Australian Government
Department of Agriculture, Water and the Environment

CRDC

Rural R&D for Profit Program

More Profit from Nitrogen

RRDP1720 (July 2016 - June 2021)

Optimising nutrient management for improved productivity and fruit quality in mangoes

Final Report

31 August 2021

Report prepared by: Dr Joanne Tilbrook[1], Alan Niscioli[1], Dr David Rowlings[2] and Dr Constancio Asis[1]

[1]**Northern Territory Department of Industry, Tourism and Trade**

[2]Queensland University of Technology.

Hort Innovation
Strategic levy investment

MANGO FUND

NORTHERN TERRITORY GOVERNMENT

QUT Queensland University of Technology

AUSTRALIAN MANGOES

This publication (and any material sourced from it) should be attributed as: Tilbrook, J., Niscioli, A., Rowlings, D. & Asis, C., 2021, Optimising nutrient management for improved productivity and fruit quality in mango (RRDP1720), *Final report submitted to the Cotton Research and Development Corporation - More Profit from Nitrogen Program*, CRDC, Narrabri NSW 2390, Australia.

This publication is available at https://www.crdc.com.au/more-profit-nitrogen

Acknowledgements

This project was supported by funding from the Australian Government Department of Agriculture, Water and the Environment as part of its Rural R&D for Profit program, Hort Innovation using the mango research and development levy and funds and contributions from the Australian Government, Northern Territory Department of Industry, Tourism and Trade, Queensland University of Technology and the Australian Mango Industry Association.

We would like to acknowledge Dr Mila Bristow, who initiated the collaboration with Dr Dave Rowlings and negotiated support and funding for the project. She was project leader from 2016-2018, creating the direction and structure of the project and contributed significantly to the outcomes.

Thanks are due to all Northern Territory commercial mango growers for their continuing support and assistance during this and other mango research work. In particular: Nutrano Produce Group-Katherine Farm, Acacia Hills Farm, Tou's Garden, Jabiru Tropical Orchards, NTLD Darwin and Katherine.

Acknowledgement and particular thanks to Dallas Anson and Alan Niscioli who contributed to every part of the project in multiple ways and worked many hard hours in the heat and humidity. Thank you to Heather Wallace, Dr Danilo Guinto and Heshan Jayasekara of Plant Industries, NT DITT. Thanks also to (Hemant) Raj Pandeya, Sarah Carrick, Ella Dewilde and Dr Johannes Friedl of Sustainable Agriculture, Centre for Agriculture and Bioeconomy, QUT for field and extensive laboratory work. The work was wide ranging and highly appreciated. Finally, Dr Mark Hearnden, Dr M. Sohail Mazhar and Dr Matt Hall of NT DITT for reviewing the report.

Project team details

Provide details of all personnel involved in the project.

Name	Position	Organisation	Role	Duration of involvement
Mila Bristow	Project leader	DITT	Overall responsibility for project governance including contractual arrangements. Approves the Project Plan, lends support at senior levels, and ensures that the necessary resources (both financial and human) are available to the project. Responsible for recruitment, resolving planning and implementation issues and monitoring the project's progress and budget, Implementation of the communications strategy.	Dec 2016 – Aug 2018
Matt Hall	Project leader Extension	DITT	As above Assist with extension of project output, grower liaison and communication.	Aug 2018 – July 2021 April 2018 – Aug 2018
Constancio (Tony) Asis	Plant nutrition scientist	DITT	Lead in the conduct of field experiments in the research stations and commercial growers' orchards. Ensure methodologies comply with best practice and are relevant to the region. Assist in the preparation of report.	Dec 2016 – July 2021
David Rowlings	Soil scientist and QUT project leader	QUT	Responsible for the QUT component of the project including science direction, PhD supervision, lab methodologies and analysis, and preparation of report.	Dec 2016 – July 2021
Joanne (Jo) Tilbrook	Project scientist	DITT	Cooperatively design and implement experiments to address the research objectives. Collect and analyse the data for discussion. Prepare report for communication to the stakeholders.	Feb 2017 – July 2021
Danilo Guinto	Research scientist	DITT	Assist in the conduct of field experiment in Katherine and coordinate with mango growers.	May 2018 – Jan 2020
Alan Niscioli	Senior technical officer	DITT	Technical leadership across the project, coordinate technical staff on all aspects of the project and maintain grower liaison/ industry engagement.	Dec 2018 – July 2021
Dallas Anson	Technical officer	DITT	Provide technical assistance with trial implementation and management.	April 2017 – March 2020
Heshan Jayasekara	Technical officer	DITT	Technical assistance with trial management.	Aug 2018 – Jan 2020
Cliff Hansen	Executive officer	DITT	Financial accounting and financial report preparation.	Dec 2016 – July 2021

Contents

Abbreviations and glossary

ANOVA, analysis of variance

B74, Calypso ® mango variety

BBCH Scale, Biologische Bundesantalt, Bundessortenamt und Chemische Industrie Scale

CDU, Charles Darwin University

CEC, cation exchange capacity

$CO(NH_2)_2$, urea

CPRF, Coastal Plains Research Farm

C, carbon

dff, derived from fertiliser

dfi, derived from infusion

DM, dry matter

DW, dry weight

EF, emission factors

FW, fresh weight

IPCC, Intergovernmental Panel on Climate Change

KRS, Katherine Research Station

LSD, least significant difference

N, nitrogen

$(NH_4)_2SO_4$, ammonium sulfate

N_2O, nitrous oxide

NT, Northern Territory

NUE, nitrogen uptake efficiency

RE, resorption efficiency

RP, resorption proficiency

sem, standard error of the mean

Plain English Executive Summary

Nitrogen (N) is an essential tree crop nutrient. N fertiliser inputs are a significant portion of farm production costs and influence fruit production and quality. Mangoes are a significant crop in tropical climates of Australia with a value of over $200 million annually. Half are produced in the Northern Territory (NT). Almost no data is available for Australian mango growing regions, either on the relative importance of the soil N process or on total N losses from current management practices. 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 management options.

This project used an integrated approach to quantify plant N demand and cycling through the soil-plant-atmosphere system to increase understanding of how N impacts on the quantity and quality of mango yields. The objectives were to build an understanding of how growers might optimise nitrogen use efficiency, and how N supply through mineralisation in the soil can improve resource use in mango crop production.

To do this, stable isotopes of N were used to quantify N uptake of soil-applied fertiliser, N uptake across the leaf cuticle, and to track N as leaf litter decomposed. To measure the timing and amounts of N released into forms available for the roots to access, intact soil cores were excavated over a year in orchards and analysed in laboratories. A field laboratory was constructed in a mango orchard and gaseous emissions collected and analysed on site, with data remotely accessible in real time. Leaf litter and pruned materials were collected in commercial orchards and analysed to understand the quantities of nutrients cycling in orchards annually. Varying amounts of N fertiliser were applied in orchards to measure how fruit yield and quality were affected. From this work we found:

- The N uptake efficiency of soil-applied fertiliser of mature mango trees decreases as the quantities applied increase.
- Spraying a dilute solution of N onto mango tree leaves is a comparatively efficient way to supplement N into trees. Any N not taken up can be recycled within the orchard.
- Nitrogen in mango trees is highly mobile and is transported around trees rapidly via xylem and phloem, including leaves to roots.
- In a mature orchard, litter and pruned material contains about 20 kg N per hectare. This decomposes annually (100 % in Darwin and 85 % in Katherine). The litter N becomes available in the top 20 cm of soil during the build-up and wet season (~11 kg per hectare in Darwin and 17 kg per hectare in Katherine). It is a short-term N bank for trees to access. What is not used is lost each year.
- Emissions of the greenhouse gas nitrous oxide (N_2O) from litter and fertiliser are well below the Tier 2 Intergovernmental Panel on Climate Change (IPCC) limits for intensive horticulture in Australia.
- Harvested fruit takes about 0.8 to 1.0 kg N per tonne as it leaves the orchard. Supplying too much N for a particular harvest yield causes the skin of mango fruit to stay green when ripe. Fruit from trees receiving no applied N contain 0.4 kg N per tonne.
- Soils in NT mango orchards have minimal texture and structure, with a low capacity to store N or carbon over the medium or long term.

This work directly quantifies, for the first time, N uptake and cycling in NT mango orchards so N inputs can be refined in terms of quantity and timing. Recommendations to the industry are made in the four R context as set out below.

Right time

Fertiliser should be applied to soils post-harvest, during the active growth phase of trees, and approaching the monsoon period. This coincides with the reactivation of soil macro and microfauna as moisture levels increase with break of season rains. Avoid applying N to soils or via fertigation (dissolved fertiliser delivered to trees through the watering system) when soils are waterlogged during the wet season, and during the dormant or quiescent period as trees approach flower induction. Foliar application of N (a solution of dissolved fertiliser applied to leaves as a spray) can occur at any time when rain is not expected.

Right form

Commercially available fertilisers are recommended. Minimal N_2O emissions were measured from decomposing litter and urea. Enhanced efficiency fertilisers show limited economic or environmental benefit in NT weather conditions. Soil amendments such as zeolite or biochar mixed into topsoil show some potential to retain nutrients over time but are currently cost prohibitive.

Right place

Placement of fertiliser depends on the type being applied. Soil-applied fertiliser should be placed under the drip line of the canopy, where tree feeder roots can easily access it. Avoid placing close to tree stems. Fertigation will depend on the orchard irrigation in place, pressure in the system and the radius of the sprinkler throw. Foliar applications should be made using spray equipment that is correctly calibrated to deliver the desired volume of N to the canopy of each tree.

Right amount

This will vary according to location, soils, leaf and soil analysis results, seasonal conditions and yield history. Soil-applied N uptake efficiency decreases markedly as the quantities of applied N increase. Extra or 'insurance' N is washed beyond tree roots during the wet season but may still impact on fruit quality. For each orchard, growers need to know the relationship between excess N supply, yield and 'stay green' skin when ripe. Consider how much N left the orchard in fruit, how much N is cycling in litter and available in the top 20 cm of soil during active growth, and predicted yield for the next season. Fertigation and foliar application are efficient ways to add N in orchards when soils are not waterlogged. Monitor soil and tree health as usual to avoid nutrient mining or a negative nutrient balance in orchards over time.

This research provides new evidence for when and where N is available in soils for uptake and when it is lost, how much N is taken up by foliar application, and how much N and other nutrients are cycling annually in orchards. This information can now be developed further for the industry and a calculator constructed to help growers reconsider what are necessary and economic N inputs.

1 Project rationale

This project was led by the Northern Territory Government Department of Industry, Tourism and Trade (DITT) in partnership with Queensland University of Technology (QUT). It used an integrated approach to quantify N demand and cycling through the soil-plant-atmosphere system of mango crops. Management strategies to increase the quantity and quality of mango yields, while effectively mitigating loss of N to the environment, were developed.

Mangoes are a significant tree crop grown in tropical and subtropical climates of Australia. The mango industry in northern Australia relies heavily on the commercial cultivar KP, which has erratic flowering, excessive vigour and low productivity. Recent breeding has led to hybrids, such as the Calypso™ variety, which has higher productivity, but this remains far lower than international mango production. While the exact reasons for this are unknown, it can be attributed to the irregular bearing nature of the tree, seasonal conditions and mineral nutrition problems of the plant.

Nitrogen is essential for mango tree development, fruit production and quality. It is an important regulator involved in many biological processes, including carbon metabolism, amino acid metabolism and protein synthesis. However, the response of mango to N fertiliser application is also influenced by its source, rate, timing, and method of application, tree-growth stage, climate, edaphic conditions, soil moisture status, and cultivar vigour.

Improper application of N may lead to nutrient deficiencies and toxicities which result in reduced tree growth, yields and fruit quality. 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. It also significantly increases the risks of reduced air and water quality, and higher greenhouse gas emissions. Presently, limited data on the relative importance of soil N processes, total N loss from current management practices and profitable use of N in the plant is available for Australian mango growing regions.

Thus, improving our understanding of the impact of management tactics on nitrogen uptake efficiency (NUE) is an essential prerequisite in giving producers the confidence to reduce N application rates and still maintain, or even improve, current yields. The research will use stable isotopes to quantify plant N demand, soil supply and NUE to develop best management recommendations for optimising N fertiliser use, including the use of soil amendments. The project aims to maximise NUE in the Australian mango industry to increase productivity, profitability and good environmental management.

The objectives of the project were:

- To determine the dynamic of N concentrations in the different parts, phenology and production season of mango
- To estimate the loss pathway of applied N and determine how these losses can be reduced to drive profitable outcomes for mango growers
- To quantify plant N use, availability and timing of N released from crop residues and soil organic matter mineralisation, and the contribution of N mineralisation to the total N demands of mangoes

- To evaluate overall mango nutrition and the differences between the regions and soils
- To provide information to growers regarding N dynamics and seasonal availability to guide their decisions for a better economic outcome
- To evaluate a range of soil amendments under laboratory conditions to identify which may have the potential to retain nutrients in NT mango orchard soils.

