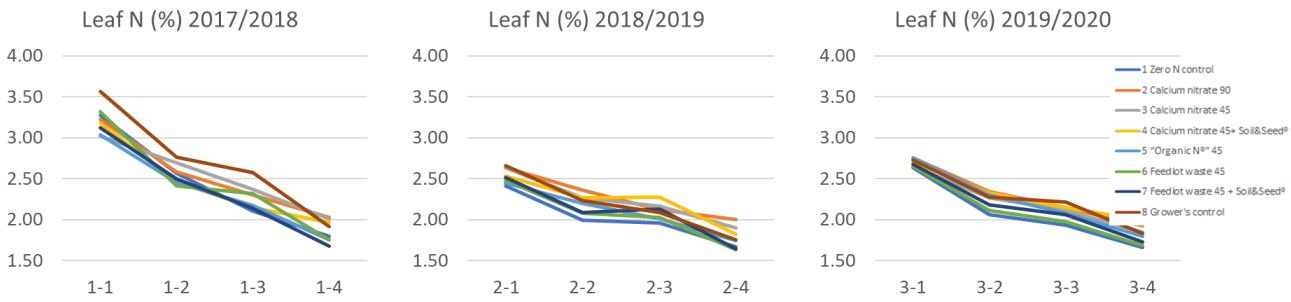

Figure 18 Leaf N content (% in dried sample) over three seasons at key dates; S1 – season 1 (2017-2018), S2 – season (2018-2019), S3 – season 3 (2019-2020); 1 – immediately prior to the first pre-harvest fertiliser application, 2– one week after the last pre-harvest application, 3 – at harvest and 4- post – one week after the last post-harvest fertiliser application under eight nitrogen treatments.

Leaf N was highest at the beginning of each season (*Figure 18*). The highest initial contents were measured in season 1, i.e. before commencement of the trial. At the beginning of the second and third seasons, leaf N content was around 2.5%, regardless of previously applied N treatment. At this stage, N is provided by remobilisation from internal N storage reserves (Grassi, Millard, Gioacchini, & Tagliavini, 2003). Fertiliser application commenced when uptake of soil-applied N via roots was likely to occur, i.e. in early November. Leaf N contents declined throughout each season. The steepest decline was observed in the Zero N control and feedlot waste treatments. The decline of leaf N in seasons 3 was less steep with increasing N application rates, confirming a trend observed in season 2. At harvest, leaf N content was around 2%, with no significant treatment effects.

For fruit N content, with calcium nitrate as N source (conventional), increased application of calcium nitrate resulted in increased fruit N contents, with the season 2 full rate N application (90 kg N / ha, fruit N = 1.36 %) being significantly higher than any other treatments in that season. The relationship is linear for applications of 0, 45 and 90 kg N / ha with R^2 of 0.978 and 0.970 for seasons 2 and 3, respectively. Increased fruit N contents with increased N application and a linear relationship have previously been reported (Kadir, 2018; G. Neilsen, Kappel, & Neilsen, 2004, 2007).

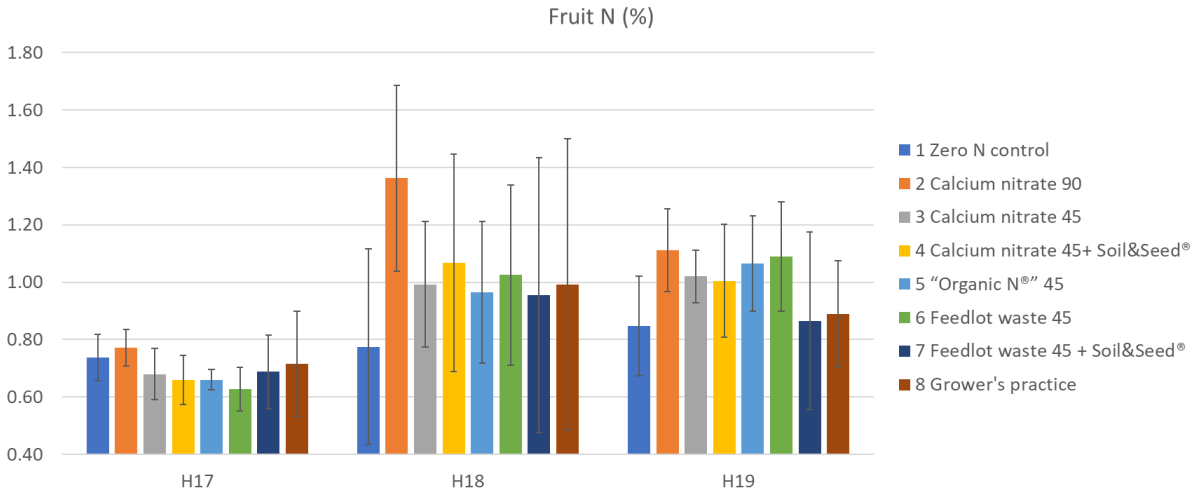

Figure 19 Fruit N (% of the dried edible portion) under conventional and alternative nitrogen applications for harvest of 2017, 2018 and 2019 seasons. Error bars represent ± 1.96 × standard error, n = 4.

There were no significant differences in the fruit N contents between alternative N sources at an annual rate of 45 kg / ha and calcium nitrate applied at the same rate. Fruit N contents were in the range of 0.87-1.09 %. This indicates that fruit N content is independent of the N source. Interestingly, unaltered management practice (Grower's control) which received the highest N application (ca. 180 kg N / ha) did not result in the highest fruit N contents, but contents equivalent to or lower than a half-rate N application. This indicates that a considerable amount of N is either used for vegetative growth or lost to the environment. Personal communication with the growers confirms that a considerable amount of N is applied very early in the season. Early-season, i.e. during the first 6–8 weeks after bud burst (mid-September), tree development is characterised by a remobilisation of internal N storage reserves (Grassi et al., 2003). In that time, root uptake of soil-applied N is limited, and its onset depends on internal N stores. Fertiliser applied too early, may be prone to being lost to the environment due to leaching or as nitrous oxide emission. This highlights the importance of adjusting the timing of fertiliser application to tree physiological processes.

Fruit size and weight in season 3 were significantly lower compared to season 2. No treatment effects on fruit size and weight were observed in season 2. Figure 20 summarizes fruit size outcomes for both seasons. In season 3, a reduced rate of calcium nitrate resulted in increased fruit weight and size compared to a full rate N application and the grower's control. This is in alignment with work conducted with 'Lapins' on 'GiSelA 5' in Canada, reporting the highest fruit size at low annual N applications (63 kg / ha) compared to high application rates (G. Neilsen et al., 2004, 2007).

For the parameters of fruit size and weight, feedlot waste performed best among the alternative treatments. Interestingly, the addition of Soil&Seed® resulted in smaller fruit, regardless of whether it was applied in combination with calcium nitrate or feedlot waste.

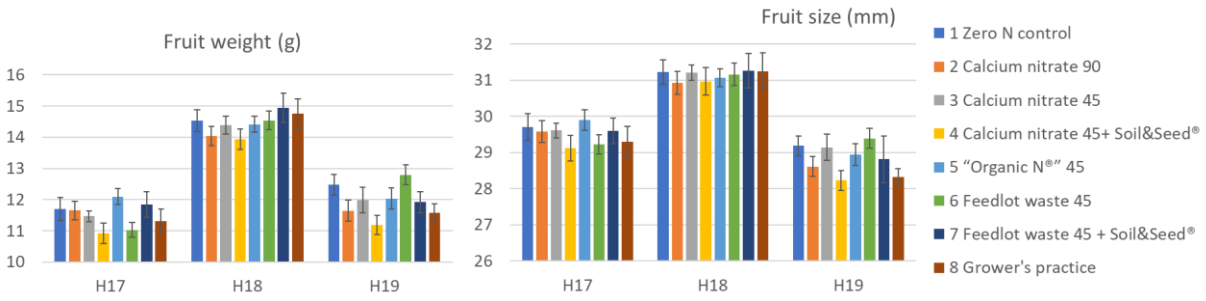

Figure 20 Fruit weight (left) and fruit size (right) under conventional and alternative nitrogen applications. Error bars represent ± 1.96 × standard error, n = 100.

Fruit colour is a main factor for setting the harvest date. Cherries with a minimum of "3" on a colour chart with ratings from "1" (pale red) to "6" (dark red) on an industry standard colour chart are considered ready for picking. Harvested on the same day as in the previous season, season 3 cherries appeared lighter in colour, indicated by significantly lower chroma scores. This was also confirmed by parameters measured with a colourimeter such as chroma (Figure *21*). It was evident in both seasons that fruit grown with no additional N (zero control) showed more intense, darker colouration than fruit grown under the higher N rates. Interestingly, the feedlot manure treatment showed a similar effect. Studies conducted in other species indicate that N depletion results in an accumulation of anthocyanins, the red and purple pigments responsible for colouration in cherries.

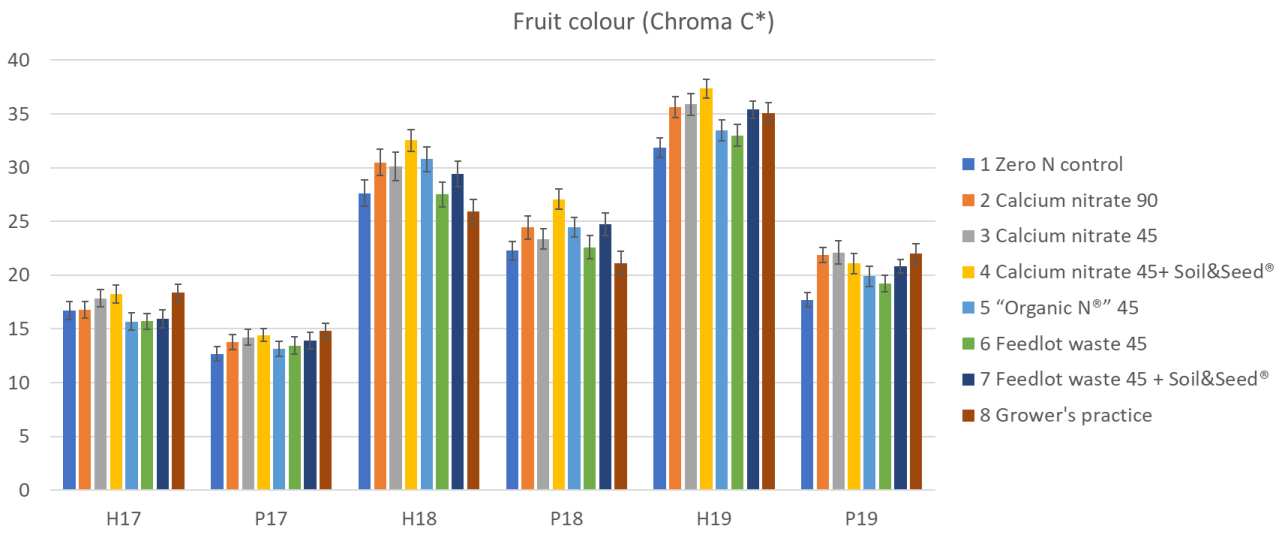

Figure 21 Fruit colour under conventional and alternative nitrogen applications in the 2017, 18 and 19 seasons. H refers to measurements recorded at Harvest and P refers to measurements recorded post-harvest after 30 days in storage. Error bars represent ± 1.96 × standard error, n = 100.

N has been associated with effects on fruit firmness, however, there are contradicting reports for sweet cherry. In a previous study, fruit firmness was affected by different N applications, with fruit from the highest N treatment showing decreased firmness compared to fruit grown under lower N treatment (N. D. Swarts, Mertes, & Close, 2017), whereas (G. Neilsen et al., 2007) did not observe effects on fruit firmness. In the current study, the effect of N application rate and source on firmness parameters were inconclusive (Figure 22). The positive influence of Soil&Seed® application on fruit firmness as indicated in season 2 was not confirmed in season 3.

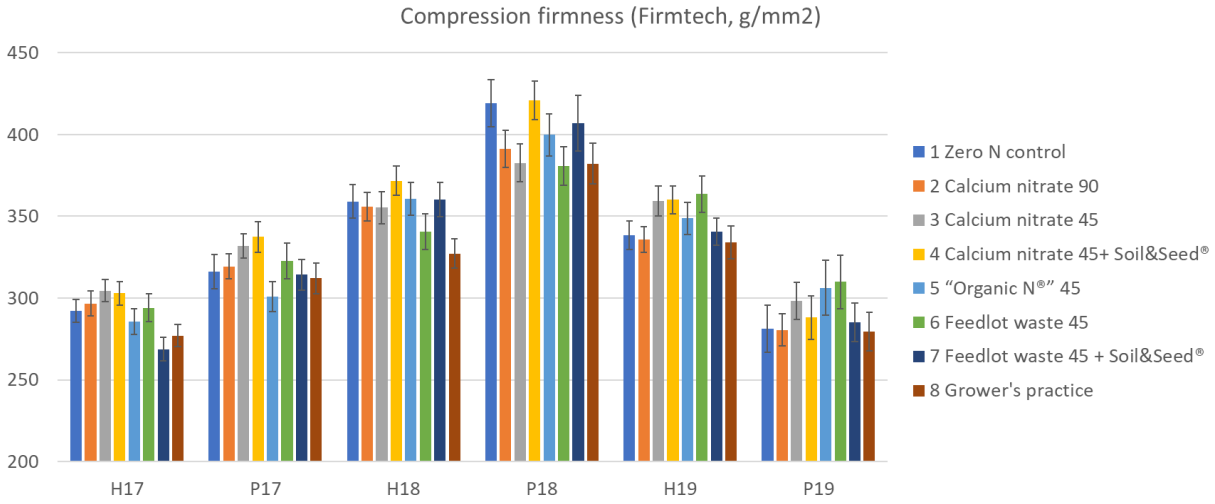

Figure 22 Fruit firmness under conventional and alternative nitrogen applications in the 2017, 18 and 19 seasons. H refers to measurements recorded at Harvest and P refers to measurements recorded post-harvest after 30 days in storage. Error bars represent ± 1.96 × standard error, n = 100.

Although there were differences between seasons for other fruit quality parameters such as packout, fruit dry weight, weight loss in storage, Total soluble solids (sugar content), no direct effects of N treatments were observed for these quality factors.

Alternative biological based fertiliser treatments at the N rate applied performed (45 kg N/ha) in general, comparably to the conventional calcium nitrate-based fertiliser applied at the same rate over the three seasons trialed. The feedlot waste was a relatively cheap and simple source of biologically based N and fruit quality and yield outcomes were satisfactory over the three-year period. There is likely to be some variation in N rate between batches of feedlot waste so regular monitoring of source material is required. We recognise that there is a labour requirement to distribute the waste over the orchard and the volume required to supply the necessary N to meet tree requirements may not always be available from the supplier. However, that the application only needs to be done once or twice over a season means that this is achievable from an overall management perspective. Certainly, this form of N could be complimentary to either conventional forms of N or the Organic N which is significantly more expensive yet comparatively easier to apply. The liquid based Organic N can be directly applied through existing fertigation infrastructure, however for growers considering this source of N as a viable alternative, some longer-term studies investigating the soil health benefits of this form on top of fruit quality outcomes would be necessary given the high input cost. Complementing the conventional N and feedlot waste forms with Soil and Seed as a nutrient uptake facilitator showed some early evidence of being beneficial, however the positive effect wasn't repeated in seasons 2 and 3. There was some evidence that Soil and Seed delayed ripening possibly due to more efficient accumulation of N as seen in the leaf N concentration charts, yet this was not conclusive either. The biological based forms of N tested here clearly provide an effective alternative to conventional based fertilisers, yet based on ^{15}N recovery trials, we would recommend applying at a greater rate than the 45 kg N /ha trialed here for ongoing tree health and adequate nutrition. This additional cost would need to be offset by further evidence of improved long-term soil and orchard health to encourage industry to adopt these N management approaches.