2 Method and project locations

There is no N budget available for mango growing in the NT, so this project went back to basics and planned a range of work designed to quantify local N inputs, cycling, mineralisation in soils and losses. This will inform, and potentially modify, the N application practices of commercial mango growers.

Uptake of soil-applied nitrogen into trees

This study was designed to quantify how much soil-applied fertiliser is taken up by tree roots into mango trees as they grow and mature into commercial production. In the environment, N occurs naturally in two stable isotopic forms, ^{14}N and ^{15}N. Over 99.6 % occurs in ^{14}N form and less than 0.4 % as ^{15}N, which has an extra neutron and is heavier. This natural ratio can be enriched, increasing the ^{15}N component in fertilisers such as ammonium sulfate and potassium nitrate (KNO_3). This enriched or labelled form of N can be measured in plant tissues using mass spectroscopy techniques.

We mixed the labelled ammonium sulfate fertiliser with standard ammonium sulfate and applied it to the soil in a developing mango orchard. Over three years, we quantified how much of that fertiliser was taken up by trees by measuring the ^{14}N:^{15}N ratio in the tree tissues. From these direct measurements, we estimated the NUE. Detail of this work may be found in Appendix C.

Is nitrogen taken up across the leaf cuticle?

In commercial mango orchards, dilute solutions of KNO_3 are usually applied before the flowering period to support floral induction and to ensure sufficient supplies of potassium (K) to maximise fruit set and retention to harvest maturity. There is no direct evidence or published data on how much N is taken up into mango leaves across the leaf cuticles. Does it occur, and how efficient is it as a way of applying N in orchards? To answer these questions, ^{15}N enriched KNO_3 was prepared and applied to mature, sun-hardened leaves of potted, grafted mango trees. Foliar uptake of N was quantified directly, and varietal differences were observed. Details of this work are in Appendix C.

Nitrogen movement within the tree

The leaf N content as a percentage of the leaf dry weight (leaf % N) reduces during flowering and fruit set and it is well known that leaf % N is not linked to fruit yield. To increase knowledge of tree N content and movement within mango, trees were infused with a known quantity of ^{15}N-labelled ammonium sulfate. N movement was tracked and recovered on a whole-tree basis at four phenological time points over a year: during rapid growth in the wet season, the quiescent period where growth is minimal, flowering and fruit set, and post-harvest. N storage and movement in mango trees were quantitatively assessed. Details are provided in Appendix C.

Nitrogen impacts on fruit yield and quality

To assess whether N applications could impact fruit yields, and to identify the level of N that would cause the skin of ripening fruit to stay green in NT growing conditions, experiments were designed and conducted in a commercial KP mango orchard in the Katherine region. The fertiliser was applied during the active growth period but prior to mechanical tree pruning. N application levels ranged from 0–50 kg per hectare (ha) at a tree planting density of 250 trees ha^{-1}.

Fruit from trees was harvested, counted and weighed, then ripened. As fruit ripened, skin colour was measured using a colorimeter and the CIEL*a*b system until stage 4 (Soft) was reached, then destructive measurements of flesh colour, texture and sugar content were carried out. Differences in skin colour while mangoes ripened were seen in response to the varying N amounts applied. The following year, the orchard yield was double and the skin 'stay green' effect was not repeated. However, ethylene application to ripening fruit shortened the time for fruit to change skin colour from green to yellow and for the fruit to soften by five days. It was notable that the ethylene-treated fruit did not reach the same sugar content when ripe as untreated fruit. Details of the trial may be found in Appendix C.

Nutrients in litter and pruned material

The amount of litter and its nutrient content have not been quantified and are unknown elements of the N cycle in mango orchard systems. To capture the falling material, litter traps were constructed, placed under trees in four commercial orchards, and litter was collected over a year. It was sorted, dried, weighed, processed and quantities calculated for orchards. For material pruned from trees, tarpaulins were laid under tree canopies and the pruned material collected as for litter. Nutrient content of the materials captured was calculated and found to contain significant quantities of N. Further detail about N and other nutrients cycling annually in mango orchards may be found in Appendix C.

Leaf nutrient economics

Trees grown in low nutrient environments often reabsorb nutrients from leaves and reuse them seasonally. To understand how much N is reabsorbed back into mango trees and reused, sets of mature attached green leaves and senescing, yellow but attached leaves were sampled from a number of mango varieties growing in a managed orchard. The macro and micronutrient content of the two groups were analysed and compared to calculate the differences between them. The results quantify the nutrients which are recycling within the tree and in the litter that is dropped annually onto the orchard floor and decomposed. Read more about this work in Appendix C.

Leaf litter decomposition, soil N fixation and gaseous losses in mango orchards

Field and laboratory-based studies were designed to measure annual leaf litter decomposition, N_2O gas emissions and how the application of synthetic fertilisers affected them over time. Work was conducted in regional research orchards for over a year to capture data for an entire season. Gas samples were collected manually (Darwin) or automatically (Katherine) using multiple lidded chambers. Gases were analysed using an isotope mass spectrometer and daily flux rates were calculated. Soil mineral N, and organic N and carbon (C) measurements were assessed throughout the year in soil cores taken in the orchard and linked with air temperature, soil moisture and soil temperature dynamics. Detailed results for this work are in Appendix C.

Soil amendments to improve nitrogen retention

As NT soils have poor structure and texture, and retain few nutrients, a range of soil amendments were trialled to assess their effects on leaching of inorganic N, N_2O emissions, nutrient retention and alternative N loss pathways. Also examined were any changes in soil physiochemical properties. The amendments trialled were biochar and hydrochar (produced from the pyrolysis of organic materials), zeolites (aluminosilicate minerals with high adsorption properties that can increase N retention) and

tree leaf litter. This work was undertaken as the NT weather conditions mean that enhanced efficiency fertilisers in their current forms cannot be used as they are in the CRDC cotton and sugarcane programs. The results of how the soil amendments performed are in Appendix D.

Preparing mango growers for precision agriculture technologies

This work is an ongoing collaboration with colleagues at the University of New England in New South Wales to examine whether high resolution satellite imagery can be used to estimate crop yields in terms of weight and fruit number. Alternatively, the method can be used to estimate crop losses. The prediction accuracy of this method for mango in NT orchards is a 7 % chance of underestimating and a 1 % chance of overestimating. To 'ground truth' the satellite image analysis, this project has measured trees, estimated fruit and yield, then counted and weighed harvested fruit from trees and compared these results with predicted yields. Results for this ongoing work may be found in Appendix C.

Research Site Type	Name	Location	Coordinates	Active Site Period	Experimental treatments
Orchard	Coastal Plains Research Farm	Middle Point	S 12^0 33' 39.38" E 131^0 18' 17. 05"	2015–2019	Soil-applied ^{15}N-labelled fertiliser uptake efficiency experiments Tree growth in response to N N movement in mango trees In situ soil N mineralisation, litter decomposition and gaseous emissions studies
Orchard	Jabiru Tropical Orchards	Arnhem Highway	S 12^0 33' 10. 37" E 131^0 15' 46.16"	2017–2018	Soil sampling for laboratory analysis of N mineralisation, leaching and amendment experiments
Orchard	Acacia Hills Mango Farm	Acacia Hills	S 12^0 44' 52. 80" E 131^0 10' 38.97"	2016–2021	Remote sensing collaboration using satellite imagery to predict mango and other crop yields – ongoing. Collecting ground-based data to validate predictions Litter and pruned material collection
Orchard	Tou's Garden	Acacia Hills	S 12^0 47' 28.07" E 131^0 09' 30. 67"	2016–2021	Remote sensing collaboration using satellite imagery to predict mango and other crop yields – ongoing. Collecting ground-based data to validate predictions Litter and pruned material collection
Orchard	NTLD Katherine	West of Katherine	S 14^0 35' 05.80" E 132^0 23' 33.22"	2018–2020	Litter and pruned material collection
Orchard	Katherine Research Station	Katherine	S 14^0 27' 59. 91"	2017–2018	In situ soil N mineralisation, litter decomposition and gaseous emissions studies Generated ^{15}N-labelled leaf litter for decomposition studies Conducted leaf nutrient resorption studies

			E 131^0 18’ 45.39”		
Orchard	Nutrano-Eumaralla Farm	South-east of Katherine	S 14^0 32’ 24. 90” E 132^0 28’ 06.20”	2018–2020	Two-year trial designed to quantify N impacts on fruit yield and quality Litter and pruned material collection
Laboratory	Katherine Research Station		S 14^0 27’ 58.48” E 132^0 18’ 58.48”	2017–2019	Mango orchard outdoor laboratory: automated gas sampling from stainless steel chambers with insulated acrylic lids, quantified in situ using a gas chromatograph to measure N_2O. Litter decomposition rates were measured and soil cores taken periodically for analysis of N mineralisation and other parameters. Laboratory: estimating mango fruit dry matter as a % of the fresh weight of the fruit (fruit % DM), using a calibrated F-750 near infrared spectroscope (Felix Instruments, Washington State, USA).
Laboratory	Coastal Plains Research Farm		S 12^0 35’ 36.77” E 131^0 18’ 14.30”	2017–2020	A manual chamber system was set up in an established orchard to collect gas samples for analysis of N_2O emissions. Litter decomposition rates were measured and soil cores taken periodically for analysis of N mineralisation and other parameters. KP orchard planted and ^{15}N-labelled fertiliser applied over time to assess N uptake efficiency by destructive sampling of the trees. ^{15}N-labelled fertiliser was infused into replicate trees to assess N movement at phenological timepoints. Destructive sampling of trees allowed the recovery of the labelled fertiliser across the tree parts. Ripening chambers were used onsite in 2019, and imaging of fruit was carried out in the laboratory.
Laboratory	Berrimah Farm	Darwin	S 12^0 26’ 38.79” E 130^0 55’ 46.61”	2017–2020	Agriculture laboratory-washing, drying and milling plant and soil samples, preparation for analyses Post-harvest laboratory-ripening fruit, cold store of material, post-harvest assessments, destructive measurements of fruit
Nursery	Berrimah Farm nursery	Darwin	S 12^0 26’ 37.47” E 130^0 55’ 52.29”	2018–2019	Grow, graft and maintain mango seedlings for ^{15}N foliar uptake experiments

Laboratory	Queensland University of Technology	Brisbane	S 27^0 28' 37.98" E 153^0 01' 39.37"	2017–2020	All laboratory-based soil experimental work including cores sampled in the NT orchards and trialling of impacts of soil amendments Analysis of plant material and soils, including ^{15}N content using isotope ratio mass spectrometry (IRMS) and inductively coupled plasma mass spectrometry (ICP-MS)

All results of this research are applicable to the major mango growing regions of the NT, near Darwin and Katherine. Some results may be transferrable to other tropical regions of Australia; however, different climate, soil types and management practices would need to be considered.

3 Project outcomes

Mango orchard systems are similar to other cropping systems in that 30–70 % of N inputs are lost to the environment. N is highly mobile and can be applied and taken up by trees at most times of the year in one form or another. Visible N deficiency in mangoes is likely to reflect a longer-term lack of N and a degree of soil nutrient mining. Thus, routine soil and leaf sampling assessments should continue as they indicate a range of nutrient deficiencies and excesses. Along with fruit N content, they are part of an assessment of whether the N application decisions being made are reasonable.

Taking the results from this project and other work into account, the following industry recommendations are made in the four R context.

Right time

Soil-applied N

- Apply to soil or fertigate immediately post-harvest (October to December) while the tree is actively growing. This takes advantage of break of season rains for immediate uptake, but before available N is flushed out of range of roots. Avoid applying during monsoon periods or when soil is waterlogged.
- Alternatively, consider the end of the wet season when growth is still active, soils are moist and before the last rains. This is difficult to time correctly, and risks late leaf flushes (perhaps at the expense of flowering) and a strong reliance on applications of paclobutrazol and foliar N to manage and mature the vegetative shoots.
- Split applications can be considered.

Post-harvest, tree N is significantly depleted with trees losing a significant portion of their N content in harvested fruit and litter (including flowers, which have a high N content). At this time, trees are entering an active growth phase which requires N.

The dry-to-wet-season transition period and the beginning of the wet season is when high levels of N mineralisation in orchard topsoils were measured, concurrent with break of season rainfall events.

N mineralised from leaf litter provides ~11 kg N ha^{-1} in the top 20 cm of soil in the Darwin region, and ~17 kg N ha^{-1} in the Katherine region. Feeder roots of trees can access this layer of soil and rain will facilitate uptake.

Soil-applied and litter-supplied N are available for rapid uptake at this time of the year, before nutrient leaching monsoon rains begin.

Fertigation

- This method can be used through most of the year, but should cease during the dormant or quiescent period to allow shoots to mature prior to flowering. Do not apply N to soils as the flowering induction period approaches.
- This is a convenient method to split N into smaller, more frequent applications to match tree needs and reduce N losses.
- Do not fertigate waterlogged soils.

Smaller applications of soil-applied fertiliser resulted in higher NUE. This implies that uptake of smaller applications of N via fertigation would follow the same trend; however, fertigation NUE was not trialled in this work.

Foliar application

- Conventionally prepared as a dilute solution of KNO_3 with a surfactant, it is usually applied before flowering induction and may continue periodically during the flowering and fruit set period. It needs to be prepared at a low concentration that will not damage leaves, usually 1-4 % KNO_3.
- This method can be used to apply N at any time of the year unless rain is expected in the following 24-48 hours.

Quantitative measurements of foliar uptake of N in mangoes show that between 27 and 44 % of the N applied to leaves is taken up across the cuticle over 1–2 days, depending on variety. NMBP 1201 and NMBP 1243 have the least efficient uptake and NMBP 4069 the most efficient uptake. KP and Calypso® are mid-range. These NUE compare favourably with soil NUE, but there is extra value with foliar application. It is usually applied in the dry season, and remnant solution is likely to stay on the leaves until washed off by rain, diluted by dew and falls onto the litter below, or the leaves drop and become litter. In this way there are two opportunities for the N to be taken up into the tree. Additional N in litter can contribute to increasing the rate of litter decomposition.

Right form

Commercially available fertilisers are recommended.

- In this work, ammonium sulfate [$(NH_4)_2SO_4$] and urea [$(CO(NH_2)_2$] were used for soil, litter and infusion trials, and KNO_3 for foliar application.
- Minimal N_2O emissions were measured with decomposing litter and/or $CO(NH_2)_2$ applied to soils.
- Preliminary work on soil amendments found that zeolite mixed with topsoil maintained water and nutrient content better than the other combinations tested. Biochar mixed with topsoil also performed well.
- There were no studies comparing the performance of different forms of enhanced efficiency fertilisers as they are considered unsuitable for the wet-dry tropical climate.

Soil amendments are likely to have a place in NT mango orchard management if an affordable, available form is found.

Right place

Placement or delivery of fertiliser will vary according to the method of application.

- Soil-applied fertiliser should be placed on soil under the drip line of the canopy, where tree feeder roots can easily access it. Avoid placing close to tree stems.
- Fertigation will depend on the orchard irrigation in place and the water/solution pressure within the system. Ideally, sprinklers would throw the solutions evenly under canopies to deliver fertiliser to tree feeder roots.
- Foliar applications should be made using spray equipment that is correctly calibrated to deliver the desired volume of N to each tree across the orchard.

Placement of N was not specifically researched in this project.

Right amount

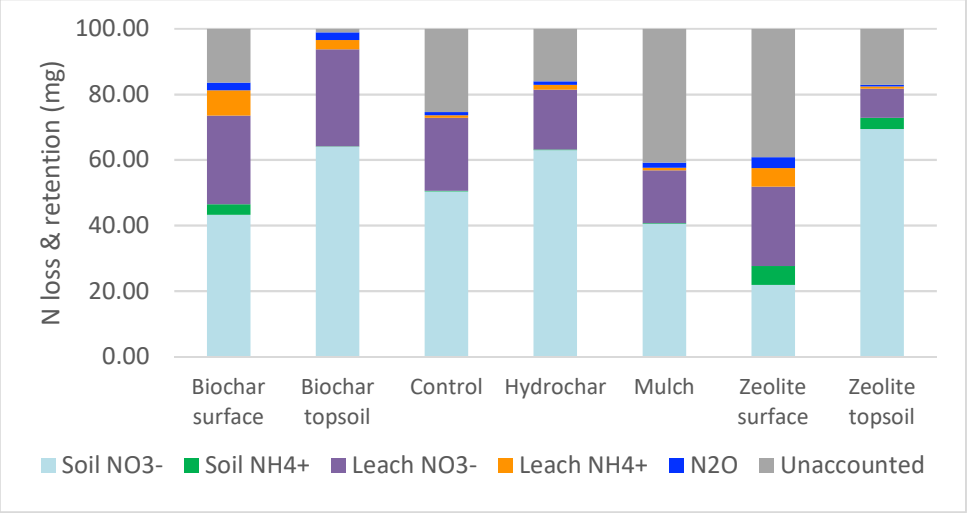
This will vary according to location, soils, seasonal conditions and yield. It assumes that litter and pruned material are left on the orchard floor. Information to consider includes:

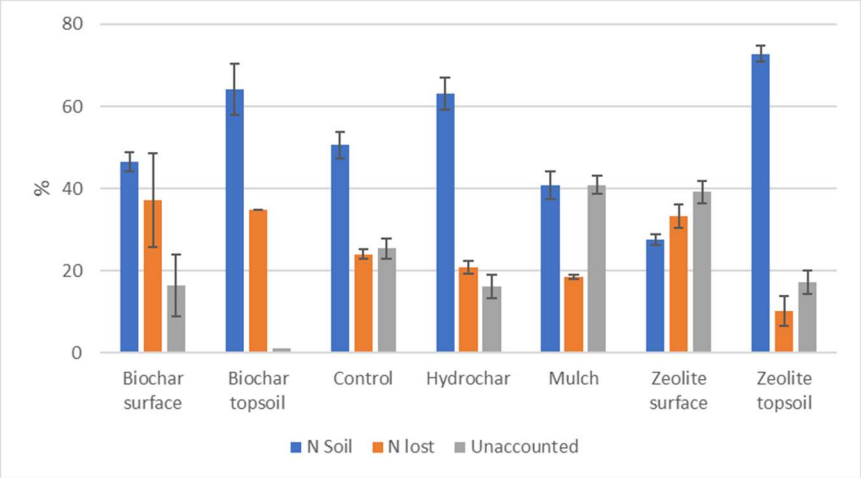
- Most available N is lost from soils annually between the break of season rains and the end of the wet season.
- Litter contains up to 20 kg N ha^{-1} in a commercial orchard annually. Decomposition of litter in response to rain events releases ~11 kg of available N ha^{-1} in the top 20 cm of soil in the Darwin region each season and ~17 kg N ha^{-1} in the Katherine region. These amounts cycle each year. What is not taken up by trees is effectively lost.
- Mangoes in managed orchards contain ~0.8–1 kg N $tonne^{-1}$ of fruit harvested. This amount of N leaves the property and needs to be replaced. Trees receiving no N inputs over four years had fruit with 0.4 kg N $tonne^{-1}$ of fruit harvested.
- Growers need an understanding of the relationship between yield, excess N application and 'stay green' skin on ripe mangoes in their orchard.
    - For example, at a commercial KP orchard, with a yield of 20 tonnes fruit ha^{-1} and 250 trees ha^{-1}, fruit from trees receiving 25 kg N ha^{-1} had blotchy green skin when ripe, and at 50 kg N ha^{-1} the fruit stayed green when ripe. Fruit from trees receiving 12.5 kg N ha^{-1} or 50 g $tree^{-1}$ ripened normally.
    - There were no differences in yield, number of fruit, % dry matter (% DM), or fruit N content in response to 0, 12.5, 25 or 50 kg N applied ha^{-1}.
    - The same N rates applied after that harvest generated yields the following year that approached 40 tonnes ha^{-1}, and no fruit ripened with 'stay green' skin. The N content in fruit did not vary in response to applied N levels. Fruit numbers increased, fruit size was the same and there was no yield response to the range of N levels applied.
- How much N a maturing mango tree takes up from soil-applied fertiliser is linked to how much is applied.
    - In our work, ~75 % of 5 kg N ha^{-1} was taken up.
    - 35 % of 10 kg N ha^{-1} and 20 % of 15 kg N ha^{-1} was taken up.
    - The NUE reduced as more N was applied.
- Avoid applying 'insurance' N. Most of it will be lost and inaccessible to trees.
- Additional N can be applied via fertigation at most times, but avoid when soils are waterlogged, during monsoon rainfall and the period of quiescence or dormancy prior to flower induction.
- Foliar application of N can occur at any time in clear weather.

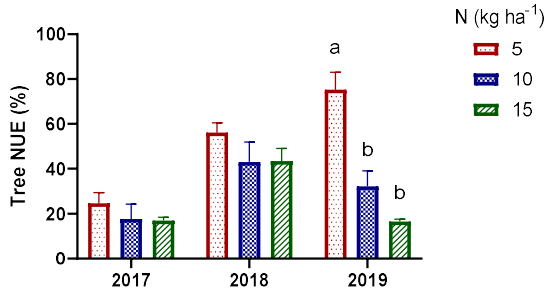
Each of the 'right amount' points is supported by data collected as part of this project, and can be found in Appendix C and Appendix D.

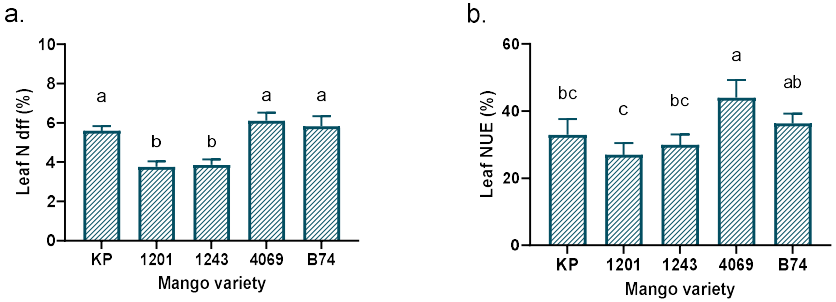
3.1 Project level achievements

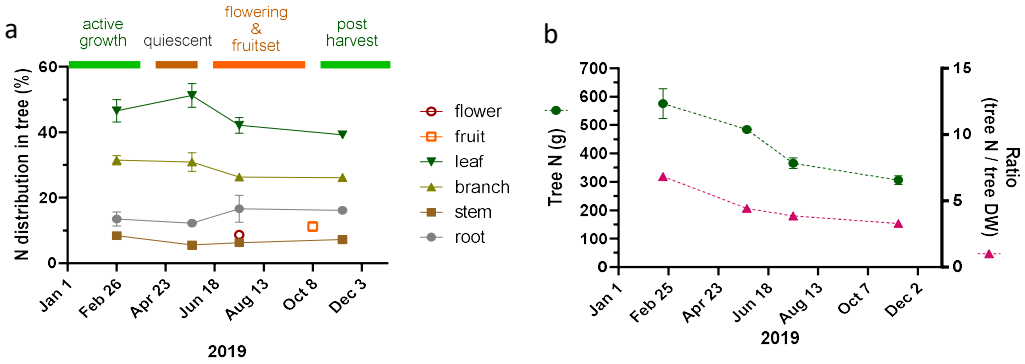
KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI
KPI 8.6 and 8.7: Provide a final account of the evaluation of the best performing enhanced efficiency fertilisers in mango crops.	*30/6/21*	*Activity B4* *4 l*	Enhanced efficiency fertilisers show limited economic or environmental benefit in NT weather conditions and appropriate biological fertilisers were unavailable locally. There was biochar being produced locally, and an alternative program was designed to assess the potential of soil amendments to improve N retention in local horticultural soils. The work was conducted in a laboratory setting using zeolite, biochar, hydrochar or leaf litter with urea and simulated rain events on soil column incubations over time. Overall, soil nitrate levels increased over the period while ammonium levels decreased as the fertiliser was hydrolysed and nitrified. Zeolite mixed into the topsoil retained the most mineralised N, being significantly more effective than zeolite placed on the surface. The same result was seen with biochar mixed into the topsoil compared to surface placement. After rain events, leachate was collected from the columns and zeolite mixed with topsoil released the least nitrate and ammonium leachates. Soil emissions of N_2O varied significantly between treatments but were minor, showing that the main loss pathways were leaching of nitrate and ammonium (Figure 1).

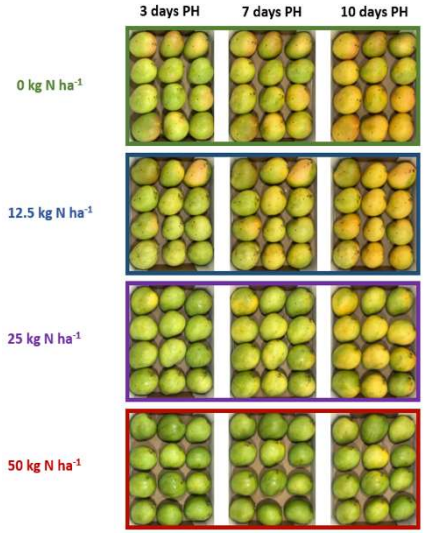
KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI
			 *Figure 1 Breakdown of N loss pathways and N retention in soils for each treatment based on final (Day 100) values. (100 mg of N applied)* A summary comparing the N retained in soils, N lost via leaching and unaccounted for each amendment combination shows zeolite mixed with topsoil having the highest N retention, closely followed by biochar mixed with topsoil and hydrochar (Figure 2). Also, zeolite nutrient losses were close to zero after ~30 days of 100, suggesting potential for longer-term retention capacity.