Trial 2: Young trees at Reid Fruits' Honeywood Orchard at Jericho, Tasmania

Background

To investigate the influence of conventional and alternative treatments on young trees, in a similar manner to the experiment described above, a trial was established on a young orchard.

Methods

A three-year trial, using one-year-old *Lapins* cherries growing on Colt rootstock, planted in July 2016, was established at Reid Fruits' Honeywood Orchard at Jericho in the Tasmanian Midlands (42.383° S, 147.280° E) in the spring of 2017. The 180-tree trial compared application of forms of organic N with an equivalent, and different rates, of the standard mineral N fertiliser, calcium nitrate [$Ca(NO_3)_2$]. The trees were on a gently sloping site, spread over 11 orchard rows (*Figure 23*), running east-west with 4.5 m between rows and 2 m between trees, for about 1,110 trees/ha. Nine treatments were applied (

Table 17) over three full seasons (except for clover, refer below) , arranged in a randomised complete block design of four replicates, each replicate consisting of five trees, the middle three being 'trial' trees and the outer two 'buffer' trees. The projected orchard application of 150 kg $Ca(NO_3)_2$ /ha/year was taken as a standard N rate, equivalent to 120 g N/tree/year within the rows of about 1 m width, and typical of that applied in many cherry orchards. Fertigation was applied by a

dedicated dripper system, using dripper line (Netafim®) with a dripper every 0.5 m and with a line either side of the trees. Fertigation treatments (

Table 17) commenced in early November in each season and were applied at three-weekly intervals, continuing until February. The soil microbial inoculant *Soil & Seed* was supplied by the manufacturer, BioAg Pty Ltd, Narrandera NSW and mixed with the fertiliser prior to application. The feedlot waste, freshly sourced for each season, in each year had a total N content of 1.45 % by weight, with mineral N constituting 76% and 93% of that being in the form of nitrate (average of three samples each year, with minimal variance). It was applied to the surface of the raised growing beds in mid-November of each season, measured so that the dry weight equivalent had a total N content equal to 120 g per tree. The subterranean clover, which grew strongly following transplanting early in the 2017/18 season, was in scant evidence by the following spring. Re-seeding in the second season failed to produce any substantial coverage and this treatment was deemed incompatible with the existing orchard management practice, and so discontinued thereafter. Irrigation throughout the whole orchard is by micro-sprinklers supplied with water from a local irrigation scheme. No other N was applied to the trees during the duration of the trial. All non-N fertiliser application, weed and pest control, and pruning were overseen by management in accordance with standard orchard practice.

The region is of a cool, temperate climate, well suited to the chill requirements of cherry production. Monthly average temperatures and rainfall are shown in *Figure 24* and *Figure 25*, respectively, recorded at Oatlands Post Office, 12 km to the north-east of the trial site and of very similar climatic conditions.

Table 17 Treatments and method of application of nitrogen.

Treatment code	Total treatment (per tree)	Method of application	Number of applications per year
A	0 g N	n/a	n/a
B	60 g N as $Ca(NO_3)_2$	Fertigation	6
C	120 g N as $Ca(NO_3)_2$	Fertigation	6
D	240 g N as $Ca(NO_3)_2$	Fertigation	6
E	120 g N as cattle feedlot waste	Spread on surface	1
F	120 g N as '*Organic N*'	Fertigation	6
G	120 g N as '*Nitro Humus 323*'	Fertigation	6
H	Subterranean clover	Coverage of surface	Established in first year, re-seeded in second.
J	120 g N as $Ca(NO_3)_2$ plus '*Soil & Seed*' microbial inoculant*	Fertigation	6

* Initial application of 4.8 mL/tree (6 L/ha) and thereafter 2.4 mL/tree, as recommended by the manufacturer.

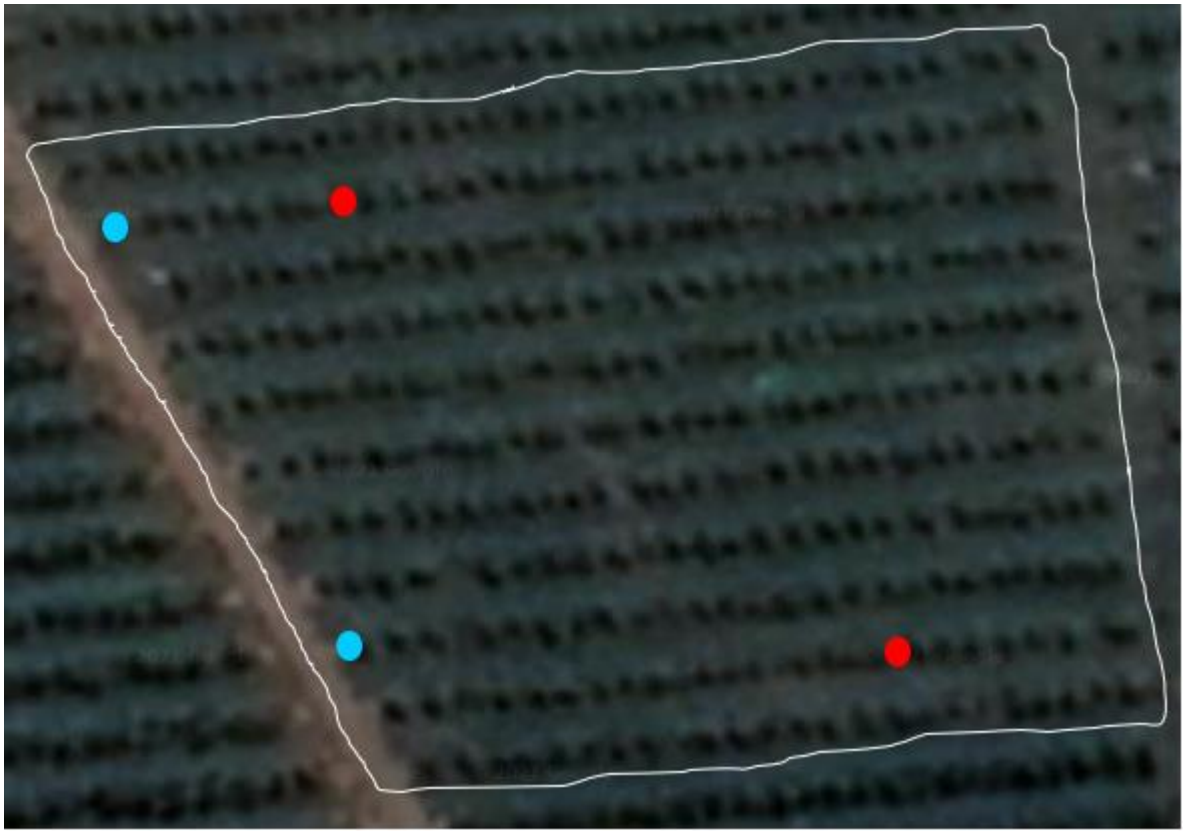

Figure 23 The 11-row trial site with a gentle downhill slope from top to bottom of the image. Soil moisture/temperature probes (*Sentek* Drill & Drop) with data loggers were installed at two points (red dots) and irrigation flow meters at two points (blue dots), the upper one fitted with a data logger (*Tinytag*, Hastings UK).

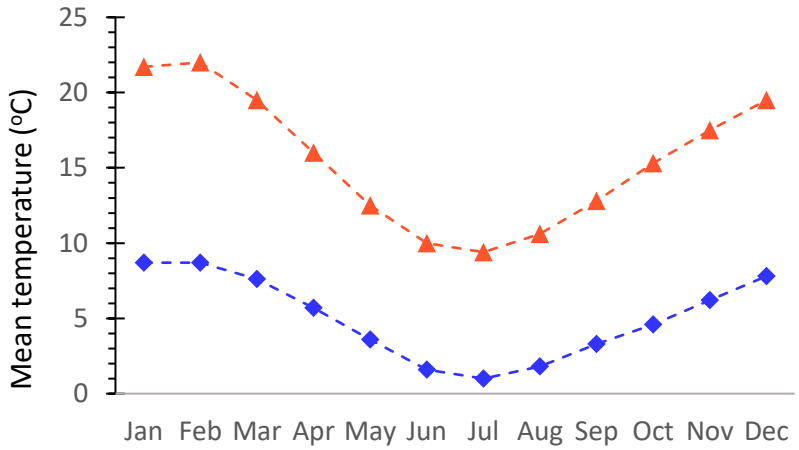

Figure 24 Mean monthly temperatures at Oatlands PO (1957 – 1993, the most recent available) (Bureau of Meteorology, 2021b).

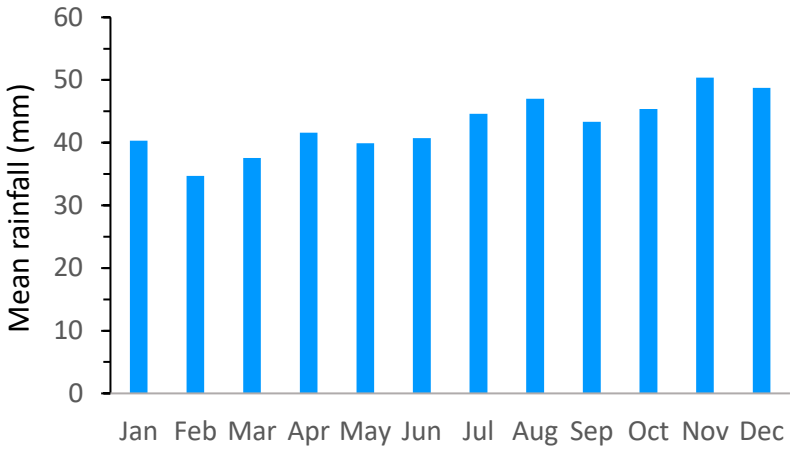

Figure 25 Mean monthly rainfall totals at Oatlands PO (1960 – 2020) (Bureau of Meteorology, 2021a)

On the establishment of the trial in the spring of 2017 lysimeters were installed directly below a fertigation dripper, at an upper (inlet) depth of 400 mm, in each replicate of four N treatments (A, C, D & J in

Table 17 to measure applied NO_3^- that was leached below the trees' root zone. A problem that was not identified until the following winter hampered extraction of water from most of the lysimeters prior to that time. From then onwards, all leached water was extracted monthly, volumetrically measured, and analysed for NO_3^--N concentration.

Two soil moisture and temperature probes fitted with data loggers (Drill and Drop, 90 cm; *Sentek*, South Australia) were installed at the points shown in *Figure 23* Initially set to log half-hourly, this frequency was reduced to hourly to extend the time needed between data downloads. Irrigation flow meters were installed at two points (*Figure 23*), one automated to log a reading every 10 minutes. Readings from both were recorded at the time of monthly extractions of lysimeter water.

Extension growth of each of the 120 trial trees (i.e., the inner three of each five making up a replicate for each treatment) was measured in early March of each of years 2018 and 2019. The measurement was made on each of five scaffold (main) branches, close to equally spaced around the tree, by measuring the total length of the branch, including that of all lateral growth, and summing the totals for the five branches. Trunk circumference of the same trees was measured in July 2018 and again, at the same marked points, in July 2019. Restrictions on movement related to Covid-19 prevented measurement of extension growth and trunk circumference in the autumn/winter of 2020.

All fruit was removed from trial trees on 23-01-2019 and weighed for a yield comparison only, the trees at this growth stage not yet ready for commercial harvest. The first harvest of commercial quantities was on 6/02/2020: fruit from each three-tree replicate was combined for weighing on site and returned to the laboratory for quality analysis the following day, after being stored overnight at 2 °C. The method of fruit quality analysis is detailed in the *Assessment of quality and bioactive properties* section of this report.

Results

Growth parameters

Extension growth, measured in early March of 2018 and 2019, is shown in *Figure 26* While for most treatments there was a significant increase (at 95% confidence interval) in extension growth from 2018 to 2019, there were no significant differences between treatments within each year. Unfortunately, many trees were affected by bacterial canker in the 2018/19 season. Consequently, there was some minor pruning carried out by orchard management during October to December of 2018, prior to the measurement of extension growth in the following autumn. This may have had a small influence on comparative measurements between treatments, although the effect of canker did not appear to be at all related to treatment type. Measurements of trunk circumference (*Figure 27*) showed a significant increase for all treatments from 2018 to 2019, but within each year no significant differences between treatments.

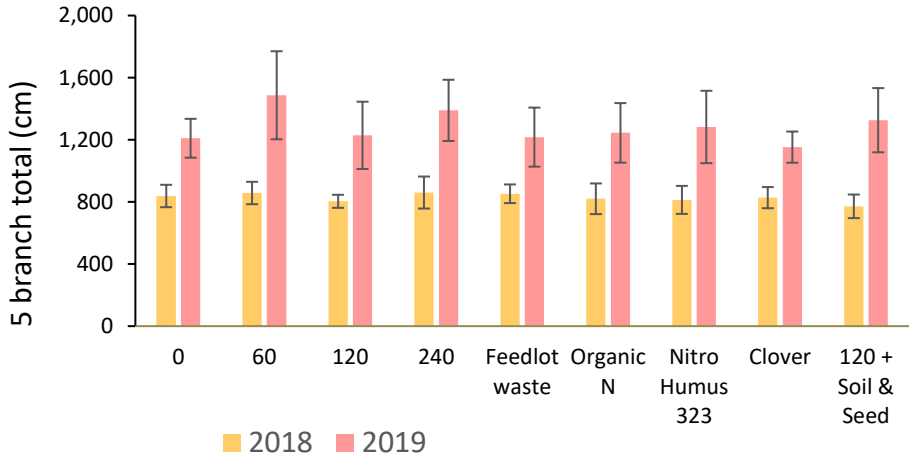

Figure 26 Extension growth measured in autumn of each year, on 5 representative branches around trees for the treatments shown in

Table 17 (error bars represent ± SE; n = 4 replicates × 3 trees per replicate = 12).

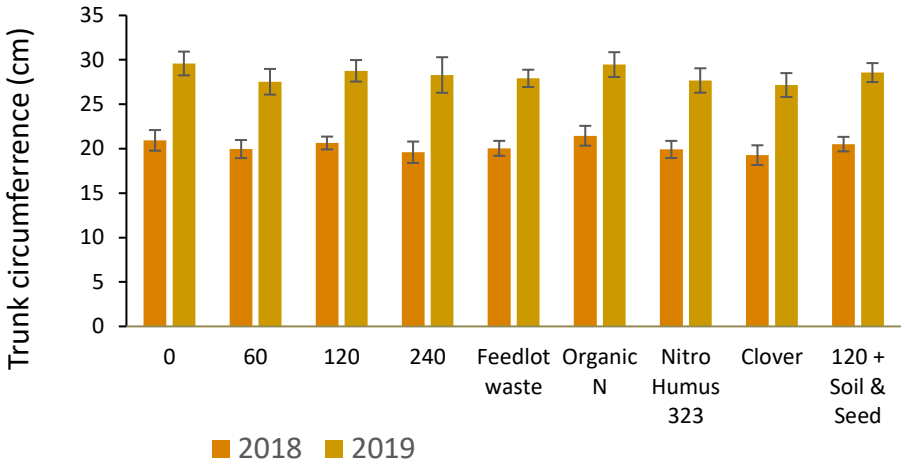

Figure 27 Trunk circumference, measured at the same point in each year, for treatments shown in *Table 17* (error bars represent ± SE; n = 4 replicates × 3 trees per replicate = 12).