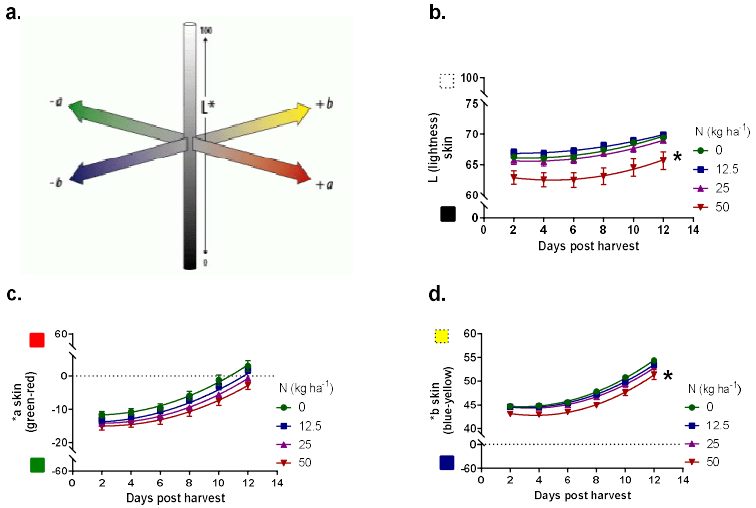
KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI
			 *Figure 2: Percentage and comparison of soil N retained, N lost and unaccounted for loss pathways at the end of incubation for all treatments. Bars indicate standard error. N loss includes leachate and N_2O emissions.* For the soil tested (Kandosol, sampled from Coastal Plains Research Farm in the Darwin region), zeolite mixed into topsoil was the best performing amendment. While zeolites do not break down in the environment, the longer-term impacts on soil physiochemical properties are unknown. *Industry outcomes:* An application of zeolite on a broad scale would cost about \$50,000 ha^{-1}. Biochar is being produced on a small, domestic scale in the NT, and is costed at \$17,600 ha^{-1} at an application rate of 2 L m^{-2}. Neither of these are viable in dollar terms; however, the results do indicate that soil amendments can make large differences in N retention if an economic option can be found. It is new, baseline research for industry to build upon. Refer to Appendix D for further details.

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 8.9: Provide a brief and final account of calculating NUE for mango nitrogen use.	*30/6/21*	*Activity B5 5 d*	*Measuring nitrogen uptake efficiency in mangoes* In the environment, N occurs naturally in two stable isotopic forms, ^{14}N and ^{15}N. Over 99.6 % occurs in ^{14}N form and less than 0.4 % as ^{15}N, which has an extra neutron and is heavier. This natural ratio can be enriched, increasing the ^{15}N component in fertilisers such as ammonium sulfate ($(NH_4)_2SO_4$) and potassium nitrate (KNO_3). This enriched or labelled form of N can be measured in plant tissues using mass spectroscopy techniques. We mixed the labelled $(NH_4)_2SO_4$ fertiliser with standard $(NH_4)_2SO_4$, and applied it to the soil in a developing mango orchard. Over three years, from the juvenile phase to mature and entering commercial productivity, we quantified how much of the applied fertiliser was taken up by trees by measuring the ^{14}N:^{15}N ratio in the tree tissues. From these direct measurements, we found that N uptake efficiency (NUE) in a maturing mango tree reduces as the amount applied increases (Figure 3). Not only did the uptake efficiency reduce, the amount of N taken up reduced. There were no differences in fruit yield, number of fruit, % DM of fruit, fruit N content or tree size in response to the N treatments.  *Figure 3: Nitrogen uptake efficiency (NUE) of trees changed over time, as trees matured and began commercial production in 2019. Trees with the lowest quantity of N applied showed significantly higher uptake than the two larger N applications in 2019 (letters indicate significant difference), time series analysis of variance (ANOVA), Tukey's post-test, p=<0.0001, n=3).* Foliar uptake of N applied to fruit trees has never been measured directly. Also, it is usually applied as a dilute KNO_3 solution for the potassium to maximise flowering and fruit retention on panicles. The spray adds about 2.2 kg N ha^{-1} if a

KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI
			2 % solution is applied twice. A method was developed using a ^{15}N-labelled solution of KNO_3 and potted, grafted mangoes. N was absorbed through the leaf surface over two days, and the NUE into the leaves varied from 27 % to 44 %, depending on the mango variety (Figure 4).  *Figure 4: The amount of N derived from the KNO_3 dipping solution showed significant varietal differences with letters indicating similarities and differences (a) ANOVA, p=0.0009, mean, standard error of the mean (sem), n=10, LSD post-test). Varietal differences were also significant when N uptake efficiency was calculated (b). Means with different letters are significantly different at 5 % LSD (p=0.033, mean, (sem), n=10).* To assess seasonal use and movement of N in mango trees, a method was developed to infuse ^{15}N-labelled $(NH_4)_2SO_4$. This provided evidence that N is moved rapidly within trees and the labelled N was evenly distributed in every tissue, including the roots, xylem and phloem within 70 days of infusion. Leaf N content increased during the quiescent period, as trees approached the flowering induction period, then dropped as high N content flowers developed, using almost 10 % of total tree N (Figure 5a). Much of the reduction in tree N over the season (Figure 5b) is accounted for by N measured in flowers and fruit (Figure 5a), but not all. The flower N can be recycled on the orchard floor but fruit N will leave the orchard.

KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI
			 *Figure 5 Nitrogen (N) distribution in tree components over time (a) and total N in trees over time (b)* Excess application of N can have negative effects on the colour of skin while fruit is ripening. A grower may not be aware they are picking fruit with this ripening defect as it is not possible to see when fruit is harvested at the mature, green stage. While it will depend on the soil, season, orchard history and how much N is applied, we established some guidelines to work within to reduce the incidence of 'stay green' skin. For example: at a commercial KP orchard, on a low yield year of 20 tonnes fruit ha^{-1} and 250 trees ha^{-1}, fruit from trees receiving 25 kg N ha^{-1} had blotchy green skin when ripe, and at 50 kg N ha^{-1} the fruit stayed green when ripe (Figure 6). Fruit from trees receiving 12.5 kg N ha^{-1} or 50 g $tree^{-1}$ ripened normally. The same rates applied after that harvest generated yields approaching 40 tonnes ha^{-1}, and no fruit harvested ripened with 'stay green' skin. The difference in skin colour when ripening was quantitatively measured (Figure 7).

KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI
			 *Figure 6: Fruit from trees with a range of levels of N were harvested on the same day and imaged every second day to visually track ripening progress. At each level of N application, 0, 12.5, 25 and 50 kg ha^{-1} (above, top to bottom), the same tray of replicate fruit from a single tree is shown 3 days post-harvest (left column of images above), 7 days post-harvest (centre column of images) and 10 days post-harvest (right column of images above).*

KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI
			 *Figure 7 Colour components of skin colour were measured on ripening mangoes with a Konica-Minolta colorimeter using the CIEL*a*b system (a). Skin lightness showed significant variability in response to tree-applied N levels (p=0.007). Significant interaction between N level and time occurred, and LSD post-tests indicate that fruit skin from trees with 50 kg N ha^{-1} applied was significantly darker over the period compared to fruit from trees with lower N treatment (* in b above). Measurements of skin *a (green-red) showed no significant differences in response to N treatments (p=0.052); however, there was a strong trend implying that as N application to trees increases, fruit skin colour tends to be greener as it ripens (c). Values for *b (blue-yellow) show significant differences in response to N levels applied and no significant interactions (p=0.008), LSD post-test indicates that skin of the fruit from trees with 50 kg N ha^{-1} is less yellow than from trees with lower levels of applied N (* in d above). ANOVA, mean, sem, n=4.* Industry outcomes will be summarised at the end of the table as they related to the results as a whole.

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 8.10: Develop and test algorithms for remote sensing of leaf N content (mangoes) for the mango industry.	*30/6/21*	*Activity B5* *5 c, d*	Remote sensing and satellite image analysis is expanding into precision agriculture associated with tree crops, including mangoes. This collaboration between the University of New England (UNE), Central Queensland University, Queensland Department of Agriculture and Fisheries, University of Sydney, peak bodies for mango, macadamia and avocado, and others is ongoing. To assist the mapping project, we provided a desktop generated layer of all mango orchards to the UNE project and directly to Queensland Department of Science, Innovation and IT (DSITI), (NTMango Layer version1). Queensland DSITI have used this information to generate the draft Australian mango map. The current version (not linked with this project) is here: ATCM Dashboard Experience (arcgis.com) Imagery analysis can reliably predict yield and number of fruit. The models are refining with work over time, and we continue to provide field estimates of number of fruit and annual yield, then harvest the fruit to validate the modelling. It has the potential to assess tree health and biosecurity issues in the future. This milestone was aspirational, but was ultimately not financially viable.
KPI 8.11: Develop NUE guidelines for the horticulture industry to target. *KPI 8.14: Quantify the timing and amount of N released in crop residues.*	*30/6/21*	*Activity B5* *5 f* *Activity B6* *6 d*	By collecting litter and pruned material in commercial orchards, we found that significant quantities of N are recycling on the orchard floor annually, 17–27 kg N annually (Figure 8).

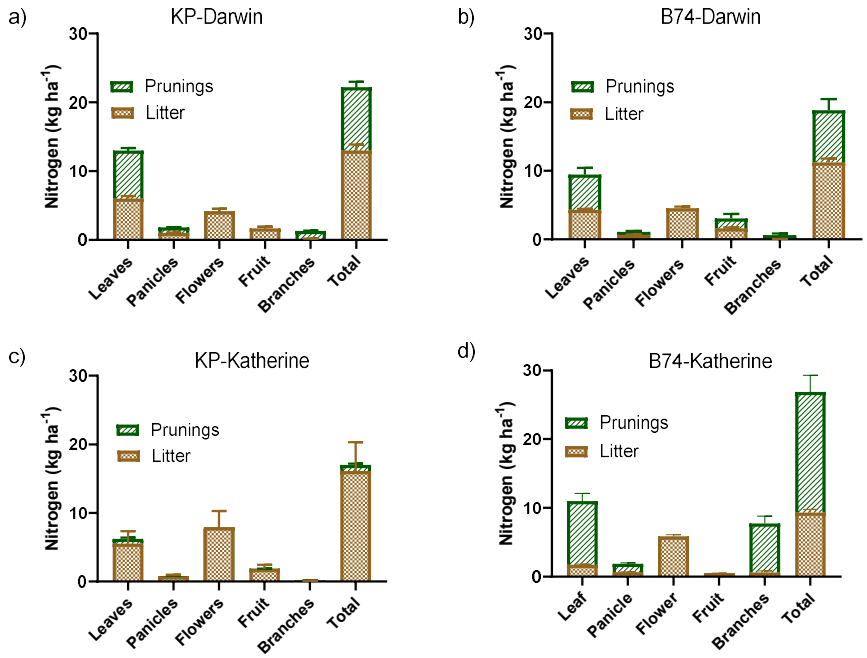


Figure 8: The N content of in-orchard annual litter and pruned material in the Darwin region litter was similarly proportioned (a, b). In the Katherine region, the KP orchard with large, mature trees and minimal branch tip pruning, shed most N in litter over the year 2018–19 (c). In contrast, the B74 were pruned heavily and reshaped, with most N accumulated in the prunings (d). Mean, sem, n=10 collection trays at each site. Data is standardised to a tree density of 250 trees ha^{-1}.

Litter and pruned material also deposits ~40 kg ha^{-1} of calcium (Ca) annually along with other macro and micronutrients.

In the Darwin region, 100 % of leaf litter decomposes annually over the build-up and wet season, and in the Katherine region the figure is 85 %. The difference is attributed to reduced rainfall in the Katherine region.

Decomposition of litter in response to rain events releases ~11 kg of available N ha^{-1} in the top 20 cm of soil in the Darwin region and 18 kg N ha^{-1} in the Katherine region. These amounts cycle each year. Soil samples suggest that any available N that is not taken up by trees is leached and lost annually. There is little or no capacity for sequestration of N in soils.

Minimal N_2O emissions (~0.2 kg N ha^{-1}) were measured with decomposing litter, $CO(NH_2)_2$ applied to soils and in combination.

KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI

			Summary of industry guidelines:
			
			The new knowledge to incorporate into mango orchard management in the NT will expand grower knowledge of how much N is in mango orchards, how it cycles annually, how much is taken up and how much is lost. Further extension work and materials are in preparation and it will encourage growers to review N application and, for many, will encourage a reduction in N application and associated savings.
			
			New knowledge includes:
			• How much N a tree takes up from soil-applied fertiliser is related to how much is applied.
			  ○ If it is less than the tree needs, ~75 % of applied N can be taken up.
			  ○ Otherwise, it is 20–35 % uptake of applied N and less N overall.
			• Foliar uptake of N into leaves has an efficiency similar to soil-applied fertiliser. Uptake is fast, N is rapidly transported around trees, and there is a second uptake opportunity as the N is recycled on the orchard floor in rainfall or litter.
			• Most available N is lost from soils annually between the break of seasonal rains to the end of the wet season.
			• Apply fertiliser to soils immediately post-harvest to take advantage of first rainfall events, before the monsoon period.
			  ○ Avoid applying 'insurance' N, it is wasted.
			  ○ If N is need during the dry season, fertigation or foliar application is preferred.
			• Decomposition of litter in response to rain events releases ~11 kg of available N ha^{-1} in the top 20 cm of soil in the Darwin region and 18 kg N ha^{-1} in the Katherine region. These amounts cycle each year.
			• Mangoes contain ~0.8–1 kg N $tonne^{-1}$ of fruit harvested. This amount of N leaves the property and needs to be replaced.
			• An understanding of the relationship between yield, excess N application and 'stay green' skin on ripe mangoes.
			  ○ For example: at a commercial KP orchard, on a low yield year of 20 tonnes fruit ha^{-1} and 250 trees ha^{-1}, fruit from trees receiving 25 kg N ha^{-1} had blotchy green skin when ripe, and at 50 kg N ha^{-1} the fruit stayed green when ripe. Fruit from trees receiving 12.5 kg ha^{-1} or 50 g $tree^{-1}$ ripened normally.

KPI no. and description	KPI due date	Relevant CRDC FRP milestone number/s	Summary of final outcome of the research concluded by this KPI
			○ The same rates applied after that harvest generated yields approaching 40 tonnes ha^{-1}, and no fruit harvested ripened with 'stay green' skin.

3.2 Contribution to MPfN program objectives

The objective of the More Profit from Nitrogen (MPfN) program is to enhance NUE, improving profitability and sustainable use, through better understanding the influence of contributing factors. These results will:

1. Generate a greater knowledge and understanding of the interplay of factors to optimise nitrogen (N) application, rate and timing for tropically grown mango crops in the Northern Territory.
2. Provide clear information about the timing and quantity of N availability, cycling and losses within mango orchard systems in the NT. It has also shown that significant quantities recycle, that excess available N in the system is lost annually, and the level of N at which there may be a reduction in harvested fruit quality. The N uptake efficiency of soil and foliar application were quantified, and advice on best timings and rates provided (Activity 5).
3. Establish the timing and amount of mineralisation of N in mango orchards, with reference to the two mango growing regions of the NT (Activity 6).
4. Show the potential of a range of soil amendments were tested using local soils, in a laboratory situation. The nutrient holding capacities, leaching of nitrates and ammonium, and nitrous oxide gas emissions were quantified. There is potential for soil amendments to be used in commercial situations depending on cost and availability (Activity 4).

3.3 Demonstrate more profit from nitrogen

The research has quantified N uptake, N cycling and N loss pathways in NT orchards.

It has demonstrated that applying more N than necessary risks harvesting fruit with ‘stay green’ skin, a post-harvest defect that can reduce the value of a crop. Excess available N is lost annually over the wet season.

The significance of litter has been quantified in terms of nutrient content and nutrient recycling. A value for that litter being maintained in the orchard system can be calculated along with the N losses associated with wet season rainfall.

An economic analysis was prepared by Ag Econ, contracted by the MPfN project and will be available October 2021 (More Profit from Nitrogen | CRDC). The potential fertiliser cost savings are estimated at $140 per hectare. Also, reducing N inputs maintained fruit quality at “first grade”, with less likelihood of fruit being downgraded to “composite”. This maximised the harvest return, potentially worth $2,949 per hectare.

4 Collaboration

The most productive collaborations are those with NT commercial mango growers. A research program that generates meaningful results is only possible with their generous assistance and access to their orchards. There are many that have participated in multiple projects over the years. Thank you to all of you for past and future collaborations.

5 Extension and adoption activities

Description	Title	Date
Project launch: Mango Matters	Project funding success – More Profit from Nitrogen	10/07/2016
Project announcement: NT Farmers newsletter	RD4P project funding success – More Profit from Nitrogen	1/12/2016
NT Department of Primary Industry and Resources (DITT) e-newsletter article	Project starts research work	24/03/2017
Australian Mangoes Industry Association (AMIA) e-newsletter article	Project starts field work	14/04/2017
DITT webpage article	NT mango research hits the national stage	10/05/2017
Fresh Plaza e-newsletter of the Fresh Produce Industry: Project article	New research to better use nitrogen in mango production	12/05/2017
Presentation to Chinese delegation from Baise City, Guangxi	Understanding nitrogen in mangoes, improving profitability	17/12/2016
Abstract for XII International Mango Symposium, China	Precision farming in mango to manage nutrition	15/03/2017
Abstract for XII International Mango Symposium, China	Optimising foliar nitrogen uptake of mango: Effect of adjuvant, leaf position and time of potassium nitrate spray	15/03/2017
Presentation at AMIA conference, Bowen	More Profit from Nitrogen: Understanding the role of nitrogen in mango production	4/05/2017
Presentation at AMIA conference, Bowen	Multi-scale monitoring tools for managing Australian tree crops – phase 2	5/04/2017
Top Paddock article	2017 Australian Mango Industry Conference a blast	15/09/2017
Participation in mango magpie geese project – mango grower meeting at CDU	Mango magpie geese project – mango grower meeting	25/05/2017
Mango Matters article – Winter 2017	Growers can actively lead new cutting-edge research into optimising nutrient management for the mango industry	12/07/2017

Presentation at XII International Mango Symposium, China	Precision farming in mango to manage nutrition	12/07/2017
Participation in AMIA mango pre-harvest meetings	AMIA mango pre-harvest meetings	15/08/2017
Master's thesis: Maddison Clonan, Charles Sturt University	Site-specific variation of nitrogen availability across four soil types found on Top End mango farms	15/11/2017
MPfN mango grower update – November 2017 newsletter	Mango grower update	16/11/2017
Top Paddock article	Quantifying nitrogen use efficiency in tropical mango production systems	15/12/2017
DITT and NT Farmers Association mango R&D forums for industry (Darwin and Katherine)	Optimising nutrient management for improved productivity and fruit quality in mangoes	9/05/2018
International Society of Horticultural Science Congress, 12–18 August 2018, Turkey	Ionome balance analysis of mango fruit from an orchard with and without resin canal discolouration	12/08/2018
Nitrogen Natters article	Nitrogen management for improved mango productivity and quality	31/07/2018
Journal article *Acta Horticulturae* 1299, p 269–274	Optimising foliar nitrogen uptake of mango: Effect of adjuvant, leaf position and time of potassium nitrate spray	22/09/2018
Journal Article *Remote Sensing* 10, 1866	Exploring the potential of high resolution WordView-3 imagery for estimating yield of mango	22/11/2018
Post-harvest review meeting, NT Farmers	Post-harvest review meeting Darwin and Katherine the following day	21, 22/03/2019
Research group meeting on mango-based research across horticulture, entomology and biosecurity. Berrimah Farm. DITT	More profit from nitrogen	30/05/2019
MPfN mango grower update – May 2019 newsletter	Mango grower update	30/05/2019
Top Paddock article	Mango leaf litter and pruning: A large and hidden nitrogen input	3/06/2019

AMIA 12th Biennial Australian Mangoes Conference, 2019 Darwin-presentation	Advances in mango production 2019	15/05/2019
AMIA 12th Biennial Australian Mangoes Conference, 2019, field day presentation	Optimising nutrients-nitrogen in mango systems of the NT	17/05/2019
Project presentation RRDP1720: 2019 Australian Fertiliser Industry Conference/MPfN program partner forum field excursion	Optimising nutrient management for improved productivity and fruit quality in mangoes	6/09/2019
Conference proceeding, TROPAG 2019, Brisbane	Exploring the potential of high-resolution satellite imagery for yield prediction of avocado and mango crops	11/11/2019
Northern Territory Natural Resource Management Awards. DITT MPfN project finalist	More efficient nitrogen fertiliser use in mangoes in tropical climates	12/11/2019
Master's thesis: Dakshina Yadav, Charles Darwin University	Effect of different scions on macronutrient resorption of mango Kensington Pride rootstock	1/12/2019
Top Paddock article	DITT Territory Natural Resource Management (TNRM) Award finalists	9/12/2019
Honours thesis: Benjamin Vickery, Queensland University of Technology	The limit of soil organic carbon sequestration in tropical soils	2/12/2019
American Geosciences Union Fall meeting, San Francisco, USA	The interaction between nitrogen fertiliser and leaf litter application drives nitrous oxide emission from tropical mango orchards	9/12/2019
Nitrogen Natters article	Mango leaf litter and prunings: A large and hidden nitrogen input	20/12/2019
Australian Tree Crop Magazine article	'Floored' by mango's benefits	26/02/2020
Zoom presentation to Nutrano Farm managers	Mango orchard N cycling and use	30/04/2020
2020 MPfN program partner update and exchange	Optimising nutrient management for improved productivity and fruit quality in mangoes	23/04/2020
2020 International Nitrogen Initiative (INI) conference in Berlin, abstract and poster	Nitrogen recycling in mango orchards from litter and biomass	27/05/2020

accepted (deferred due to Covid-19)		
Mango Matters magazine – Spring 2020 article	Skin deep: How ethylene affects mango quality	15/10/2020
Australian Tree Crop magazine – Winter 2020 article	Mango litter linked to fruit colour penalty	30/06/2020
Nitrogen Natters article	Is there leaf N uptake from foliar spraying of potassium nitrate at mango flowering and fruit set?	14/08/2020
Nitrogen Natters article	The mango team creatively extend their work to mango growers of the NT	18/11/2020
Participation in mango growers pre-harvest roadshow, Darwin	Mango growers pre-harvest roadshow, Darwin	11/08/2020
Participation in mango growers pre-harvest roadshow, Katherine	Mango growers pre-harvest roadshow, Katherine	12/08/2020
Journal article *Nutrient cycling in agroecosystems*, September 2020	Combined effect of nitrogen fertiliser and leaf litter carbon drive nitrous oxide emissions in tropical soils	23/09/2020
Journal article Submitted for review	Leaf litter decomposition dynamics in tropical soils: The effect of N fertilisation and precipitation	Submitted
Factsheet Will be available electronically, DITT	Foliar uptake of nitrogen	In preparation
Factsheet Will be available electronically, DITT	How nitrogen affects mango fruit quality	In preparation
Factsheet Will be available electronically, DITT	Annual nitrogen cycling in NT mango orchards	In preparation
Factsheet Will be available electronically, DITT	Nutrient resorption in mangoes	In preparation
Mango orchard N balance for the NT – in preparation	Best practice guide for N application in NT mango orchards-in preparation for inclusion in MG17000	Continuing

Please note that some planned extension events were cancelled due to Covid-19 restrictions and associated uncertainties in 2020.

5.1 Extension of the research to the end user

The 'story' has come together at the end of the project and while a number of articles were written and updates provided in talks and meetings along the way, the big picture was in development as components were gradually completed. There were significant delays due to Covid-19 in 2020 with laboratories shut down for extended periods and catching up with the subsequent backlog of analyses. However, the project has generated interesting and new information for growers to consider and incorporate into their orchard management.

To ensure this story containing new and different information is heard, it is being further developed for delivery to NT growers.

This will from part of a collaborative project (MG17000 with Hort Innovation, AMIA, DITT, CQ University, Queensland Department of Agriculture and Fisheries (QDAF), and the Western Australian Department of Primary Industries and Regional Development (WADITTD) that is underway building 'best practice' resources for the mango industry. The factsheets resulting from our N research are being prepared for local release. This will expand the reach and extension of this work significantly.

5.2 Recommendations to industry on adoption of the research outcomes

The data generated in the MPfN mango project is consistent with the presented outputs of the cherry, cotton and sugarcane industries. There is an excess of N being applied generally across horticultural crops.

In mangoes, there is a fruit quality penalty to excess application of N. In this work, an indication of what constitutes too much N in NT conditions for post-harvest ripening defects has been provided for growers.

Other consequences of excess N application for mango growers, but not canvassed in this work, are increased fruit susceptibility to fungal pathogens and associated fruit rots during the post-harvest period.

As emissions of the greenhouse gas nitrous oxide (N_2O) from litter and fertiliser are well below the Intergovernmental Panel on Climate Change (IPCC) limits for intensive horticulture in Australia, NT mangoes have the potential to be promoted as a low emissions horticultural industry.

By adopting these recommendations, the industry will benefit from higher profits due to lower inputs and higher quality fruit.

6 Lessons learnt

6.1 Research level

It is always a challenge researching annual crops, particularly large and valuable trees. Field research is preferred and if you are lucky, there is time and some sacrificial trees available to develop new methods. Nature waits for no-one, and gathering high-quality data and samples can be an exciting and occasionally stressful occupation.

We learnt that mango trees transpire slowly and develop only slightly negative xylem pressure in the wet season when humidity is around 90 % and the ground is waterlogged. This contrasts dramatically with the low humidity of the dry season when there is no rain for months.

Mangoes contain about the same amount of N as apples, and trees require comparatively low amounts of N to produce high-quality fruit.

On a lighter note, tree netting is not suitable to catch litter in NT mango orchards as pythons can become trapped and are unable to reverse out of a tight situation. This was discovered before experiments began and several pythons were released. The revised shade cloth and screen litter nets captured every leaf and flower while pythons were seen passing through.