Fruit harvest

A minor (non-commercial) fruit harvest was undertaken on 23/01/2019, the first 'real' harvest not being until the following season. Average yields from this minor harvest are shown in *Table 18*.

Table 18 Average fruit yields per tree from the minor harvest of 2019 (standard errors are in parentheses, n = 4 replicates × 3 trees per replicate = 12).

Treatment	Treatment (per tree)	Average yield per tree (g)
A	0 g N	964 (63) a, b
B	60 g N as $Ca(NO_3)_2$	709 (270)
C	120 g N as $Ca(NO_3)_2$	765 (121) c
D	240 g N as $Ca(NO_3)_2$	843 (67) d, e
E	120 g N as cattle feedlot waste	996 (192)
F	120 g N as '*Organic N*'	1285 (78) b, c, e, f
G	120 g N as '*Nitro Humus 323*'	574 (23) a, d, f
H	Subterranean clover	714 (206)
J	120 g N as $Ca(NO_3)_2$ plus '*Soil & Seed*' microbial inoculant*	952 (348)

Values accompanied by the same letter are significantly different (*p* range of < 0.02 to 0.0001).

The first fruit harvest of a commercial scale took place on 6/02/2020, with trees from the discontinued subterranean clover treatment excluded. Average yields from this harvest are shown in *Figure 28*, including significant differences between treatments. The pack-out of Class 1 fruit (*Figure 29*) was not significantly different between any treatments, nor was N content, at an average 1.19%.

Weight and size measurements of fruit are shown in *Table 19*, with there being some slight significant differences – the only ones being the treatment with 240 g N applied/tree was significantly greater than that with 120 g N applied/tree along with *Soil & Seed*, in weight ($p = 0.034$), and width ($p = 0.031$). The measures of skin puncture force, fruit and flesh firmness and stem retention force (*Table 20*) saw no significant differences between treatments.

Fruit colour parameters are shown in *Figure 30*. For each parameter there were no significant differences between treatments.

Fruit was analysed for titratable acidity and total soluble solids (*Figure 31*), with many significant differences between treatments found in the former – for example, the titratable acid in the fruit from the 0 N treatment was significantly different ($p \leq 0.0001$) from that of all treatments, other than the 120 g N/tree with *Soil & Seed* treatment ($p < 0.01$) and the 240 g N/tree treatment, from which there was no significant difference. For other treatments, where the 1.96 × standard error bars in *Figure 31* do not overlap, a significant difference in titratable acidity is indicated. There was much less evidence in significant differences between treatments for total soluble solids, with only that of the 0 N treatment being significantly less than that of the feedlot waste treatment (p = 0.0332), and that of the 240 g N/tree treatment being significantly less than that of the 60 g N (p = 0.0345), and very significantly less than the feedlot waste (p = 0.0060), and the Nitro Humus 323 (p = 0.0009) treatments, respectively.

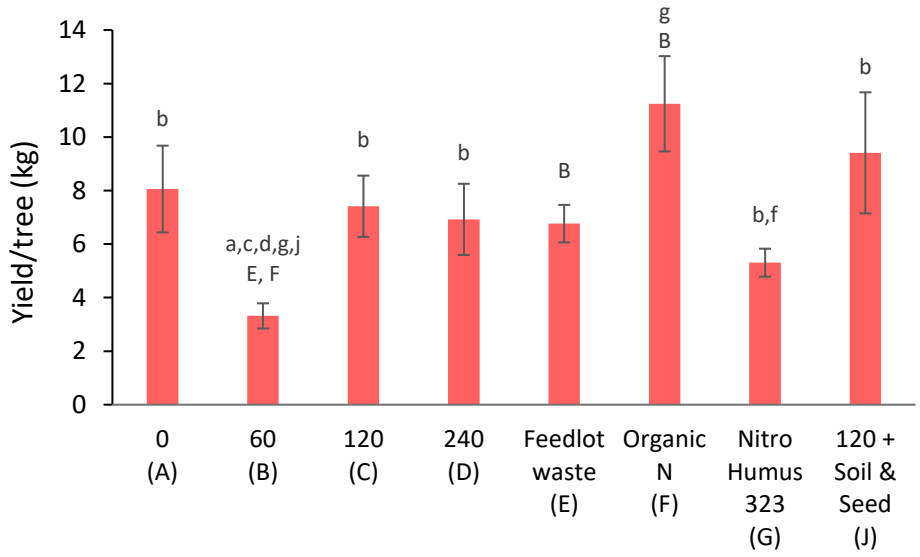

Figure 28 Average fruit yields per tree from the harvest of 2020 (error bars represent ± SE; n = 4). Treatments are labelled with letter codes as in

Table 17 – accompanying letters above error bars indicate a significant difference from those treatments, when lower-case ($p < 0.05$), or upper-case ($p < 0.01$).

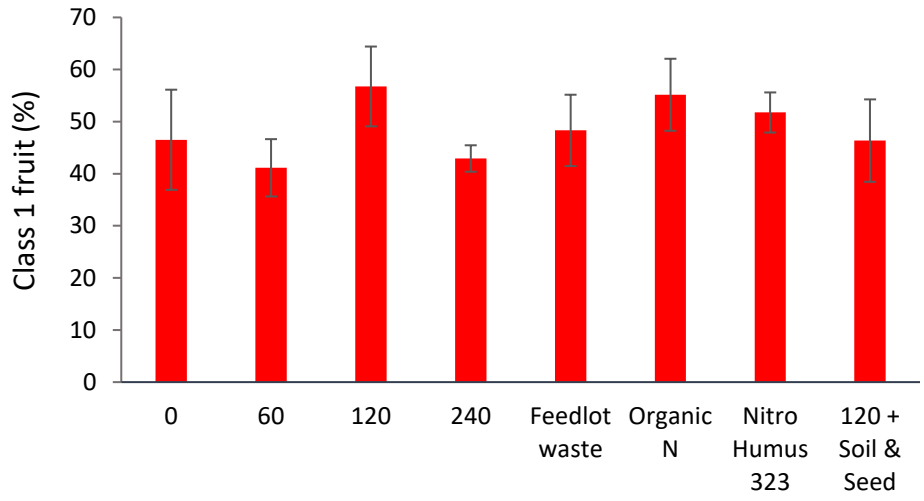

Figure 29 Packout of Class 1 fruit from the harvest of 2020 (error bars represent ± SE; n = 4).

Table 19 Manually measured weight and width, and size (Firmtech) of fruit harvested in 2020 (n = 4 replicates × 25 fruit per replicate = 100).

Code (*Table 17*)	**Treatment (per tree)**	Weight (g)		Width(mm)		Size(mm)	
		Mean	SE	Mean	SE	Mean	SE
A	0 g N	13.13	0.35	30.18	0.32	31.01	0.34
B	60 g N as $Ca(NO_3)_2$	13.79	0.29	30.63	0.25	31.56	0.25
C	120 g N as $Ca(NO_3)_2$	13.46	0.28	30.36	0.24	31.20	0.25
D	240 g N as $Ca(NO_3)_2$	14.21	0.35	31.06	0.29	31.72	0.28
E	120 g N as cattle feedlot waste	13.46	0.33	30.40	0.29	31.32	0.30
F	120 g N as '*Organic N*'	13.21	0.30	30.15	0.28	31.01	0.29
G	120 g N as '*Nitro Humus 323*'	13.60	0.28	30.59	0.23	31.46	0.24
J	120 g N as $Ca(NO_3)_2$ plus '*Soil & Seed*' microbial inoculant*	12.97	0.29	29.97	0.26	30.81	0.27

Table 20 Manually measured skin puncture force, fruit flesh firmness and stem retention force, and firmness (Firmtech) of fruit harvested in 2020 (n = 4 replicates × 25 fruit per replicate = 100).

Code (*Table 17*)	**Treatment (per tree)**	Skin puncture force (g)		Fruit flesh firmness (g)		Stem retention force (g)		Firmness (g)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	0 g N	0.51	0.016	0.164	0.0044	473	39.3	424	12.4
B	60 g N as $Ca(NO_3)_2$	0.54	0.015	0.174	0.0056	537	41.2	416	12.2
C	120 g N as $Ca(NO_3)_2$	0.52	0.015	0.170	0.0056	462	39.2	425	12.5
D	240 g N as $Ca(NO_3)_2$	0.52	0.015	0.168	0.0054	455	36.7	435	13.4
E	120 g N as cattle feedlot waste	0.52	0.014	0.168	0.0052	389	37.6	422	10.1
F	120 g N as '*Organic N*'	0.50	0.014	0.155	0.0046	421	28.2	417	10.6
G	120 g N as '*Nitro Humus 323*'	0.51	0.015	0.164	0.0038	488	40.9	420	10.5

J	120 g N as $Ca(NO_3)_2$ plus '*Soil & Seed*' microbial inoculant*	0.52	0.014	0.164	0.0042	486	30.9	416	11.3

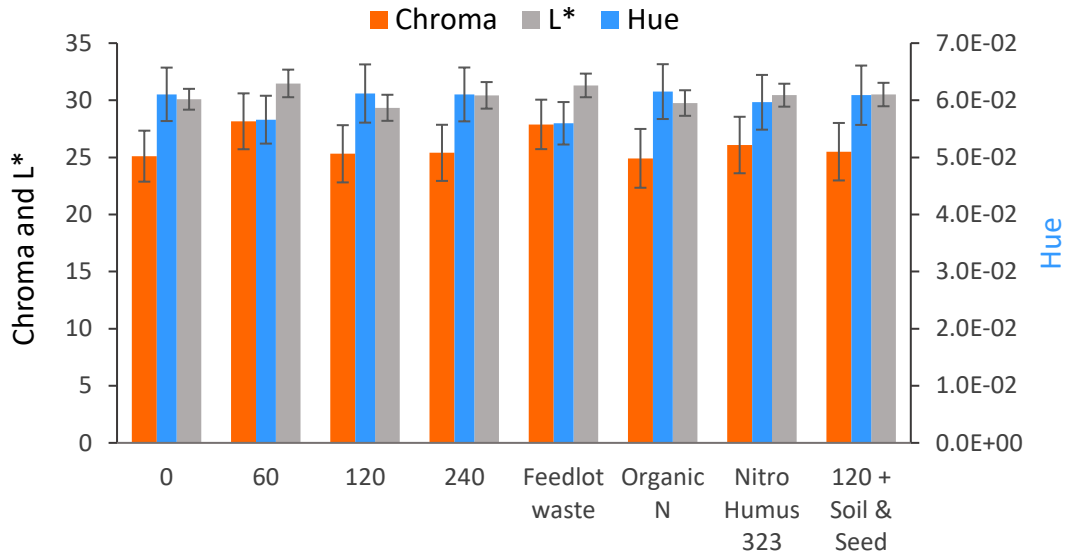

Figure 30 Fruit colour parameters measured in the CIE L*C*h* colour space (error bars represent ± SE; n = 4 replicates × 25 fruit per replicate = 100).

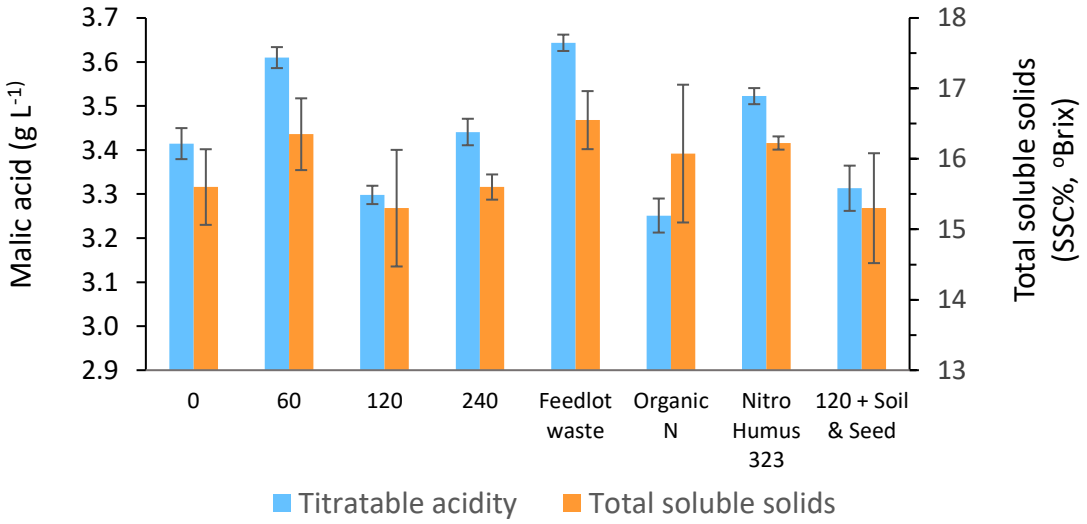

Figure 31 Titratable acidity and total soluble solids of fruit from 2020 harvest (error bars represent ± 1.96 × SE; n = 4 replicates × 2 samples = 8 for acidity and n = 4 for solids).

Leached nitrate

The water extracted approximately monthly from lysimeters was analysed for NO_3^--N over a period covering more than a full season – 17/08/2018 to 22/10/2019 – thus following the six, three-weekly fertigation applications of the previous season (November 2017 to February 2018) and including the span of the six, three-weekly fertigation applications that commenced on 7/11/2018 and continued until 20/02/2019. The NO_3^--N extracted is shown in Figure 32 and it clearly declined substantially

from February 2019, as generally did the volumes of water. Consequently, any water extractions during this latter period of less than 140 mL (those all being < 100 mL) were deemed inconsequential, relative to numerous earlier volumes in the range of 2 – 13 L, and not analysed. For each date of extraction there were no significant differences between treatments in NO_3^--N yielded, due at least in part to the large variance between replicates. Likewise, there were no significant differences between the totals of NO_3^--N extracted over the full period from lysimeters associated with treatments that received 0 g N, 120 g N, 120 g N with Soil & Seed, or 240 g N, these totals being 1435, 2048, 2242, and 3022 mg NO_3^--N, respectively. If plotted over the 14-month period, these four values are found to follow a very close to linear relationship with the N applied:

Leached NO_3^--N (mg) = 6.613 × NO_3^--N applied/tree (g) + 1390 (R^2 = 0.98).

The lysimeters were installed directly below drippers in the fertigation array, and with the equivalent of eight drippers per tree (*Methods*) the potential losses of N through leaching below the root zone, in relation to these previous values, would range from 14.4 to 30.2 kg NO_3^--N/ha. For those treatments with NO_3^--N applied, an average of 12.9% of was leached into the lysimeters over the 14-month period. From the control plots (0 g N applied) , soil samples were taken from each replicate in late autumn 2018 (four homogenised samples/plot) – these having not received any N fertiliser for over 12 months. The samples were analysed shortly thereafter (Moody, 2018) and found to have mean concentrations of 52.4 mg NO_3^--N/kg soil (SE = 21.5, n = 4) at 0 -10 cm and 24.4 mg NO_3^--N/kg soil (SE = 13.4, n = 4) at 20 -30 cm were found. These values were not atypical of a reasonably fertilised, healthy orchard soil and with perhaps 350 kg of soil m^{-2} to a depth 350 mm, they demonstrate the amount of NO_3^--N that might be available for potential leaching.