6.2 Industry level

NT grown mangoes have the potential to be promoted as a crop associated with low nitrous oxide (a greenhouse gas) emissions. The emissions measured in this project were well below the Tier 2 IPCC limits for intensive horticulture. Compared to many crops, mangoes require low N inputs.

The impacts of ethylene-accelerated ripening on sugar accumulation in mangoes are notable. In this project, skin colour and flesh softening processes responded to ethylene treatment, but sugar accumulation did not. Industry needs to be aware of this; it has the potential to extend the time to ‘next purchase’.

6.3 Service provider/ Primary producer level

Talking to producers is always a learning opportunity. Finding out how they manage crops and the reasons why they do certain things at certain times can make a researcher re-evaluate priorities or redesign an experiment. It also provides a better understanding of the commercial pressures they operate under, such as product pricing and timing, labour needs and costs of inputs. All these things contribute to better directed research programs.

Appendix A: Additional project information

A.1 Project material and intellectual property

Journal papers published

Pandeya H, Friedl J, De Rosa D, Asis C, Tilbrook J, Scheer C, Bristow M, Grace P, Rowlings D (2020) Combined effect of nitrogen fertiliser and leaf litter carbon drive nitrous oxide emissions in tropical soils. Nutrient Cycling in Agroecosystems 118, 207-222. https://doi.org/10.1007/s10705-020-10094-6

Rahman M, Robson A, Bristow M (2018) Exploring the potential of high resolution WorldView-3 imagery for estimating yield of mango. Remote Sensing 10, 1866. https://doi.org/10.3390/rs10121866

Sarkhosh A, Shahkoomahally S, Asis C, McConchie C (2021) Influence of rootstocks on scion leaf mineral content in mango tree (*Mangifera indica* L.). Horticulture, Environment, and Biotechnology. https://doi.org/10.1007/s13580-021-00355-w

Journal papers in preparation and review

Pandeya H, Friedl J, Mitchell E, De Rosa D, Asis C, Tilbrook J, Scheer C, Bristow M, Grace P, Rowlings D. Submitted for peer review. Leaf litter decomposition dynamics in tropical soils: the effect of N fertilisation and precipitation.

Conference papers

Anson D (2019) Optimising nutrients in mango systems of the NT. 12th Australian Mango Conference, AMIA, Darwin, Northern Territory.

Asis C, Meschiari L, McConchie C (2019) Ionome balance analysis of mango fruit from orchard with and without resin canal discolouration. Acta Horticulturae 1244, 221-228. https://doi.org/10.17660/ActaHortic.2019.1244.33

Asis C, Alexander T, Sarkhosh A, Umar M, McConchie C (2020) Optimising foliar nitrogen uptake of mango: effect of adjuvant, leaf position and time of potassium nitrate spray. Acta Horticulturae 1299, 269-274. https://doi.org/10.17660/ActaHortic.2020.1299.40

Bristow M, Asis C, Niscioli A, Robson A, Rowlings D (2017) Precision farming in mango to manage nitrogen nutrition. XII International Mango Symposium, China.

Bristow M, Asis C, Tilbrook J, Rowlings D, Robson A (2017) More profit from nitrogen: understanding the role of N in mango production. 11th Australian Mango Conference, AMIA, Bowen, Queensland.

McConchie C (2019) Advances in mango production: 2019. 12th Australian Mango Conference, AMIA, Darwin, Northern Territory.

Rahman M, Robson A, Salgadoe S, Walsh K, Bristow M (2019) Exploring the potential of high resolution satellite imagery for yield prediction of avocado and mango crops. Proceedings 36, 154. https://doi.org/10.3390/proceedings2019036154

Pandeya H, Friedl J, Asis C, Scheer C, Grace P, Rowlings D (2019) The interaction between nitrogen fertiliser and leaf litter application drives nitrous oxide emission from tropical mango orchards. AGU Fall meeting, San Francisco USA.

Thesis

PhD thesis

Pandeya H (2021) Carbon and nitrogen flux dynamics in highly weathered tropical soils: interaction effect of leaf litter and fertiliser. Queensland University of Technology. Submitted for assessment May 2021.

Master's theses

Clonan, M (2017) Site-specific variation of nitrogen availability across four soil types found on Top End mango farms. Master of Environmental Mangement, Charles Sturt University.

Yadav, D (2019) Effect of different scions on macronutrient resorption of mango Kensington Pride rootstock. Master of Environmental Management, Charles Darwin University.

Honours thesis

Vickery, B (2019) The limit to soil organic carbon sequestration in tropical soils. Queensland University of Technology.

Intellectual property

Nil

A.2 Equipment and assets

Item purchased	Date of purchase	Purchase price (GST exclusive)
Nil	NA	NA

A.3 Media and communications material

Description	Title	Date
Project launch: Mango Matters article	Project funding success – More Profit from Nitrogen	10/07/2016
Project announcement: NT Farmers newsletter	RD4P project funding success – More Profit from Nitrogen	1/12/2016
DITT e-newsletter article	Project starts research work	24/03/2017
AMIA e-newsletter article	Project starts field work	14/04/2017
DITT webpage article	NT mango research hits the national stage	10/05/2017
Fresh Plaza e-newsletter of the Fresh Produce Industry: project article	New research to better use nitrogen in mango production	12/05/2017
Top Paddock article	2017 Australian Mango Industry Conference a blast	15/09/2017
Mango Matters article – Winter 2017	Growers can actively lead new cutting-edge research into optimising nutrient management for the mango industry	12/07/2017
MPfN mango grower update – November 2017 newsletter	Mango grower update	16/11/2017
Top Paddock article	Quantifying nitrogen use efficiency in tropical mango production systems	15/12/2017
Nitrogen Natters article	Nitrogen management for improved mango productivity and quality	31/07/2018

Journal Article *Acta Horticulturae* 1299, p 269-274	Optimising foliar nitrogen uptake of mango: Effect of adjuvant, leaf position and time of potassium nitrate spray	22/09/2018
Journal Article *Remote Sensing* 10, 1866	Exploring the potential of high resolution WordView-3 imagery for estimating yield of mango	22/11/2018
MPfN mango grower update – May 2019 newsletter	Mango grower update	30/05/2019
Top Paddock article	Mango leaf litter and pruning: A large and hidden nitrogen input	3/06/2019
Top Paddock article	DITT Territory Natural Resource Management (TNRM) Award finalists	9/12/2019
Nitrogen Natters article	Mango leaf litter and prunings: A large and hidden nitrogen input	20/12/2019
Australian Tree Crop magazine article	'Floored' by mango's benefits	26/02/2020
Mango Matters magazine – Spring 2020 article	Skin deep: How ethylene affects mango quality	15/10/2020
Australian Tree Crop magazine – Winter 2020 article	Mango litter linked to fruit colour penalty	30/06/2020
Nitrogen Natters article	Is there leaf N uptake from foliar spraying of potassium nitrate at mango flowering and fruit set?	14/08/2020
Nitrogen Natters article	The mango team creatively extend their work to mango growers of the NT	18/11/2020
Journal article Nutrient cycling in agroecosystems – September 2020	Combined effect of nitrogen fertiliser and leaf litter carbon drive nitrous oxide emissions in tropical soils	23/09/2020
Journal article Submitted for review	Leaf litter decomposition dynamics in tropical soils: The effect of N fertilisation and precipitation	Submitted

A.4 Resource outputs for industry

Title	**Author**	**Date finalised**	**Host platform for ongoing use**
Poster: Mango leaf uptake of foliar applied nitrogen	Tilbrook, Anson, Niscioli, Rowlings and Asis	In preparation, ready for editing and publication	Electronic: DITT
Poster: Nutrient cycling in mango orchards	Tilbrook, Niscioli, Anson, Jayasekara, Guinto, Bristow,	In preparation, ready for editing and publication	Electronic: DITT

	Rowlings, and Asis		
Poster: Leaf nutrient resorption in mango	Tilbrook, Yadav, Adabor and Asis	In preparation, ready for editing and publication	Electronic: DITT
Poster: How nitrogen affects mango fruit quality	Tilbrook, Anson, Niscioli, Guinto, Jayasekara, Rowlings and Asis	In preparation, ready for editing and publication	Electronic: DITT
Poster: Mango trees and nitrogen movement within	Tilbrook, Anson, Niscioli, Rowlings and Asis	In preparation, ready for editing and publication	Electronic: DITT
Best practice guide for N application in NT mango orchards	Mango extension group DITT	In preparation for inclusion in MG17000	Electronic AMIA DITT

A.5 Budget

Reconciliation and final budget details are pending final submissions and approvals.

Appendix B: Current management practices of commercial mango orchards in the NT

B.1 Introduction

Mango is the largest fruit crop by volume and value in the Northern Territory (NT). It is grown in two main regions: Darwin and Katherine. Both regions have distinct wet and dry seasons; they are prone to intense rainfall and flooding during the wet season, followed by droughts and fires. The average annual temperature is around 27 °C. Over the course of the year, the temperature in Darwin typically varies from 20 °C to 33 °C and is rarely below 17 °C or above 35 °C, with a minimum humidity of 30 % during the dry season (Figure B.1a). In Katherine, the temperature ranges from 14 °C to 37 °C and is rarely below 10°C or above 40°C, and humidity in the dry season is typically less than 10 % (Figure B.1b). Mean annual rainfall ranges from 1,724 mm in the Darwin region and 1,009 mm in the Katherine region, with the most rain occurring during the distinct November–March wet season.

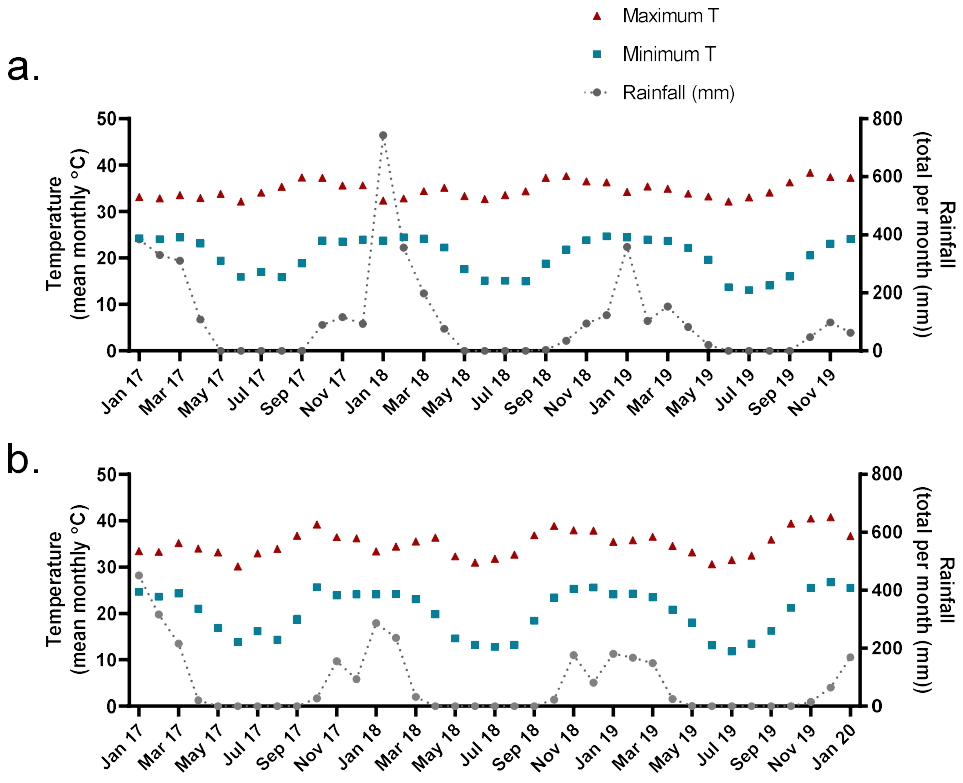


Figure B.1. Temperature and rainfall records for the Darwin mango growing region (Middle Point) (a) and Katherine mango growing region (RAAF, Tindal) (b). Figures are redrawn from data sourced in 2020 (BOM).

The annual mango production cycle in the NT is dictated by the arrival of cool season weather between April and August. Any variation in the timing of cool weather will influence when flowering occurs. The fruit growth component of the production cycle is also influenced by temperature. Temperatures that are too cool or too hot can limit fruit growth. Warm temperatures throughout the off-season period (December to March) coincide with monsoonal rainfall which encourages vegetative growth and tree recovery.

A typical NT mango harvest starts in the Darwin region in September and ends in Katherine in December. Fruit is sold in the domestic markets of Brisbane, Sydney, Melbourne and Adelaide with a small crop volume exported.

This is a summary of current tree and fertiliser management practices in commercial mango orchards across the NT. It highlights the variability within the industry where growers have site-specific practices that are influenced by cultivar, microclimate and soil variability. This information was collected over time from a range of sources as part of the More Profit from Nitrogen project (MPfN) and includes grower engagement, field experiments conducted during MPfN, published information, observations, industry small group extension activities and recent and historic grower surveys.

An examination of grower practices, in conjunction with MPfN project research results, will help understand how nitrogen fertilisers are being used in the local mango industry and focus on nitrogen use efficiency in the NT environment to deliver the benefit to growers in terms of a higher return on investment.

B.2 Variety

In the NT, Kensington Pride is the most commonly cultivated mango variety and accounts for around 70 % of production. Australia-wide it accounts for ~50 % of production but is reducing as new varieties are planted commercially. Other prominent commercial varieties are Calypso, R2E2 and Honey Gold, with Nam Doc Mai and Keow Savoey grown for the green mango market (Table B.1). Kensington Pride and common mango rootstocks are generally used for grafted trees and the type of rootstock used generally comes down to its availability. The release of three new mango hybrids developed by the Australian National Mango Breeding Program is pending. Currently known as NMBP 1201, NMBP 1243 and NMBP 4069, they were developed to produce superior fruit in Australian conditions.

Table B.1: Orchard characteristics vary across the NT, depending on varietal and spacing recommendations at the time of planting. The mango varieties, age and planting densities indicate the range of practices observed across the Darwin and Katherine mango growing regions (personal observations of Alan Niscioli, DITT).

Variety	Average tree age (yr)	Tree density (trees ha^{-1})	Tree spacing	
			Row (m)	Tree (m)
Kensington Pride	20-25	100–180	9–10	8–9
Calypso	15	125–300	8–10	4–6
R2E2	15	125–200	8–10	6–8
Honey Gold	10–15	125–300	8	4–8
Nam Doc Mai	10–15	125–300	8–10	4–6
Keow Savoey	10–15	125–300	8–10	4–6

B.3 Soil

Mangoes are grown on a range of soil types from light sandy loams to red clay loams that are common in the growing regions of Darwin and Katherine. The soils are weakly structured and tend to lack any textural contrast. They are generally characterised as having a high sand content of approximately 50 % along with an average clay content of around 10–30 %. Soil chemical properties are acidic to neutral,

trending into alkaline in Katherine, the drier of the two regions. This difference in pH is correlated to the cation exchange capacity (CEC) of soils and the higher calcium content of Katherine soils (Table B.2). The soils in these regions have been classified as Kandosols with grades of Rudosols and Tenosols (Vickery 2019).

Table B.2: The physicochemical properties of soils collected from six sites in the Northern Territory, Australia. Native vegetation and orchard sites from the Darwin and Katherine region were paired with adjacent sites containing mango orchards of different ages. Soil properties include soil texture, cation exchange capacity (CEC), pH, electrical conductivity (EC), bulk density (BD), total nitrogen (N), and total carbon (C). The displayed BDs were calculated after the removal of gravel > 2 mm. Standard deviation is shown (n=3) (Vickery 2019).

	Native vegetation	±SD	Orchard	± SD	Inter-row	± SD
Clay (%)	9.2	1.6	8.7	1.8	8.8	0.8
Silt (%)	40.2	4.0	37.3	5.2	37.1	1.7
Sand (%)	50.6	4.9	53.9	6.6	54.0	2.4
Bulk density (g cm^{-3})	1.2	0.1	1.3	0.1	1.3	0.0
CEC (cmol kg^{-1})	4.0	1.1	4.5	1.1	3.3	0.2
pH	6.5	0.2	7.3	0.3	6.8	0.0
EC (mS cm^{-3})	24.6	10.1	33.2	9.6	25.1	1.4
Total N (t ha^{-1})	1.6	0.4	1.6	0.3	1.6	0.1
Total C (t $ha^{-1)}$	31.6	11.2	31.3	8.7	28.5	1.1
Stable C (t ha^{-1})	22.8	7.2	21.4	5.3	20.2	0.8
Labile C (t ha^{-1})	9.0	5.0	9.8	4.2	19.3	0.3

B.4 Fertiliser management

Nutrition can be a significant constraint to the productivity of mangoes in the tropics. Mangoes respond well to macro elements such as nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S) and micronutrients such as boron (B), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and molybdenum (Mo) (Levin 2018).

N has the greatest influence on the growth and development of mango plants and is an essential element for chlorophyll, enzymes, protein and hormones. Deficiency of this element impacts on vegetative and reproductive growth (Marschner 2012).

P is needed for energy storage and transfer and is associated with stem and root development. It is also important in fruit set and ripening. Deficiencies of P impact a wide range of metabolic processes and can lead to poor growth and small fruit (Marschner 2012; Nasreen et al. 2014).

K regulates osmosis and the movement of assimilates. It helps strengthen plants by thickening their cell walls which improves fruit set, crop quality and shelf life. The strengthening of cell walls improves tolerance to stress and diseases. K deficiency harms physiological functions and can affect fruit development and reduce yield (Marschner 2012).

Ca is essential for plant processes including signalling, cell wall construction, and cell division. A lack of calcium can reduce plant stress responses and weaken tissue development, causing physiological disorders in fruit (de Freitas and Mitcham 2012; Hocking et al. 2016).

Mg is a central constituent of the chlorophyll molecule and is essential for carbohydrate metabolism and nucleic acid synthesis. Deficiencies of this element are detrimental to metabolic processes and affect photosynthesis and carbon assimilation (Marschner 2012).

Microelements, B, Zn, Fe, Mn, Cu and Mo all have roles in cell growth and enzymatic activity in mangoes (Winston undated). B for instance is associated with germination and development of apical growing points. It has a strong relationship with Ca. Deficiencies in B can lead to reduced fruit set while Zn is important for growth regulation and other metabolic processes, formation of chlorophyll, proteins and carbohydrates. A lack of this micronutrient can lead to stunted growth, fruit abscission and reduced yield (Winston undated; Marschner 2012).

Mango fertiliser/nutrition practices are determined by soil type, tree size and cropping history. Climatic conditions, phenology cycle and annual leaf and soil analysis are usually considered when fertilising. Nutrient management in mangoes is not highly standardised and a mix of methods are used to determine fertiliser programs. Some growers develop programs based on technical advice provided by agronomists while others base their programs on the interpretation of leaf and soil analysis (Hunt, unpublished). Some growers experiment with various amendments and compounds to ameliorate soils and improve nutrient productivity or to address a nutrient deficiency. Such products are used intermittently and usually not for sustained periods. They range from organic and inorganic compounds through to biological amendments and stimulants (Table B.3).

A number of fertiliser application methods are used in mango production including granular surface application, fertigation and foliar spray application. Most fertilisers are generally applied after harvest as a way of replacing nutrients removed from the farm when the fruit is harvested. A base-level mix of fertiliser is administered either as surface-applied granules, which are broadcast within the irrigation zone, or through fertigation using soluble forms of N, P, K, Ca and B. They can be applied singly or in appropriately blended formulations (Table B.3).

Table B.3: Examples of commercially available fertilisers which are soluble and suitable for fertigation or application via irrigation systems.

Fertiliser	Principle nutrients
Urea	Nitrogen
Calcium nitrate	Nitrogen, calcium
Potassium nitrate	Nitrogen, potassium
Potassium sulfate ('K spray')	Potassium
Monoammonium phosphate (MAP technical grade)	Nitrogen, phosphorous
Magnesium sulfate (Epsom salts)	Magnesium
Solubor	Boron
Boric acid	Boron
Zinc sulfate heptahydrate	Zinc
Iron sulfate	Iron
Iron chelate	Iron
Manganese sulfate	Manganese
Copper sulfate heptahydrate	Copper

Foliar fertiliser sprays are generally applied to add trace elements or to top up nutrient supplies at critical times in mango phenology such as flowering and setting fruit. In biennial bearing or less reliable mango cultivars, the foliar application of N- and K-based solutions has become normal practice for promoting and synchronising floral development, which depends on maturation or hardening of flushes during inductive periods.

Atmospheric sources of nutrients

Atmospheric nutrient deposition, both wet and dry, can be important sources of critical or limiting nutrients in natural ecosystems. An examination of the precipitation chemistry for Katherine over four years (1980–1984) (Likens et al. 1987) found that wet season rainfall was responsible for the deposition of 0.59 kg ha^{-1} dissolved nitrate and 0.42 kg ha^{-1} ammonium forms of N. Rainfall was also found to contain 0.39 kg ha^{-1} of dissolved Ca, 0.18 kg ha^{-1} of Mg and 0.44 kg ha^{-1} of K. The study indicated that higher rates of deposition occur early in the wet season. The concentration of chemical constituents in precipitation decreases in the later months of the wet season when monsoonal rainfall is heaviest, suggesting a dilution of ions in the atmosphere. It was postulated that these relatively high concentrations were driven mostly by the combustion products of wildfires burning in the region. The Darwin region experiences significant wildfires and rainfall over the same periods, so nutrient deposition may also be fire-linked, with around 1 kg ha^{-1} N deposited annually.

B.5 Water management

The irrigation of mangoes is highly varied between orchards. Almost all commercial mango enterprises in the Katherine and Darwin regions irrigate their mangoes with water derived from bores that recharge in the wet season. The quality of water varies from low pH and low mineral content water, usually

derived from shale or quartz-based aquifers, to high pH and high Ca and Mg content water that comes from dolomitic or limestone based aquifers (Blaikie and Cavenagh 2003).

In soils irrigated with water of low pH, such as in the Darwin region, nutrients tend to be available for uptake into plants, but are also more mobile and subject to leaching (Table B.4). Regular applications of Aglime and dolomite are needed to maintain the desired cation balance, while fertiliser additions may be required to compensate for lost nutrients. Water with a high pH is generally derived from limestone aquifers, as in the Katherine region. The soil in the region tends to have a high pH and can require large applications of dolomite or gypsum to maintain nutrient availability (Slattery et al. 1999)

Table B.4: The characteristics of bore water can vary greatly depending on the source. The slightly acidic (lower pH) water in the Darwin region has little to no Ca or Mg so they are applied as fertiliser components. Bore water in the Katherine region tends to be alkaline and the soil is alkaline which can limit the availability of Ca and Mg to tree roots in orchards. Applications of dolomite or gypsum can reduce soil pH, making these nutrients more available for uptake. Table redrawn (Blaikie and Cavenagh 2003).

Location	pH	EC	Ca	Mg	HCO3
		(µS cm^{-1})		(mg L^{-1})	
Darwin	5.2	19	5	1	3
Katherine	7.4	655	79	29	232

Delivery and irrigation scheduling

Micro sprinkler systems with high delivery rates, usually 70 L ha^{-1} or more, to irrigate orchards are common across the industry. A wide range of irrigation schedules are used by growers. Some growers use a fixed schedule throughout the flowering and fruiting period where the same weekly amount is applied across the irrigation season, while others have a varying schedule that considers the physiological stages of mango such as flowering, fruit growth, fruit load and seasonal conditions.

In mature orchards (established fruiting trees), most growers prefer to start irrigating at low or restricted volumes at the pre-flowering stage, equivalent to 25-50 % of the average rate of evaporation (Blaikie and Cavenagh 2003). This is usually from the early-to-mid dry season until flowering development begins (April–June). This period of low soil moisture or mild water stress is believed to encourage earlier and more synchronous flowering. The supply of small volumes of water ensures trees are not excessively stressed and encourages enough active growth for bud development and differentiation. Some growers prefer to increase irrigation after 50 % of the tree is in flower and at least 50 % of the flowers are open. This can cause flower drop and reduced fruit set. However, most producers will increase the irrigation from the commencement of visible flower panicle development in an attempt to speed up the flowering and fruit-setting process (Diczbalis et al. 2006). Post flowering, trees generally receive irrigation at a level of around 70–85 % of the rate of evaporation, which is usually continued until harvest. Some growers encourage the earlier development of 15 % fruit dry matter (minimum market standard) by manipulation of irrigation inputs whereby irrigation volumes are reduced up to 1–2 weeks prior to harvest, usually at 20–40 % of evaporation. The desire for the early flowering of trees is driven by the financial benefits of early access to interstate markets and the associated reward of high prices.

After harvest, some growers will reinitiate irrigation to promote growth and early flush. This is usually applied after pruning and fertiliser operations are complete, especially if there is a delay in wet season rain.

Irrigation rates

The irrigation requirements of mangoes are highly variable and depend on many factors including the variety grown, tree age, planting density, rainfall, evaporation and evaporation replacement. The amount of water taken up by the roots differs considerably from about 100 L per week in young trees to around 700 L per week in mature trees. When losses of soil water such as evaporation, runoff and deep drainage are considered, the weekly irrigation requirement to avoid plant drought stress during times of peak demand can be in the range of 1,000–1,500 L for large trees (Blaikie and Cavanagh 2003).

Irrigation volume and frequency depend on soil type (water holding capacity), effective root depth, bore capacity and output. Many growers calculate their crop irrigation requirements based on crop coefficients that have been published for mangoes. The crop coefficient (Kc) is calculated from data about the production site and plant development phase, along with rainfall and evapotranspiration (ET) estimates or forecasts and soil water holding capacity. Combined, they are used to calculate the mango irrigation requirement for a specified area. Typical crop coefficients and factors for mango grown in the Darwin region are outlined in the irrigation calculator (Table B.5).