Soil water content measurements at four depths, from one of the installed probes (the upper one in shown in *Figure 23* – see also *Soil water content and* temperature, below) are shown in Figure 33, along with daily rainfall (Figure 34) and irrigation water applied (Figure 35), all covering the period for which lysimeter NO_3^--N contents are shown in Figure 32. It is clear that the period of August to October 2018, when considerable NO_3^--N was collected from all lysimeters, corresponded to high soil water content at measured depths, the deepest of these at 45 cm being below the level at which soil water would drain into the lysimeters (*Methods*). Such conditions of very wet, possibly saturated soil would favour drainage of soil water containing dissolved NO_3^--N from the trees' root zone (0 to approx. 35 cm) into the lysimeters. This would be encouraged by rain falling onto very wet soil during times of tree dormancy and low rates of evaporation due to low atmospheric and soil temperatures (Figure 36). The decrease in soil water content from early October 2018, particularly at the depth of 5 cm, corresponded to a period of little rainfall, far from intense irrigation, and early spring tree growth. As such, soil conditions did not favour drainage of water through the profile and the reduced NO_3^--N collected from lysimeters on 16-11-2018 supported this. The increases in soil water contents at all four recorded depths, and the wide and regular variation at the depths of 5 and 15 cm in particular, during December 2018 and January 2019, corresponded with a period of maximum irrigation intensity (Figure 35) and addition of further NO_3^--N to the soil through the resumption of the seasonal fertigation program (*Methods*). Such conditions favoured the increased leaching of NO_3^--N in to the lysimeters seen in those two months. The reduction in irrigation intensity from late January 2019 and the corresponding decrease in soil moisture would likely explain the decrease in NO_3^--N collected from lysimeters in February 2019.

From March to October 2019, relatively little NO_3^--N was collected from any lysimeters when compared with most of the previous months for which lysimeter water was analysed. Much of this contrast could be attributed to a reduction in water entering the lysimeters – an average of 0.33 L per lysimeter, compared with 4.09 L for August to October 2018, or 1.75 L for January 2019 – but a reduction in NO_3^--N dissolved in soil water may have been a contributing factor. Clearly, the generally lower soil water contents measured at all four depths during June to October 2019 than in the corresponding period of the previous year would have led to much less drainage of water through the soil profile. Nonetheless, with the resumption of fertigation in November 2019, and increasing irrigation intensity, it might be expected that leaching of NO_3^--N would again increase. An increase in N demand from trees of increased maturity could have been a counteracting influence.

Irrigation

The irrigation data shown in Figure 35 is taken from the 'upper' flow meter (Figure 23), with logger attached. Although determination of short-term irrigation volumes cannot be taken from Figure 35, it does give an overview of irrigated periods and their intensity. At each date of lysimeter water extraction, approximately monthly for the three years of the trial, readings were taken from both the 'upper' and 'lower' flow meters (Figure 23), the latter having no logger attached. A comparison of these readings confirmed that, except for the brief period of irrigation malfunction (*Soil water content and temperature*), trees in rows 'two' and 'nine' of the trial where the meters were situated, with different numbers of trees per row, were receiving the same volumes of water. It was therefore assumed that all trees across the 11-row site were similarly supplied.

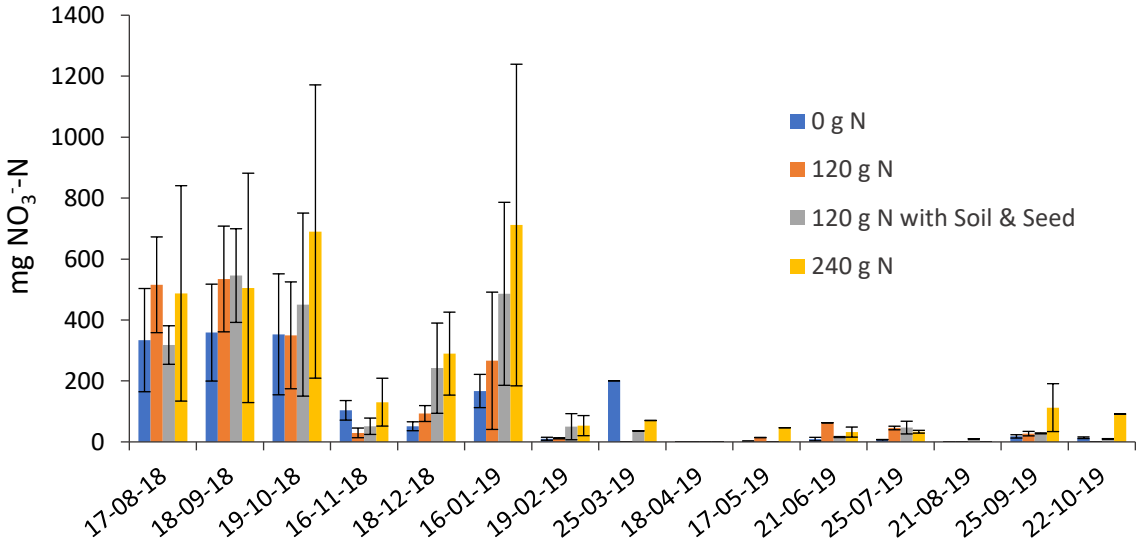

Figure 32 Nitrate content of water withdrawn from lysimeters installed below drippers of treatments A, C, J, and D (

Table 17 (error bars represent ± SE, n = 4).

Soil water content and temperature

The two combined soil moisture and temperature probes installed at different positions in the trial area (Figure 23) showed some variation in soil temperatures and water contents measured at corresponding depths. A flat battery in the 'lower' probe (Figure 23) resulted in a lack of data from it for the period of 8-10-2018 to 18-12-2018, but excluding that period temperature measurements of 'upper' probe/'lower' probe ranged from 0.84 – 1.77, 0.88 – 1.37, and 0.92 – 1.09 for measurements at 5, 15, and 45 cm depths, respectively. Fluctuations in the differences, particularly at the depth of

5 cm, were to some extent diurnal and possibly related during daytime hours to varied extents of shading of the soil surface. However, differences at the deeper levels and particularly at 45 cm seemed likely to have been more influenced by differences in soil water content or structural characteristics, directly or indirectly influencing temperature measurements. Similarly, soil water content measurements showed some variation between probes at corresponding depths. Figure 37 shows a comparison between the probes of soil water content measurements at depths of 5 and 45 cm for the period 19-12-2018 t0 22-10-2019. It is clear that at a depth of 5 cm measurements from the 'upper' probe showed greater fluctuation and reached higher values than those from the 'lower' probe during the period when irrigation was most intensely applied. This was very likely due to their relative proximities to irrigation sprinklers, as during the non-irrigated part of the season the difference was much less marked, and changes followed near-identical patterns. Measured at the depth of 45 cm, it is clear that the soils at that depth had differing water-holding characteristics, but viewed over timeframes of weeks rather than days, the changes in measured soil water contents followed similar patterns, with the exception of the sudden 'spike' in water content measured by the 'upper' probe (Figure 37) in April 2019. This was the consequence of an irrigation malfunction where that section of the trial area received a great deal more water than planned for a period of 7 – 10 days and the area of the 'lower' probe none. As all trial trees were close to dormancy this event had little effect on their growth. For the 10-month period under discussion a comparison between probes of measurements taken at depths of 15 and 25 cm found that measured soil water contents, while not identical, also followed similar patterns.

Although the measurements of soil water contents differed between the two probes, because they followed similar patterns and data was unavailable from the 'lower' probe for the over two-month period of 2018 described, it was deemed appropriate to use data from the 'upper' probe only (Figure 33) in the discussion of leaching of NO_3^--N (*Leached nitrate*).

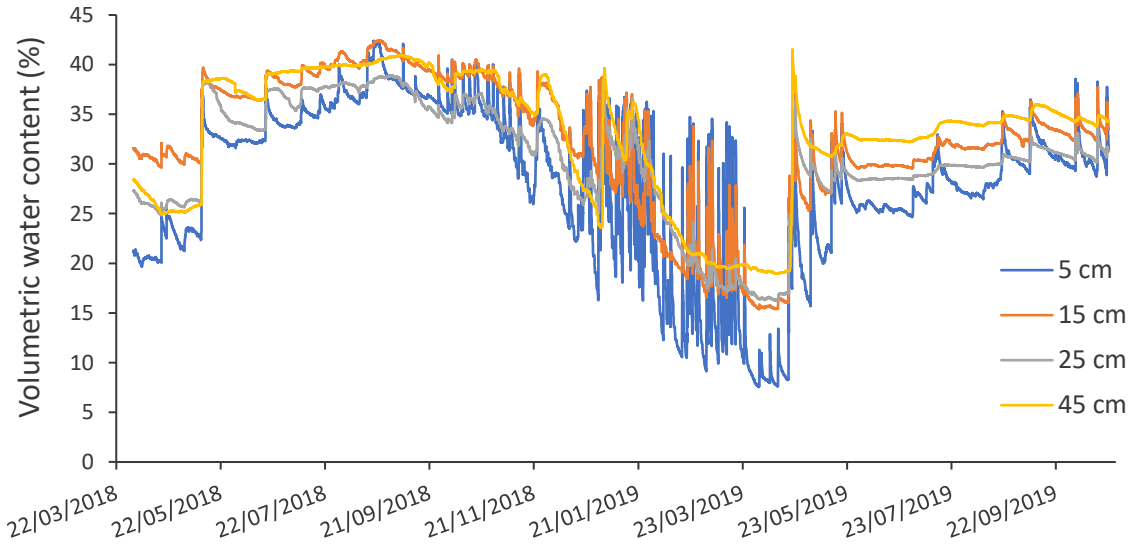

Figure 33 Soil water content at four depths, measured by the probe at the upper end of the trial site (*Figure* 23).

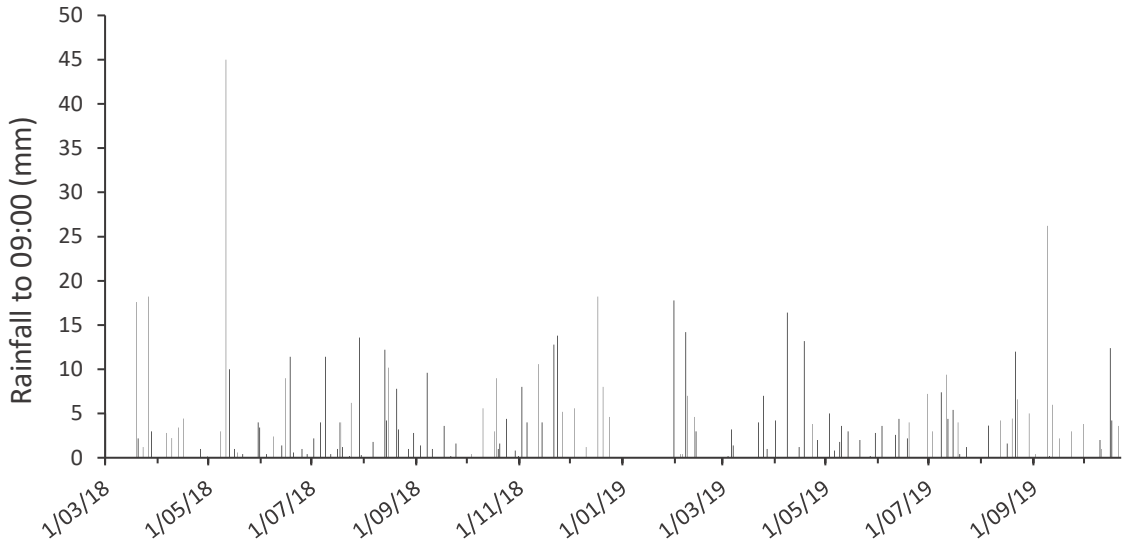

Figure 34 Regional daily rainfall measured at Oatlands Post Office (*Methods*). (Bureau of Meteorology, 2021a).

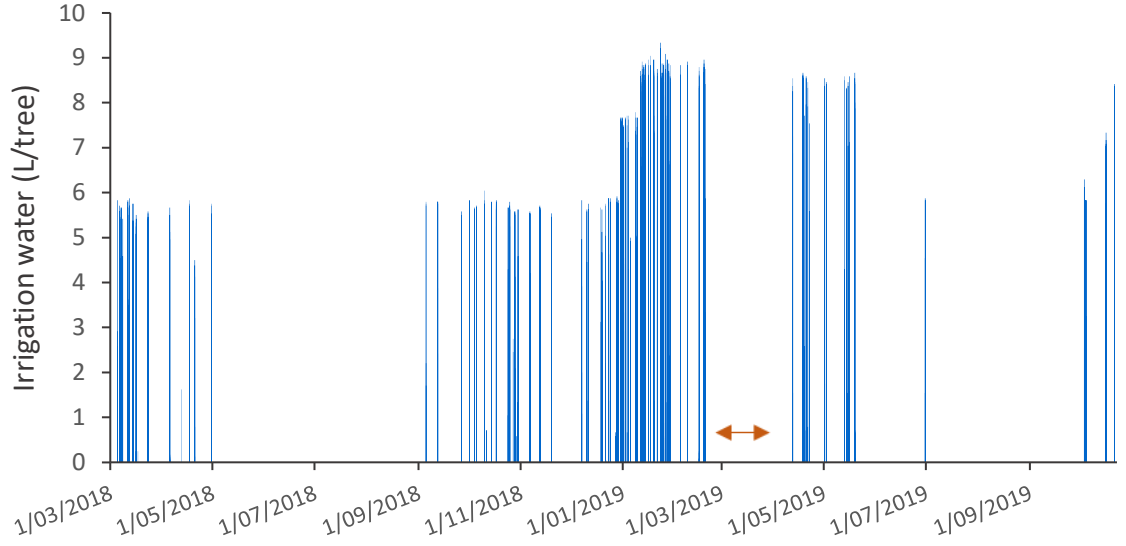

Figure 35 Irrigation water/tree, logged each ten minutes. Due to logger malfunction (a flat battery) no data was recorded for the period 19/02 to 4/04/2019, as indicated on the chart.

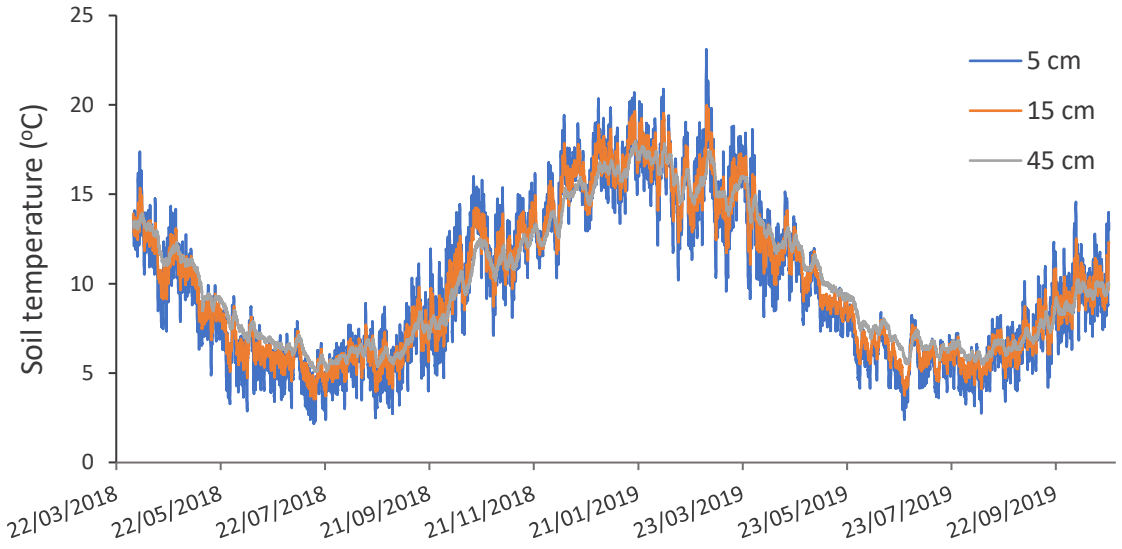

Figure 36 Soil temperature at three depths, measured by the probe at the upper end of the trial site (*Figure* 23).

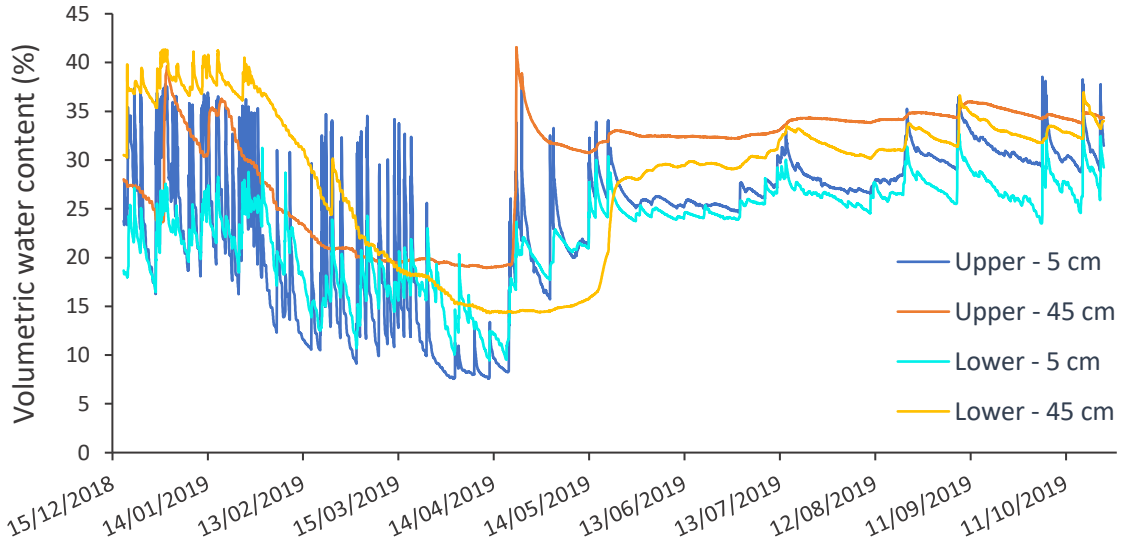

Figure 37 Comparison of soil water contents of 'upper' and 'lower' soil moisture probes (relative positions in Figure 23) at two depths.

The potential to leach N (as NO_3^-) is dependent on soil NO_3^--N content, soil structure and chemistry, and soil water content, itself affected by rainfall and irrigation. An examination of the site subsoils in the year prior to orchard establishment (AgAssist, 2015), found that "topsoil depths varied across the site with depths varying from 20cm to 40cm." The report classified the trial site topsoil as a loam and the subsoil as either a dermosol or sodosol (Isbell, 2002), with the "boundary between the dermosol and the sodosol on the bank was difficult to establish due to limited sample sites." The dermosols were classified as well drained and the sodosols as having poor drainage with waterlogging of the B horizon occurring periodically. Such subsoil variation within the trial site could easily explain the differences in soil water content, particularly at deeper depths, found between the sites of the two moisture probes (Figure 37). This could well result from different drainage characteristics at those sites. Variation in subsoil characteristics might also explain the large variance found between treatment replicates in the quantities NO_3^--N leached into their associated

lysimeters. Certainly, there can be little doubt that in sandier soils than those of the trial site, being universally recognised as freely draining, the potential for leaching of NO_3^--N would be even greater than that detected in this trial.

Discussion

The results of this three-year trial, comparing forms of organic N applied to the soil with an equivalent, and different rates, of the mineral N fertiliser, calcium nitrate, found no significant differences in the growth of the young trees in relation to the treatments applied (

Table 17). This outcome was not surprising as it was known that the orchard soil was well-prepared prior to the planting of the trees some 17 months before the first application of trial treatments. All indications were of a healthy soil, with considerable mineral N available to meet the demands of young trees (*Leached nitrate*), and soil organic matter contents, C:N ratio and pH all suited to the purpose (Moody, 2018).

The clearest difference in fruit yields between treatments (Figure 28) was that of the 60 g N/tree treatment yielding significantly less fruit than all other treatments, including the control with no N applied. There was no explanation for this surprising outcome other than that it was an aberration unrelated to N supply, there being no significant differences between the other treatments where N was also supplied as $Ca(NO_3)_2$ – either at 120 or 240 g N/tree – or indeed the control (0 g N) or the treatment of 120 g N as $Ca(NO_3)_2$ with *Soil & Seed* included (*Methods*). The only other yield difference that stood out was that of the *Organic N* treatment being, with the highest average yield of all treatments at 11.24 kg/tree, being significantly greater (p = 0.0187) than the other liquid organic treatment, the cheaper *Nitro Humus 323*, with an average yield of 5.31 kg/tree. Most fruit quality parameters (Table 19, Table 20, and Figure 30) showed no significant differences between treatments, with only some found in relation to titratable acidity and total soluble solids (TSS) (Figure 31). The considerable differences found in titratable acidity between most treatments (*Fruit harvest*) did not seem to follow any pattern, in relation to the quantity of N applied as $Ca(NO_3)_2$ for example, or whether N was applied in that form or one of the organic forms. Consequently, no conclusion could be drawn in relation to the relative acid concentrations. There was a much less pronounced difference between treatments in TSS, with its measurement in the 240 g N/tree applied as $Ca(NO_3)_2$ treatment being significantly less than in three other treatments (*Fruit harvest*) the most obvious outcome. However, again there did not seem to be any pattern in relation to quantity or form of N applied and, as with yield measurements, large variance between replicates hampered a comparison of mean values. In summary, with only the first real harvest of fruit as a basis for comparing the effects of the applied treatments on yield and fruit quality it was difficult to draw any conclusions. As the N already in the soil before treatment application seemed to have a dominant effect on tree growth, it is reasonable to assume that its effect on fruit yield and quality was at the least, an important factor. Thus, to make any fair judgement on the relative effects of the treatments on yield and quality of fruit, analysis of at least a further two harvests, with the same seasonal treatments applied, would seem a necessary requirement.

The leaching of nitrate N was clearly demonstrated, although precise estimation was more handicapped than in any other part of the trial by wide variation in mean values between treatment replicates. The difficulty in installing lysimeters and replacing an 'undisturbed' soil core above them doubtless contributed considerably to this variation, as might have variation in soil drainage characteristics across the site. Nonetheless, the data (Figure 32) did demonstrate the potential for leaching of NO_3^--N below the trees' root zone and that the likelihood that the more NO_3^--N applied,

the more would be leached. The very close to linear relationship found between NO_3^--N applied and that leached (*Leached nitrate*) certainly reinforced the relevance of the mean leached values related to each treatment, despite their associated large variances. The estimated leaching below the root zone of 14.4 to 30.2 kg NO_3^--N/ha from treatments receiving applications ranging from 0 to 240 g/tree/season of $Ca(NO_3)_2$ demonstrated the potential for substantial fertiliser wastage – equivalent to 84 to 177 kg $Ca(NO_3)_2$/ha – and a considerable, undesirable addition of N to an external environment in which N pollution is already a major problem (Galloway et al., 2003; Vitousek et al., 1997; Zhang et al., 1996). Although these values can only be regarded as approximations of NO_3^--N losses from an already well-fertilised soil, they serve to demonstrate a potential problem of which all orchard managers need be aware.

Trial 3: Foliar uptake and partitioning of applied N as an alternative to ground applied N

Background

Direct foliar application of nutrients has the potential for faster uptake of N in forms that are plant available. Thielemann 2014 showed that urea foliar sprays were effective in increasing N stored in flower spurs, shoot tips, buds with potential to improved availability for remobilisation the following spring (Thielemann2014?)" Another potential N source for spray application is the proteogenic amino acid L-proline. L-proline is a proteogenic amino acid which is a key determinant of cell wall proteins and plays an important role in plant growth and differentiation (REF).

Foliar application of L-proline has been shown to have positive effects on fruit quality parameters in Japanese pears (Takeuchi, Arakawa, Kuwahara, & Gemma, 2008), oranges (Caronia, Gugliuzza, & Inglese, 2010) and pomegranates (El Sayed, El Gammal, & Salama, 2014).

Although efficiency of foliar spray applications has been demonstrated there remains uncertainty around the mechanisms that underpin foliar nutrient uptake in addition to specific effects on sweet cherry quality. 15N-labelled foliar L-proline represents an opportunity to investigate this (proof-of-principle study)

We hypothesise that spray-applied proline is taken up and incorporated into tree components (such as leaves, buds etc) and hence can function as a N source pre- and post-harvest. We also test whether pre-harvest applied proline has an influence on fruit quality of sweet cherry. The objective of this proof-of-principle study was to measure the uptake and translocation of foliar applied 15N-labelled L-proline into fruit compartments (stem, stone, skin, and flesh) and storage of the sweet cherry variety 'Lapins'. We compared pre and post harvest foliar applied 15N-labelled foliar L-proline to investigate the efficiency of uptake/incorporation into leaves, fruit and branches as an alternative N source to soil applied N.

Methods

Study site.

A trial was conducted in a commercial sweet cherry orchard at Rosegarland in Tasmania, Australia (42.71°S, 146.94°S, 130 m above sea level) during the 2018/2019 season. This region has a cool temperate climate with an annual mean rainfall of 529 mm. Trees were mature 7-year old cv. 'Lapins' on Colt rootstock trained to a Kym Green Bush system (Green, 2005), with row spacing of 4.5 m and tree spacing of 1.7 m. The block was under bird exclusion netting and commercial orchard

management practices were maintained throughout the duration of the trial with drip irrigation providing water and nutrients (including N) to the trees.

Trial design and treatments.

The study was carried out as a randomised complete block design with four replicates per treatment. Plots were in two adjacent rows and consisted of a treatment tree and at least one buffer tree on either side of the treatment tree. Trees were selected during flowering in mid-October based on uniformity of appearance. One representative branch on the west side of each treatment tree with fruit-bearing wood older than two years was tagged. Three treatments (pre-harvest, post-harvest, control) were randomly allocated to trees within each of the four blocks.

L-proline (a total of 300 mg of 20 % enriched ^{15}N-labelled L-proline; 36.9 mg N / branch segment) was applied for three consecutive weeks (100 mg / week) commencing at either straw phase of fruit development on 29.11.2018 (pre-harvest application) or two weeks after harvest on 10.01.2019 (post-harvest application), respectively. Drinking water was applied in the control treatment. The 20% ^{15}N-enriched L-proline solution was prepared fresh for each application using isotopically labelled L-proline-^{15}N and L-proline powders ($C_5H_9{}^{15}NO_2$, g mol^{-1}, ≥ 95 atom % ^{15}N, 98 %, and $C_5H_9NO_2$; Sigma-Aldrich Pty. Ltd.) dissolved in town water. Treatments were applied to the tagged branches to wood older than two years as a fine mist from spray bottles in the morning when no rainfall was predicted for the following 24 h. At each application, 50 ml of the prepared solution was sprayed onto the leaves of the selected branches until runoff mimicking commercial spray application.

Sampling and measurements

Leaf samples

Initial leaf sampling commenced on 29.11.2018, immediately before pre-harvest application and thereafter at intervals of three weeks until leaf senescence. Samples of 10 mature leaves were randomly picked, washed with distilled water to remove surface ^{15}N-labelled L-proline and dried with paper towel. The chlorophyll content was estimated for each leaf in the first quarter closest to the petiole using a SPAD-502 Plus Chlorophyll Meter (Konica Minolta, Japan). Leaves were weighed immediately prior to drying at 60 °C and again after 48 h to determine dry weight. Shoot extension growth was removed from the top of each treated branch at time of commercial pruning/extension growth removal, i.e. seven weeks after harvest. The length of each shoot was determined before separation into three compartments: wood, leaves and buds. Total weight of each compartment was determined prior to sub-sampling and drying at 60 °C. – only leaves were analysed.

Fruit samples

All fruit from each treated branch segment was hand-picked at commercial maturity (28.12.2018), counted and weighed. To determine partitioning of ^{15}N within the fruit, 10 fruit were washed with distilled water to remove surface ^{15}N-labelled L-proline. Fruit pedicels were detached by hand; stones were removed with a manual cherry destoner (Westmark, Germany) and washed with water to remove any attached flesh; skin and flesh were carefully separated with a sharp kitchen knife. The weight of each fruit component (pedicels, stones, flesh, skin) was determined prior and after drying at 60 °C.

A subsample of 20 blemish-free fruit per branch was obtained for quality assessment, including measurement of individual fruit weight (Mettler Toledo electronic balance, Switzerland), size (Wiha DigiMax digital calipers, Switzerland), colour (Australian Cherry Colour Guide, Cherry Growers

Australia; Chroma meter CR-400, Konica Minolta, Japan), compression firmness (FirmTech 2 , BioWorks Inc., USA), flesh and skin puncture force (Güss, model S-20, South Africa), stem retention force (Mark-10 Series 5 force gauge, USA), total soluble solids (TSS, Atago PR-1 digital refractometer, Japan), titratable acidity (TA, Mettler Toledo G20 Compact Titrator, Australia) according to previously published methods (Bound, Close, Quentin, Measham, & Whiting, 2013). Total anthocyanin contents were determined by UPLC-MS/MS following methods adapted from (Blackhall, Berry, Davies, & Walls, 2018) and described in (Hölzel2021).

Branch samples

The whole treated branch was destructively harvested at winter dormancy. The section treated with ^{15}N L-proline was dissected into buds, spurs, bark and inner wood. Subsamples were weighed prior to and after drying at 60 °C.

Sample preparation and N stable isotope analysis

Dried leaves, fruit and branch samples were ground into a fine powder using a MM 200 ball mill (Retsch, Haan, Germany), in preparation for N stable isotope analysis. Ground samples were analysed for N percentage and ^{15}N atom percentage (^{15}Napc) at the Central Science Laboratory, University of Tasmania, using flash combustion isotope ratio mass spectrometry (varioPYRO cube coupled to Isoprime100 mass spectrometer), according to methods described in Tan et al. (2021).

Total N percentage (N%) and $^{15}N_{apc}$ values were used to calculate the proportion of N within a plant organ that was derived from L-proline N (NDP_{organ}) by:

$$NDP_{organ}\ (\%) = \frac{15N_{apc}\ of\ an\ organ - NA}{N_{Papc} - NA} \times 100;$$

where N_{Papc} represents the ^{15}N enrichment of applied L-proline (20 atom% ^{15}N) and NA its natural abundance as measured from leaf samples (0.3672 atom% ^{15}N) taken prior to the application of ^{15}N-enriched L-proline.

Statistical analysis

IBM SPSS Statistics Version 26 was used to analyse the results. One-way ANOVA with Duncan's/Tukey's post hoc multiple comparison was used to determine significant differences between treatments in fruit/branches.

Results

Leaf uptake

Total N percentage peaked at trial commencement (week 0) in late November 2018 at 3.1% and decreased to 2.0% at leaf senescence in late April 2019 (week 21) (Table 1). Pre-harvest application of L-proline resulted in a significant Atom-% ^{15}N increase (0.4127 Atom-% ^{15}N at first leaf collection after application, week 3) compared to the tap water control and post-harvest treatments, which were in the range of the natural abundance of 0.3672 Atom-%. N derived from L-proline was 0.22% in week 3 and decreased to 0.14% in week 21. Post-harvest application resulted in a significantly higher uptake (0.4586 Atom-% ^{15}N at first leaf collection after application, week 9) compared to pre-harvest application. NDP decreased from 0.45% in week 9 to 0.34% in week 21.

Initial leaf chlorophyll contents as estimated by a SPAD meter were in the range of 44.2 to 45.5. There were no significant L-proline treatment effects at any point in time ($p<0.05$), however there was a short-term, non significant trend towards an increase of leaf chlorophyll content in the week following L-proline application both after the pre- and post-harvest applications, i.e. in weeks 3 and 9 respectively.

Table 21: Leaf chlorophyll, N% and NDP from initial sampling prior to the first L-proline application (week 0) until leaf senescence (week 21)

	Week	Control	SE	Pre-harvest	SE	Post-harvest	SE	LSD (P<0.05)	P value
SPAD	0	45.0	0.7	44.2	0.5	45.5	0.4	ns	0.294
	3	46.2	0.7	47.2	0.8	45.6	0.5	ns	0.284
	6	45.2	1.0	46.9	0.9	44.6	1.2	ns	0.320
	9	44.8	0.8	45.0	1.1	46.9	1.1	ns	0.312
	12	44.5	0.8	43.7	0.4	43.7	0.7	ns	0.648
	15	40.7	1.1	40.6	0.8	41.0	0.9	ns	0.942
	18	42.0	0.7	40.9	1.1	41.8	0.4	ns	0.585
	21	32.9	1.3	34.8	0.8	34.2	1.1	ns	0.496
N%	0	3.11	0.04	3.12	0.11	3.13	0.08	ns	0.981
	3	3.02 b	0.13	2.64 a	0.06	2.91 ab	0.08		0.045
	6	2.69	0.14	2.40	0.08	2.58	0.07	ns	0.184
	9	2.36	0.11	2.24	0.08	2.37	0.06	ns	0.505
	12	2.51	0.08	2.32	0.10	2.32	0.04	ns	0.195
	15	2.35	0.07	2.15	0.09	2.13	0.04	ns	0.109
	18	2.38 b	0.09	2.12 a	0.01	2.12 a	0.03		0.009
	21	2.01	0.12	1.96	0.01	1.99	0.04	ns	0.897
NDP%	0	-0.002	0.001	-0.001	0.000	-0.002	0.001	ns	0.935
	3	-0.002 a	0.001	0.224 b	0.035	-0.002 a	0.001		0.000
	6	-0.001 a	0.001	0.191 b	0.030	-0.002 a	0.001		0.000
	9	-0.002 a	0.001	0.201 b	0.018	0.454 c	0.075		0.000
	12	-0.001 a	0.001	0.196 b	0.013	0.489 c	0.057		0.000
	15	-0.002 a	0.001	0.177 b	0.031	0.469 c	0.060		0.000
	18	-0.002 a	0.001	0.177 b	0.021	0.411 c	0.068		0.000
	21	-0.002 a	0.001	0.143 b	0.034	0.339 c	0.036		0.000

Fruit components

At commercial harvest in late December 2018, there were no significant differences in total N% content between treatments (Table x). NDP% significantly higher in fruit of treated branches, 0.17% for skin, 0.12% for flesh, 0.03% for the stone and 0.21% for pedicel.

Table 22: N% and NDP% of fruit components of untreated and treated (pre-harvest L-proline application) fruit at commercial harvest.

	Organ	Untreated	SE	Pre-harvest	SE	LSD (P<0.05)	P value
N%	Skin	0.96	0.04	0.91	0.02	ns	0.460
	Flesh	1.29	0.08	1.27	0.05	ns	0.853
	Stone	0.97	0.10	0.94	0.02	ns	0.855
	Pedicel	1.04	0.04	1.08	0.02	ns	0.531
NDP%	Skin	0.004	0.001	0.168	0.026		0.000
	Flesh	0.005	0.001	0.122	0.025		0.000
	Stone	0.003	0.001	0.032	0.005		0.000
	Pedicel	0.001	0.000	0.211	0.026		0.000

Branch components

At dormancy in winter 2019, there were no significant differences in total N content between treatments, however there was significantly higher NDP % in components from treated branches . Significant differences between pre- and post-harvest treatment for buds and bark (and wood) were observed as NDP % was higher in post-harvest treatment.

Table 23: N% and NDP% of branch components at dormancy

	Organ	Control	SE	Pre-harvest	SE	Post-harvest	SE	LSD (P<0.05)	P value
N%	Buds	2.00	0.30	1.95	0.20	1.85	0.19	ns	0.905
	Spurs	1.31	0.06	1.13	0.08	1.22	0.05	ns	0.204
	Wood	0.26	0.02	0.26	0.04	0.28	0.03	ns	0.880
	Bark	1.12	0.11	0.93	0.03	1.01	0.05	ns	0.233
NDP%	Buds	0.003 a	0.001	0.064 b	0.004	0.150 c	0.010		0.000
	Spurs	0.001 a	0.001	0.124 b	0.029	0.154 b	0.018		0.001
	Wood	0.004 a	0.001	0.019 a	0.002	0.043 b	0.009		0.002
	Bark	0.002 a	0.001	0.065 b	0.008	0.119 c	0.010		0.000

Fruit quality and bioactive properties.

Table 24 summarizes fruit quality parameters. Pre-harvest L-proline application commencing one month prior to harvest resulted in significant changes for parameters stem retention ($p<0.001$), the colour parameters a* ($p=0.008$), b* ($p=0.013$) and Hue° ($p=0.014$), and compression firmness ($p=0.033$). Close to significance were colour chart ($p=0.099$) and skin puncture ($p=0.051$). These parameters are associated with increased fruit maturity.

Table 24: Fruit quality parameters of untreated and treated (pre-harvest L-proline application) branches at commercial harvest.

Parameter	Untreated	SE	Pre-harvest	SE	LSD ($p<0.05$)	P value
Weight (g)	12.3	0.2	12.2	0.2	ns	0.496
Size (mm)	29.2	0.1	29.1	0.2	ns	0.442
Colour (chart)	4.7	0.1	4.9	0.1	ns	*0.099*
L*(C)	27.9	0.2	27.3	0.2	ns	0.062
a*(C)	26.1	0.4	24.3	0.6		**0.008**
b*(C)	7.1	0.2	6.2	0.3		**0.013**
hue (deg)	14.8	0.2	13.9	0.3		**0.014**
Chroma C*	23.6	0.8	25.1	0.6	ns	0.239
Firmtech (g/mm2)	305.3	3.3	293.7	4.2		**0.033**
Guss flesh (kg)	0.11	0.00	0.12	0.01	ns	0.224
Guss skin (kg)	0.39	0.00	0.37	0.01	ns	*0.051*
Stem pull (g)	895	16	796	20		**0.000**
TSS (°Brix)	15.3	0.2	15.5	0.4	ns	0.638
TA (g/L)	5.9	0.1	5.7	0.1	ns	0.410
Total anthocyanins (mg/100g)	17.6	2.5	18.4	2.1	ns	0.835

Discussion

Leaf uptake

Significantly elevated Atom-% ^{15}N was found from weeks 3 and 9, i.e. after application of the pre- and post-harvest applications, respectively and thereafter remained relatively constant. Levels were significantly higher in the post- than pre-harvest application treatments. These differences in uptake may be due to higher rainfall during and following the three weeks of pre-harvest application resulting in wash-off from the leaf before absorption and incorporation into the leaf structure, or due to re-translocation of N out of leaves to other sinks for N.

Due to the nature of application (foliar spray to mimic orchard practice), a mass balance is particularly difficult. Some L-proline may have been lost to the environment (due to wash-off, not quantifiable), whereas another portion may have been taken up by other parts of the branch.

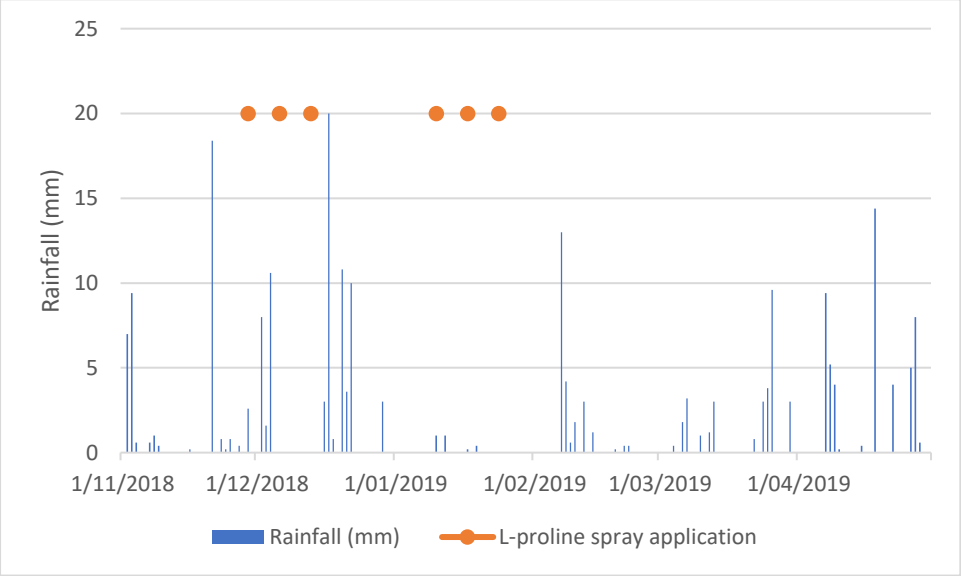

Figure 38: Daily rainfall (mm) for the trial site for November 2018 to April 2019 (source: BOM, Bushy Park Station, accessed 25.11.2020). Orange dots indicate the timing of the three weekly L-proline spray applications commencing 29.11.2018 (pre-harvest application) and 06.01.2019 (post-harvest application).

The analysis of leaves from extension growth segments, which were not subjected to direct spray application, showed no evidence of increased 15N levels (data not shown). This indicates that L-proline itself or N derived from L-proline was not translocated to these tree structures in detectable amounts during the growth phase of the tree, indicating that L-proline is incorporated directly where is has been applied.

No significant differences were observed in total N content for fruit and branch parts between treatments. As N status is effectively controlled through orchard management practices such as soil-applied N and N reserves recycled between seasons its nit surprising that L-proline in the rates applied did not enhance total N content.

Fruit quality and bioactive properties.

Differences in fruit quality parameters revealed a trend towards earlier ripening/maturity of fruit when branches were treated with L-proline (pre-harvest treatment). However, this trend needs to be interpreted cautiously due to small sample sizes (one treated branch per tree) and the general lack of information regarding the right timing and amounts of L-proline application for sweet cherry. To confirm this trend, studies with whole-tree or whole-block replicates, higher number of

treatments (timing, doses) are necessary to evaluate L-proline application as a possible tool for short-term maturity and quality management in cherry orchards.

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Nitrous oxide emissions from applied nitrate fertiliser in commercial cherry orchards

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Abstract. The application of nitrate (NO_3^-) fertiliser is important worldwide in providing nitrogen (N) nutrition to perennial fruit trees. There is little information available on N losses to the environment from commercial cherry orchards, in relation to different timings of NO_3^- application. The emission of nitrous oxide (N_2O) gas is an important greenhouse gas loss from NO_3^- application, being responsible for 6% of anthropogenic global warming and a catalyst for depletion of stratospheric ozone. In a commercial sweet-cherry orchard in southern Tasmania, we applied 373 g NO_3^--N m^{-2} (equivalent to 90 kg NO_3^--N ha^{-1}) either pre- or post-harvest, or equally split between the two, to study the resultant N_2O emissions. Emissions averaged 8.37 mg N_2O-N m^{-2} day^{-1} during the pre-harvest period, primarily driven by a heavy rainfall event, and were significantly greater ($P < 0.05$) than the average 4.88×10^{-1} mg N_2O-N m^{-2} day^{-1} from post-harvest NO_3^- application. Discounting the emissions related to the rainfall event, the resultant average 1.88 mg N_2O-N m^{-2} day^{-1} for the rest of the pre-harvest emissions remained significantly greater ($P < 0.05$) than those post-harvest. Ongoing studies will help to build on these results and efforts to minimise N_2O emissions in perennial tree cropping systems.

Additional keywords: ^{15}N, application timing, nitrate fertiliser, nitrous oxide emissions, soil moisture, sweet cherry.

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Introduction

Nitrogen (N) is essential for plant growth and is the most limiting nutrient for crop production in many of the world's agricultural systems (Fageria and Baligar 2005; Goulding *et al*. 2008). It is primarily the use of artificial fertiliser N, produced by the Haber–Bosch process, that has supported a huge increase in global food production (Fowler *et al*. 2013). It has been estimated that at the beginning of this century almost half of the human population was dependent on food produced with the use of such fertiliser (Erisman *et al*. 2008). World N fertiliser consumption in 2017 was 107.6×10^6 t, having increased from 81.2×10^6, 49.7×10^6 and 23.7×10^6 t in 1997, 1977 and 1967 respectively (International Fertilizer Association 2018). Research has shown that often less than 50% of N applied worldwide is taken up by the main cereal crops (Glass 2003; Good and Beatty 2011), with the remainder lost to the environment as various pollutants (Galloway *et al*. 2003; Cameron *et al*. 2013), one being nitrous oxide (N_2O). As a greenhouse gas, N_2O is responsible for 6% of anthropogenic radiative forcing, i.e. climate warming (IPCC 2013), and is now the most important depleting substance of stratospheric ozone (Ravishankara *et al*. 2009). Soils are the dominant source of global N_2O emissions (56–70%), with those from agricultural fertiliser use and manures constituting nearly half of that total (Butterbach-Bahl *et al*. 2013). From the measured rate of atmospheric accumulation of N_2O, it is estimated that 3–5% of N entering the agricultural cycle (including biologically fixed N) is converted to N_2O (Smith *et al*. 2012). It is reported that 60% of total N_2O emissions to the atmosphere are the product of biological denitrification in soils (Saggar *et al*. 2013). Nitrate (NO_3^-) is the most recognised substrate for this process, which is favoured by high soil moisture. It has also been found, in a study of soils across Europe, that most had their optimum of N_2O emissions under conditions wetter than 80% water-filled pore space (WFPS) (Butterbach-Bahl *et al*. 2013). Soil NO_3^- content has been found to be one of the key factors affecting N_2O emissions from agricultural soils, in combination with high WFPS and favoured by higher soil temperatures (Dobbie and Smith 2003).

Perennial fruit crops, although occupying only 1% of global agricultural land, are of considerable economic importance. The N use efficiency of such crops is generally lower than 55% and mineral fertiliser N is by far the dominant source of N nutrition (Carranca *et al*. 2018). In many, if not the great majority of instances, the N fertiliser used is calcium nitrate ($Ca(NO_3)_2$). Although a convenient and economic source of both N and calcium, it is well recognised that the NO_3^- form of N is easily taken up by the plants but can also leach through the soil profile, or be emitted as N_2O following denitrification.

Nitrate pollution of water is a serious problem for environmental and human health (Erisman *et al*. 2013; Temkin *et al*. 2019). There are few published studies related to the application of only the NO_3^- form of N fertilisers to perennial fruit trees; more relate to the application of ammonium nitrate, but its availability is now highly restricted. In the particular case of sweet cherry (*Prunus avium* L.) production, one study examined leaching of applied NO_3^-, to which it is highly prone (San Martino *et al*. 2014). A separate study measured N_2O emissions in a Tasmanian cherry orchard fertilised with $Ca(NO_3)_2$ (Swarts *et al*. 2016). Neither study compared the uptake or associated losses of NO_3^- fertilisers applied at different times of the season. In sweet cherry production, advice to growers often advocates applying a considerable portion of annual N post-harvest (e.g. Cherry Growers of Australia Inc. 2011), with the belief that it will substantially bolster winter-stored N. Two studies have shown reduced uptake of post-harvest applied N, compared with that applied pre-harvest to cherries, but neither used the purely NO_3^- form of N (Azarenko *et al*. 2008; Rivera *et al*. 2016). This study aims to better examine the fate of unexploited N by measuring N_2O emissions in relation to different timings of NO_3^- application. We compared N_2O emissions in a commercial sweet-cherry orchard following spring (pre-harvest) and summer (post-harvest) fertiliser applications to determine the main drivers of emission production in a perennial fruit tree production system.

Materials and method

Fertiliser application

The research trial was conducted in a commercial sweet-cherry orchard in southern Tasmania (42.7099°S, 146.9436°E) in a cool temperate climate. Annual rainfall for the region averages 572 mm (Bureau of Meteorology 2019) with that in summer generally less than in other seasons (Fig. 1). Sixteen five-year-old 'Lapins' cherry trees on Colt rootstock, grown with a Spanish bush training system, were provided for the trial by the grower along with a minimum of one similar 'buffer' tree on either side of each trial tree. Due to the constraints of commercial orchard management, the trial was established down one row, which ran down a gentle, north-easterly facing slope. Four randomly allocated N treatments were replicated in a four-block design within the row. Soil in the upper block (Block 1) was a Vertosol (Isbell 2002), with a relatively shallow A horizon. This continued down the hill slope, transitioning to a texture contrast soil with a deeper A horizon at the bottom of the row. A total of 67.5 g tree^{-1} of N (373 g N m^{-2}, or 90 kg N ha^{-1} on a broadacre basis) was applied by drip fertigation as 5.5 atom% ^{15}N-$Ca(NO_3)_2$, in four equal applications. Application was through a dedicated system of pressure-compensated dripper line (Netafim Uniram™ AS, 2.30 L h^{-1} @ 100 kPa, 14.2 mm ID) with four drippers per tree placed symmetrically around the trunk (Fig. 2). The system was supplied from a 12-V pump (SHURflo 4009–101-A87, 11.3 L min^{-1}), fitted with a backflow to provide constant mixing of the supply tank, that fed the line at an output pressure of 70 kPa, and tested for consistency of output per tree. Including phases of pre-wetting and line flushing with water, each dripper received 1.21 L of liquid per treatment application. Treatments were applied pre-harvest, post-harvest or split 50 : 50 between the two, with the timing of application shown in Table 1. An additional split treatment at a half-rate of 33.75 g N tree^{-1} was applied to four trees in an adjacent row, each being opposite to a tree block in the main row. No other N was applied throughout the duration of the trial, with all other nutrient application according to orchard management practice, as was pest and weed control. Irrigation was applied every second day by dripper lines (Netafim, 2.30 L h^{-1}, as above, 0.5 m dripper spacing) on either side of the tree line (Fig. 2). From before the commencement of the trial until 14 December each tree received, from each dripper line, an average of 67 L week^{-1}, then 81 L week^{-1} until 9 February and thereafter 54 L week^{-1}.

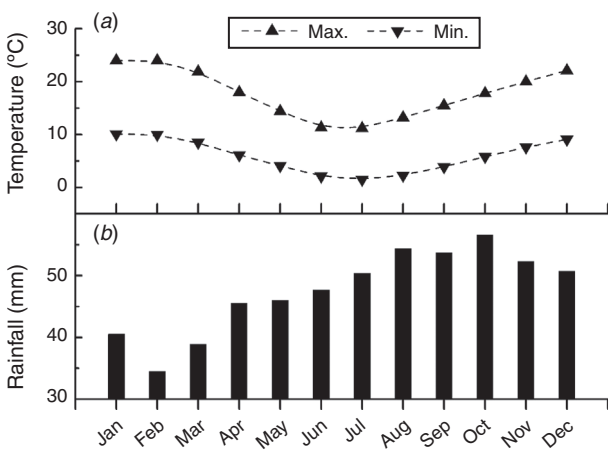

Fig. 1. Monthly climate averages: (*a*) maximum and minimum temperatures (1931–2018); (*b*) rainfall (1874–2018) (Bureau of Meteorology 2019).

Gas sampling

Gas samples were taken using static chambers (240 mm diameter × 280 mm in height), with one dripper centrally placed in each chamber and its supply tubing sealed on passage through the chamber wall. Mounting collars were inserted 100 mm into the soil, and each had three rows of 3.6-mm holes to allow for root penetration. Removable lids were covered with Mylar to minimise the influence of heating from insolation and fitted with air-stones for passive pressure compensation. Each lid was fitted with a butyl rubber septum to enable sampling of headspace gas. Background air samples were taken at $t = 0$ and from each chamber following 45 and 90 min of lid closure, with a 25-mL gas-tight syringe (SGE 25MDR-LL-GT) fitted with a 25G × $^3/_4''$ needle. All samples were immediately transferred via the needle into pre-evacuated 12-mL Exetainer® vials with rubber septa. Analysis of all samples was by gas chromatography (Shimadzu GC-2014). Samples of highest N_2O fluxes were further analysed for $^{15}N_2O$ using an automated isotope ratio mass spectrometer (Sercon Limited, 20–20, UK). The proportion of N_2O derived from N fertiliser was calculated as the ratio of the ^{15}N atom excess of N_2O and

applied ^{15}N-$Ca(NO_3)_2$. The ^{15}N abundance in N_2O further allowed determination of the contributions of denitrification and nitrification to N_2O emissions (Arah 1997), when N_2O concentration and ^{15}N enrichment were above the respective detection limits. Sampling commenced one day before each pre- and post-harvest application cycle (Table 1), until 3 weeks after the final N application of each cycle by which time related emissions were expected to be minimal (Bateman and Baggs 2005). Sampling took place at least twice weekly, with more intensive sampling during the initial period following each N application and each substantial rainfall event (≥10 mm in preceding 24 h).

Soil samples, soil moisture, rainfall and statistical analysis

Soil samples were taken at two depths within each block before treatment application (Table 2); each analysed sample being homogenised from four randomly located samples from within the tree line. Soil NO_3^- and ammonium (NH_4^+) were colourimetrically determined with a SmartChem analyser (Westco Scientific Instruments) following 2 M KCl extraction. Soil samples (20–30 mg) were finely ground using a ball mill (Retsch MM200, Dusseldorf, Germany) and analysed for total N (%) and total carbon (C) (%) using a Perkin Elmer 2400 Series II Elemental Analyser. Soil moisture was measured half-hourly at depths of 10 and 20 cm within the tree line, mid-way within the main tree row (Fig. 2, Sentek Drill and Drop). Data-logger malfunction resulted in values not being available for the early part of the trial (Fig. 3*c*). Soil cores were taken nearby from these depths to calculate soil bulk density (BD), with percentage porosity of soil (ϕ) then estimated as (1 – BD/2.65) × 100, where 2.65 g cm^{-3} was used as the mean density of soil particles (Robertson and Groffman 2007). Measured volumetric water content (θ) was converted to estimated WFPS (%) using:

$$\text{WFPS}(\%) = \theta/\phi \times 100$$

Rainfall statistics were based on a site 2.9 km to the west of the orchard, at a similar altitude (Bureau of Meteorology 2019). Gas fluxes of N_2O and CO_2 were calculated by the method of van Zwieten *et al.* (2010). The ratios of calculated fluxes for 45- and 90-min chamber closure times, as well as the correlations within each series for each treatment, were examined to test for linearity of gas emission rates. The longer time frame was used for all resultant calculations. Total gas emissions for each period were calculated by the trapezoidal method (Weideman 2002), from 17 sampling events before harvest and 12 post-harvest on account of drier soil conditions in the latter period. Mean values of calculated total emissions were compared by ANOVA in SAS® 9.4, with least significant differences calculated at a 95% confidence interval. ANOVA was used to evaluate any block effects, with the block mean square being tested, using an F-test, against the error mean square.

Table 1. Calcium nitrate fertiliser treatment applications pre- and post-harvest (harvest on 9 January 2018)

Treatment code	Time of NO_3^- fertiliser application	Number and timing of fertiliser applications
1	None – control	0
2	Pre-harvest	4, weekly (from 8 Nov. 2017)
3	Post-harvest	4, weekly (from 17 Jan. 2018)
4	Split 50 : 50	2 and 2, fortnightly (from above dates)
5	Split 50 : 50 × 0.5N	2 and 2, fortnightly (from above dates)

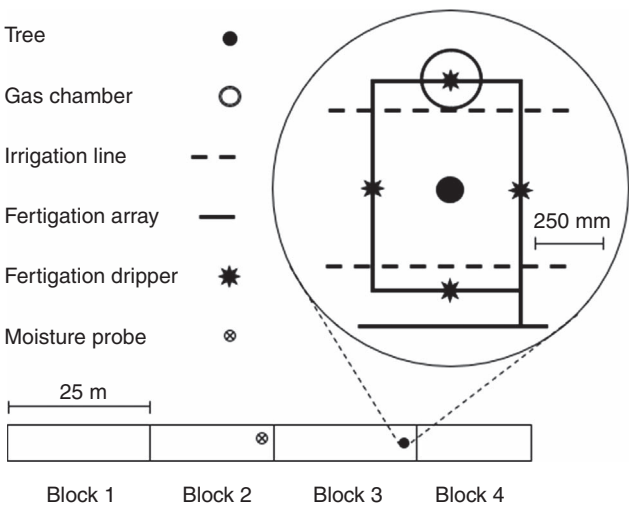

Fig. 2. Depiction of the site showing the position of the soil moisture probe within the blocks of replicates and an example of the positioning of a gas sampling chamber and fertigation drippers.

Table 2. Selected soil chemical properties at two depths of each tree block, before any treatment application

Tree block	Sample depth (cm)	pH 1 : 5, H_2O	Total C (LECO) (%)	Total N (LECO) (%)	NH_4^+-N (mg kg^{-1} soil)	NO_3^--N (mg kg^{-1} soil)
1	0–10	6.27	3.19	0.27	8.0	5.5
2	0–10	5.87	1.90	0.18	2.0	2.8
3	0–10	5.36	1.93	0.18	3.7	3.1
4	0–10	5.38	1.98	0.18	4.2	4.9
1	20–30	6.86	2.54	0.22	5.1	5.4
2	20–30	5.82	1.30	0.11	2.7	1.1
3	20–30	5.50	1.14	0.11	2.8	3.0
4	20–30	5.84	1.17	0.09	3.5	1.7

Results

Soil

Soils in Block 1 differed from the other three blocks, in that they were close to neutral pH vs mildly acidic. This block also had substantially higher concentrations of total C, total N, NH_4^+-N and NO_3^--N for both layers analysed (Table 2).

N_2O emissions

For each treatment, the ratio on each sampling day and correlations for each series of days between fluxes for 45- and 90-min chamber closure times were calculated (Table 3).

Emissions of N_2O from treatments applied pre-harvest (treatments 1, 2, 4 and 5: Table 1), along with associated rainfall and soil moisture, are shown in Fig. 3. Similarly, emissions for treatments applied post-harvest (treatments 1, 3, 4 and 5) are shown in Fig. 4. There was no significant effect on N_2O emissions across treatments related to (tree) block position. For the period of highest measured fluxes pre-harvest (Fig. 3 – 6, 8, 12 and 15 December 2017), on each of these days only the N_2O emitted from the pre-harvest treatment (2) was significantly higher ($P < 0.05$) than that from the control treatment (1), due to large variance between replicates in all treatments. There were no significant differences in measured N_2O emissions from any treatments on any other days of pre-harvest measurement. Post-harvest N_2O emissions did not show the noticeable peak of those pre-harvest but also possessed the large variation between treatment replicates. Only on the first day of post-harvest measurement (following NO_3^- application the previous day) was there a significant difference in N_2O emissions between any treatments, with that from all those with N applied being significantly greater ($P < 0.05$) than from controls but not between each other.

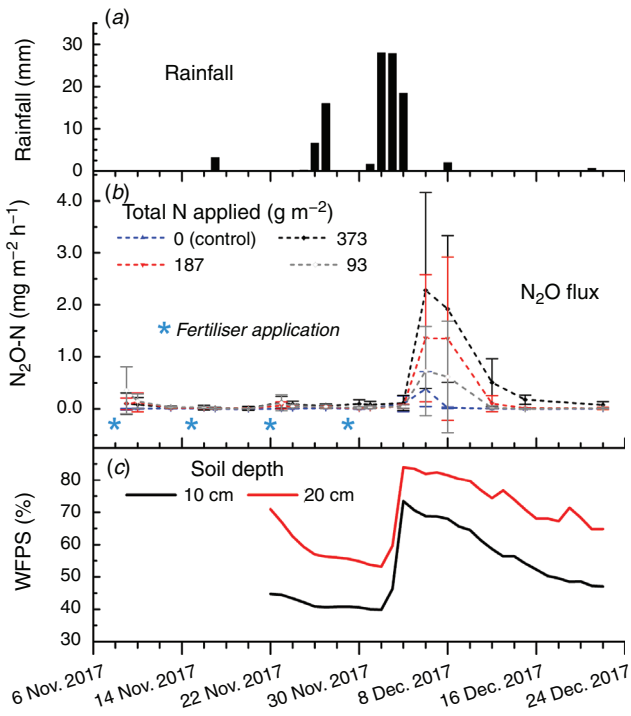

Fig. 3. (*a*) Rainfall for 24 h to 0900 hours during the pre-harvest period of emission sampling; (*b*) N_2O flux rates for each sampling day during the period (error bars represent ± 1.96 × s.e.m., $n = 4$); (*c*) soil water-filled pore space (WFPS) at 0900 hours at two depths.

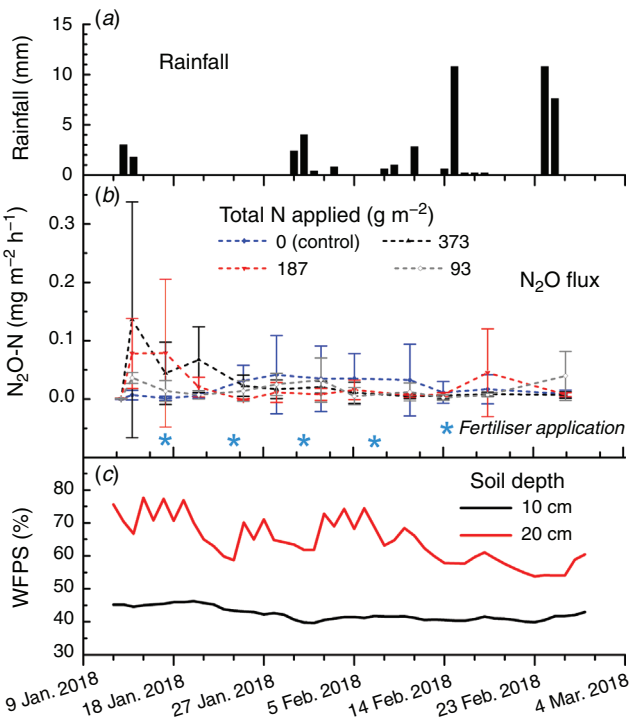

Fig. 4. (*a*) Rainfall for 24 h to 0900 hours during the post-harvest period of emission sampling; (*b*) N_2O flux rates for each sampling day during the period (error bars represent ± 1.96 × s.e.m., $n = 4$); (*c*) soil water-filled pore space (WFPS) at 0900 hours at two depths.

Table 3. Mean of the ratios between N_2O fluxes calculated for 45- and 90-min chamber closure times (F45 and F90 respectively), for each sampling period and correlations (Pearson's = R) within each series between 45- and 90-min calculated fluxes (*P*-level of R in parentheses) (Social Science Statistics 2019). Treatment codes (refer Table 1) are in parentheses following titles

Pre-harvest measurements	Control (1)	Pre-harvest (2)	Split 50 : 50 (4)	Split 0.5N (5)
Mean F45/F90 (s.e.m.)	1.16 (0.19)	1.07 (0.16)	0.98 (0.07)	1.09 (0.08)
R (*P*)	1.00 (<0.00001)	1.00 (<0.00001)	1.00 (<0.00001)	1.00 (<0.00001)
Post-harvest measurements		Post-harvest (3)		
Mean F45/F90 (s.e.m.)	–1.03 (0.83)	0.96 (0.18)	1.30 (0.34)	1.03 (1.03)
R (*P*)	–0.23 (0.4661)	0.81 (0.0015)	0.55 (0.0616)	0.99 (<0.00001)

Total N_2O emissions for the pre- and post-harvest periods are shown in Fig. 5*a* and *b* respectively. Total emissions from the pre-harvest application treatment were significantly greater ($P < 0.05$) than those from the control treatment. This was also the case when the comparison only covered the 11-day period of elevated emissions. There were no other significant differences between treatments within either pre- or post-harvest emission totals. Mean N_2O emissions per day from the pre-harvest full N application treatment (2 in Table 1), of 8.37 mg N_2O-N m^{-2} day^{-1}, were significantly greater ($P < 0.05$) than the 4.88×10^{-1} mg N_2O-N m^{-2} day^{-1} from the equivalent post-harvest treatment (3). The mean pre-harvest emissions for the 32 days outside the peak 11-day period, of 1.88 mg N_2O-N m^{-2} day^{-1} for the full N treatment, were also significantly greater ($P < 0.05$) than the mean emissions from the equivalent post-harvest treatment.

Contribution of N fertiliser to N_2O emissions and N_2O source partitioning

The contribution of N fertiliser to N_2O emissions is shown in Fig. 6. During 4–15 December 2017, 86.3, 50.9 and 16.6% of emitted N_2O was derived from the applied N fertiliser in the pre-harvest full N, split N and split 0.5N treatments respectively. During this period, denitrification from all soil sources was the main pathway of N_2O production for the pre-harvest full N treatment, accounting for 60–98% of emitted N_2O (Fig. 7). In the split N treatment, denitrification dominated N_2O production only on 6 and 8 December 2017, with nitrification as the main N_2O production pathway for the remaining days. In the split 0.5N treatment and pre-harvest control, nitrification accounted for >60% and >90% respectively of overall N_2O emissions from this period (Fig. 7).

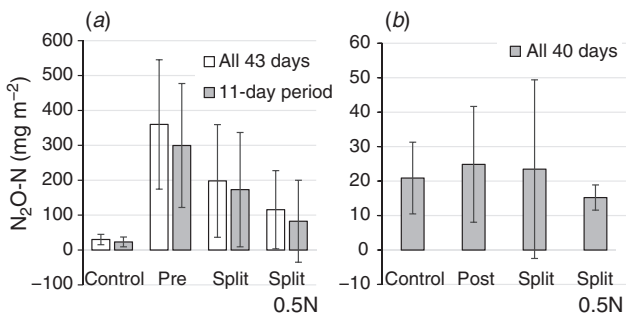

Fig. 5. (*a*) Total of pre-harvest N_2O emissions and that during the 11-day period of elevated emissions (refer Fig. 3); (*b*) total of post-harvest N_2O emissions (error bars represent ± 1.96 × s.e.m., $n = 4$).

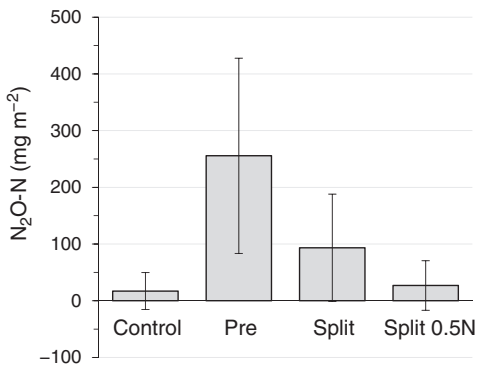

Fig. 6. Emission of N_2O-N from applied fertiliser during the period of pre-harvest peak emissions, 4–15 December 2017 (error bars represent ± 1.96 × s.e.m., $n = 4$).

Discussion

The environmental implications of N fertilisation in cool-climate perennial tree crop production, such as sweet cherry growing, are poorly understood. Nitrous oxide emissions from such production systems have received particularly little attention. As the dominant form of N fertiliser used in these systems, NO_3^- is recognised for its potential to produce substantial emissions of N_2O by the pathway of denitrification. We found that the timing of NO_3^- application relative to cherry harvest (Table 1) can significantly affect N_2O emissions through the influence of seasonal rainfall events on denitrification. However, standard orchard irrigation practices had minimal impact on emissions.

The mean daily N_2O emissions from the full N pre-harvest treatment, of 8.37 mg N_2O-N m^{-2} day^{-1}, was dominated by those from the period 4–15 December 2017 (Fig. 3), with a mean of 27.2 mg N_2O-N m^{-2} day^{-1}, leaving a mean of 1.88 mg N_2O-N m^{-2} day^{-1} for the remainder of the 43-day period. All were significantly greater ($P < 0.05$) than the mean 4.88×10^{-1} mg N_2O-N m^{-2} day^{-1} from the equivalent full N post-harvest treatment. The 11-day period of elevated N_2O emissions in all pre-harvest treatments clearly coincided with the marked increase in soil WFPS, at both monitored depths to >70% (Fig. 3*c*), following sustained rainfall of 74 mm in three days. These conditions were highly favourable to denitrification. It is well recognised that denitrification is facilitated by anaerobic soil conditions and that, depending on soil type, N_2O emissions rise sharply when WFPS exceeds ~65–70% (Dobbie and Smith 2003; Bateman and Baggs 2005; Butterbach-Bahl *et al.* 2013). Undoubtedly, this was the driving factor for 86% of N_2O emissions in this high-

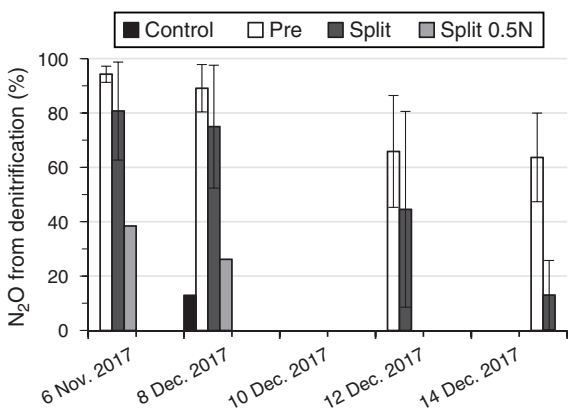

Fig. 7. The percentage (where available) of emitted N_2O resulting from the process of denitrification, as calculated by the method of Arah (1997) (error bars represent ± 1.96 × s.e.m., $n = 4$; control and split 0.5N data represent only one and two measurements respectively).

emission period from the full N treatment being from denitrification of applied NO_3^-, and 51% by the same pathway from the split N treatment. In the latter case it is worth noting that NO_3^- had been applied in a lesser quantity and to an earlier mean timeframe than for the full N treatment (Table 1), so that by the time of increased WFPS considerably less applied NO_3^- was likely to be available for denitrification than from the full N treatment. Determined by the method of Arah (1997), the majority of N_2O emissions from the split 0.5N and control treatments during the pre-harvest period of high WFPS were from a pathway of nitrification (of NH_4^+), with the presence of NO_3^- (applied or pre-existing) insufficient to make a major contribution to N_2O production through denitrification. As soil WFPS, both pre- and post-harvest, was generally below that of the period 4–15 December 2017 (Figs 3 and 4), and therefore less favourable to denitrification, it is likely that much of the lower emissions of N_2O throughout the whole trial were also produced by nitrification of soil NH_4^+ (Table 2). In the post-harvest, drier summer period, the deeper soil maintained some periods of >70% WFPS, and many of the sharp increases at that level matched the pattern of irrigation every second day (Fig. 4*c*). By contrast the shallow soil was consistently not above ~45% WFPS. This suggests, as the pre-harvest N_2O emissions from all applied N treatments were an order of magnitude higher N_2O than those from the corresponding post-harvest treatments (Fig. 5), that the higher emissions were predominantly the result of denitrification of NO_3^- in the upper soil layer. Furthermore, it further confirms that the heavy pre-harvest rainfall event drove those emissions and that regular irrigation had little such effect.

The level of ^{15}N enrichment of applied NO_3^- was insufficient to determine if denitrification produced any dinitrogen (N_2) gas during the period of highest N_2O emissions (Friedl *et al*. 2018). Nonetheless, if N_2 was produced, it seems unlikely that sufficiently high soil WFPS (and anaerobicity) was sustained long enough to produce it in quantities approaching those of N_2O (Butterbach-Bahl *et al*. 2013; Saggar *et al*. 2013).

The 1.21 L of water applied per chamber with each weekly or fortnightly fertigation session was not considered to significantly contribute to soil WFPS. It constituted 1.48–2.20% of the total amount of water applied weekly by the grower through the irrigation dripper line on the side of the tree row on which the chambers were positioned (Fig. 2). The variation was dependent upon the length of irrigation time, and half of those proportions applied when both irrigation lines were considered. Thus, it was considered that the soil moisture within the chambers was well representative of that within the broader production system and that likewise, N_2O emissions for each treatment well represented those of the broader in-row system.

In another drip-irrigated Tasmanian cherry orchard, NO_3^- was applied to chambers identical to those used in this trial (Swarts *et al*. 2016). Application was also by a centrally located dripper, over six fortnightly applications commencing in late October, at double the total rate of N applied per chamber in this trial (746 vs 373 g NO_3^--N m^{-2}). The mean in-row emissions for that trial were 1.31×10^{-1} mg N_2O-N m^{-2} day^{-1}, significantly less ($P < 0.05$) than even the mean of 1.88 mg N_2O-N m^{-2} day^{-1} for the pre-harvest period of this trial with those from the high-emission days removed. One noticeable difference from this trial was that there were no daily rain events in excess of 10 mm until the June following fertiliser application, and that from 60 days recording in that trial, 40% < WFPS < 80% at 5–10 cm depth. Consequently, it is quite possible that a considerable proportion of emitted N_2O in that trial came from nitrification of soil NH_4^+, which was not reported, rather than from the applied NO_3^-.

The calculated total N_2O-N emitted from the full N pre-harvest treatment constituted 2.1% of NO_3^--N applied, exceeding the default IPCC emission factor (EF) of 1.0% of applied synthetic fertiliser N directly emitted as N_2O-N (IPCC 2019), and outside of its uncertainty range of 0.1–1.8% ($P < 0.05$). When the high emissions for the period 4–15 December 2017 are excluded from the calculations, the EF for the full N pre-harvest treatment falls to 0.36%, emphasising the impact of the period of denitrification-driven high emissions. Although not strictly scalable from a tree-row system, on a broadacre basis the mean emissions for the full N pre-harvest treatment equate to 83.7 g N_2O-N ha^{-1} day^{-1}, far less than the 1200 g N_2O-N ha^{-1} day^{-1} from a grassland site in England (Dobbie and Smith 2003) or an even higher 13.3 kg N_2O-N ha^{-1} day^{-1} from an Australian sugarcane site (Dalal *et al*. 2003). Nonetheless, in order to lessen environmental pollution and enhance the efficient use of N it is important to attempt to decrease N_2O emissions from all cropping systems.

The substantial standard errors associated with many of the calculated mean N_2O fluxes are common to N_2O emission measurements. It is widely recognised that N_2O emissions have large spatial and temporal variation (Yanai *et al*. 2003; Butterbach-Bahl *et al*. 2013; IPCC 2013), which increases the difficulty of distinguishing significant differences between applied treatments. This trial was no exception, even when mean values between treatments appeared substantially different. A greater number of replicates might have reduced the size of standard errors, thus helping to better distinguish any significant differences between treatments. Similarly, more frequent sampling could have given a more precise estimate of total emissions for each of the two sampling periods. Unfortunately, practical considerations precluded such options.

The ratios of mean N_2O fluxes for 45- and 90-min chamber closure times (Table 3), when considered with their standard errors, were in all cases not significantly different from a ratio of 1.00. Such a value would represent, based on the two measurements, a completely linear rate of emission. In addition, the extremely high degree of correlation between the two time-closure series for each of the pre-harvest treatments and the split 0.5N post-harvest treatment suggests that, for these treatments, the emission rates of N_2O over the full 90-min period were very close to linear. The correlation values for the post-harvest full N and split N application treatments are less definite but, in consideration of the aforementioned ratios, strong enough to suggest that the overall emission rates for these treatments were close to linear.

The negative values associated with the post-harvest control treatment (Table 3) resulted from nine of the 12 values for 45-min fluxes for the treatment being negative and all of those for the 90-min fluxes being positive. Negative N_2O fluxes, suggesting the soil acted as a sink for N_2O have previously been reported (Thomson *et al*. 2012; Oertel *et al*. 2016) but are unusual. In this case we consider that the negative values in question, being of such low absolute magnitude, were likely related to insufficient sensitivity of measurement and that the 90-min fluxes calculated for this treatment must be regarded as a fair comparison with those of other post-harvest treatments. As a result, in future work we will likely dispense with an intermediate measurement, sampling only at $t = 0$ and at a maximum time, suitably determined by pre-trial tests.

We conclude that, to minimise N_2O emissions from cherry and similar tree cropping systems where NO_3^- is the form of N fertiliser applied, it is of primary importance to recognise the potential for denitrification. Clearly, application of NO_3^- fertiliser should be avoided if a substantial rain event is imminent, both to minimise its loss as N_2O and through leaching of NO_3^-. With long-term weather forecasting a difficult exercise, frequent application of smaller quantities of NO_3^- would be preferable to larger quantities applied less often. Regular soil testing, for both soil NO_3^- and NH_4^+ would aid a careful assessment of seasonal crop requirements, enabling N supply to match tree demand and avoiding build-up of excessive soil N. Such an approach can assist in reducing the harmful effects of N loss and help meet the increasingly stringent demands associated with all forms of agricultural production.

Conflicts of interest

The authors report no conflicts of interest, of financial or other kind, in the presentation of this work.

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