Table B.5: Irrigation calculator prepared by Plant Industries, NT Department of Industry, Tourism and Trade for 'Building Best Management Practice Capacity of the Australian Mango Industry' (MB17000) project, part of the Hort Innovation Australia Mango Fund.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Crop coefficient (Kc)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	
Mean monthly rainfall (mm)	301	256	246	70	1.4	0	0	0	48.6	75.2	81.6	404	
Mean monthly ET (mm)	155	159	124	136	146	129	146	164	187	168	186	115	
Soil water holding capacity (mm/m)	168	168	168	168	168	168	168	168	168	168	168	168	
Irrigated area (%)	70	70	70	70	70	70	70	70	70	70	70	70	
Total area (ha)	100	100	100	100	100	100	100	100	100	100	100	100	
Irrigation efficiency	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	
													Annual
Effective rainfall (mm)	**168**	**168**	**168**	**70**	**1.4**	**0**	**0**	**0**	**48.6**	**75.2**	**81.6**	**168**	**946.8**
Crop water requirement (mm)	**0**	**111**	**86.5**	**95.3**	**102**	**90.4**	**102**	**114**	**131**	**118**	**130**	**80.5**	**1161.2**
Water deficiency (mm)	**0**	**0**	**0**	**30.3**	**106**	**95.4**	**107**	**119**	**87.3**	**47.3**	**53.7**	**0**	**646.46**
Volume (ML/ha)	**0**	**0**	**0**	**0.18**	**0.63**	**0.57**	**0.64**	**0.71**	**0.52**	**0.28**	**0.32**	**0**	**3.8464**
Volume (ML/total area)	**0**	**0**	**0**	**18**	**63**	**56.8**	**63.7**	**71.1**	**51.9**	**28.2**	**32**	**0**	**384.64**
Volume (ML/total area/day)	**0**	**0**	**0**	**0.6**	**2.03**	**1.89**	**2.05**	**2.29**	**1.73**	**0.91**	**1.07**	**0**	

B.6 Canopy management

Annual pruning

Pruning on an annual basis is widely practised in commercial orchards. Regular pruning is used to maximise the number of fruiting terminals and control tree size, making it easy to harvest and manage while improving fruit quality.

Pruning is usually conducted as soon as possible after harvest. This allows the new flush/leaves time to fully develop in the wet season when the potential for photosynthesis is greatest and provides an

opportunity to rebuild the stored reserves of the tree before the onset of flowering and fruiting when demand for reserves is high.

In general, trees are mechanically pruned or hedged into a square shape with sides and top trimmed flat. They are typically maintained at about 4 m in height and kept in check once desired size is achieved by removing the previous season's growth at each pruning event.

Some hand pruning may be necessary after machine pruning, particularly within the tree canopy. The interior of the tree is usually opened by removing some of the branches where growth is crowded or crossing over. An open canopy will improve spray application, increase light and air circulation, and minimise both disease and pest load.

Almost all commercial mango operations recycle the material resulting from pruning operations. Cut material is generally dragged into the inter-row and mulched to small pieces or woodchip using a mulcher/flail mower. This material is left *in situ* and allowed to decompose, recycling nutrients and carbon within the orchard system.

Growth regulators

Growth regulators are used for canopy management in mangoes. Paclobutrazol (PBZ) is a plant growth regulator registered with the Australian Pesticides and Veterinary Medicines Authority (APVMA). It is widely used to reduce vigour in mango trees and to promote flowering, leading to increased yields. PBZ is registered for use on mango as a collar drench at a rate of up to 20 mL tree^{-1}, to be applied after harvest and generally no later than mid-February. However, reported usage rates vary widely. Some operators describe off-label rates of administration as high as 60–90 mL on extremely large trees. On sandy soils, some growers allow trees to commence vegetative growth after harvest prior to treatment. The chemical is diluted in several litres of water to assist in the application and spread around the root zone and is applied 90–120 days prior to when flower induction is required. The post-harvest application of PBZ to the soil can have a significant effect on flowering and fruiting following application.

B.7 Flower induction

Temperatures are inductive

In mangoes, the trigger for reproductive growth is complex, but cool weather is generally associated with the onset of flowering. Floral induction occurs when trees are exposed to cooler night-time temperatures that occur in the dry season, typically (May–June). In general, mangoes grown in the NT require temperatures below 18 °C to stimulate flowering; however, daytime maximum temperatures may also have inhibitory effects and some influence on floral induction. Recent studies suggest that these temperature thresholds are cultivar specific with different varieties exhibiting different sensitivities. Although cooler temperatures are required to induce flowering, the level of vegetative flushing will also modify or even inhibit the flowering response (Clonan et al. 2021). If trees are undergoing a flush of new leaves when cool inductive temperatures occur, then those terminals are less likely to flower compared to terminals that did not recently flush. The development of a new flush can also disrupt the signalling processes associated with flowering across the tree by altering the ratio of temperature-regulated florigenic promotors and age-related vegetative promotors during shoot initiation.

Paclobutrazol

PBZ is a plant growth regulator widely used to reduce vigour in mango trees and to promote flowering. It is believed that gibberellins act as inhibitors to flowering. PBZ acts by blocking the synthesis of these flowering inhibitors, allowing the flower-promoting factor(s) to be effective. PBZ itself does not induce flower initiation in mangoes as it is applied months before induction. It is thought that PBZ increases carbohydrate reserves through increased photosynthesis and slows the vegetative flush cycle. This allows the most recent flush to mature, making it more responsive to inductive treatments and favourable low air temperatures.

Potassium nitrate and other floral promotors

Foliar potassium nitrate (2–4 %) is commonly used by growers as a floral promotor and is thought to promote bud development, which undergoes floral induction when contemporaneous with low temperatures. Potassium nitrate is also thought to be effective in the synchronisation of flowering when temperatures are inductive. Some growers use foliar applications of both potassium and ammonium in sulfate form. Urea and foliar mixes of N, P and K are also used to mature flushes and induce flowering. Synchronised flowering means that fruit harvesting is more efficient, with most fruit maturing at the same time.

Cincturing and girdling

Girdling or cincturing is a technique used to improve the earliness and intensity of flowering in mangoes. The procedure involves cutting a ring of bark (phloem) out of the main trunk or branches of trees which restricts the movement towards the roots of carbohydrates produced in the leaves. This temporarily restricts tree growth, changes the hormone balance, and makes the tree more likely to flower. This technique is generally applied to mature or older trees as this technique can be too stressful for younger trees. Girdling was a widely used technique that is now occasionally practised in the industry. Some benefits in terms of flowering and increased yield have been shown (Chacko et al. 1982; Blaikie et al. 2000), while leaf photosynthesis is reduced (Urban and Alphonsout 2007) and vegetative growth reduced by 50–60 % (Blaikie et al. 2000). The risk of productive trees dying as a result of cincturing, and the chemicals involved, discouraged continuing widespread use of the technique.

Tip pruning for flowering and yield

Controlling growth to stimulate the formation of vegetative and reproductive buds is common practice in fruit tree management. Flowering occurs on new growth, so if there are more new shoots, more flowers are likely which means a higher potential yield in the following season.

Pre-flowering tip pruning is used to induce synchronous, uniform flowering and fruiting in mango. This pruning takes place from about the middle of May and involves the removal of apical stems to stimulate the initiation of axillary shoots. If the timing and temperature are right, it is followed by a floral rather than a vegetative flush, meaning more flowers on the tree and potentially more fruit set and higher yield. The added advantage is a reduction in tree size when two or more leaf flushes are cut back. Tip pruning is not a common practice as the time span when this pruning can be safely done is short. Unfortunately, if it is done too soon or too late in the season, the crop can be lost due to vegetative growth instead of flowering.

In this way, pruning can regulate shoot production and timing and improve productivity. Growth of mango is not continuous but occurs during the active growth period over the wet season as

intermittent, short-lasting flushes from apical or lateral buds. Under normal conditions, early initiation and cessation of growth, followed by a definite dormant or quiescent period during the early dry season will help shoots attain proper physiological maturity essential for fruit bud initiation.

Commercial mango orchard management practices are continuously reviewed and updated as research knowledge builds along with rapidly developing technologies.

B.8 References

Blaikie S, Cavenagh P (2003) Operation KP – a production guide for mango growers in northern Australia. TropHort, NTDBIRD, CSIRO, CDU and NTHA, Northern Territory

Blaikie SJ, Leonardi J, Chacko EK, Muller WJ, Scott NS (2000) Effect of cincturing and chemical treatments on growth, flowering and yield of mango Mangifera indica L. cv. Kensington Pride. Australian Journal of Experimental Agriculture 39 (6):761-770. doi:https://doi.org/10.1071/EA98178

BOM Available via Bureau of Meteorology, Australian Government. http://www.bom.gov.au/climate/data/?ref=ftr. 2020

Chacko EK, Reddy YTN, Ananthanarayanan TV (1982) Studies on the relationship between leaf number and area and fruit development in mango (Mangifera indica L.). Journal of Horticultural Science 57 (4):483-492. doi:10.1080/00221589.1982.11515082

Clonan M, McConchie C, Hall M, Hearnden M, Olesen T, Sarkhosh A (2021) Effects of ambient temperatures on floral initiation in Australian mango (Mangifera indica L.) selections. Scientia Horticulturae 276:109767. doi:https://doi.org/10.1016/j.scienta.2020.109767

de Freitas ST, Mitcham EJ (2012) Factors involved in fruit calcium deficiency disorders. In: Horticultural Reviews. pp 107-146. doi:https://doi.org/10.1002/9781118351871.ch3

Diczbalis Y, Wicks C, Owens G (2006) Mango Irrigation Management Guidelines. Agnote No. 587. Northern Territory Government, Darwin

Hocking B, Tyerman SD, Burton RA, Gilliham M (2016) Fruit calcium: Transport and physiology. Frontiers in Plant Science 7 (APR2016). doi:10.3389/fpls.2016.00569

Levin A (2018) Improving fertiliser and water use efficiency in mango cultivation. Achieving sustainable cultivation of mangoes. Burleigh Dodds Science Publishing. doi:https://doi.org/10.4324/9781351114431

Likens GE, Keene WC, Miller JM, Galloway JN (1987) Chemistry of precipitation from a remote, terrestrial site in Australia. Journal of Geophysical Research: Atmospheres 92 (D11):13299-13314. doi:https://doi.org/10.1029/JD092iD11p13299

Marschner P (2012) Marschner's Mineral nutrition of higher plants. Third edn. Academic Press

Nasreen S, Kamal AM, Siddiky MA, Rannu RP, Islam MS (2014) Effect of nitrogen, phosphorus, potassium and sulfur on the yield of mango. Bangladesh Journal of Agricultural Research 39:631-640

Slattery WJ, Conyers MK, Aitken RL (1999) Soil pH, aluminium, manganese and lime requirement. Soil analysis, an interpretation manual. CSIRO Publishing, Australia

Urban L, Alphonsout L (2007) Girdling decreases photosynthetic electron fluxes and induces sustained photoprotection in mango leaves. Tree Physiology 27 (3):345-352. doi:10.1093/treephys/27.3.345

Vickery BS (2019) The limit to soil organic carbon sequestration in tropical soils. Honours thesis, Queensland University of Technology

Winston T (undated) Understanding crop nutrition. https://www.horticulture.com.au/globalassets/hort-innovation/resource-assets/mg15006-understanding-crop-nutrition-mango.pdf Accessed 24 June 2021

Appendix C: Research technical summary. Optimising nutrient management for improved productivity and fruit quality in mangoes

The dynamics of nitrogen (N) uptake, movement and storage in mango trees over time has not been investigated in tropically grown mango in the Northern Territory (NT), which has a climate and soils that are different to conditions in other mango growing regions of Australia. To understand what constitutes efficient use of N in commercial orchards, an N budget for mangoes needs to be constructed. A budget needs to include N applied, N cycling, gaseous emissions, N losses via leaching and N leaving the orchard in harvested fruit. This will provide information to reassess N application and nitrogen uptake efficiency (NUE) in commercial orchards, thus identifying potential cost efficiencies and reduction in N loss to the environment. The experimental work was conducted in a range of locations including Coastal Plains Research Farm (CPRF) in the Darwin region, commercial orchards in the Darwin and Katherine regions, and pot-based trials at Berrimah Farm, Darwin, NT.

Yields of mango fruit generally vary from year to year. This could be attributed to the irregular bearing nature of the tree, seasonal conditions, or mineral nutrition of the plant. Among the essential nutrients needed by mango, N has the greatest effect on the growth and development of plants because of its role in morphological and physiological functions. It is a constituent of key cell molecules such as amino acids, nucleic acids, chlorophyll, adenosine triphosphate (ATP) and several plant hormones. N is also an essential regulator involved in many biological processes, including carbon metabolism, amino acid metabolism, and protein synthesis (Eckstein et al. 1999; Marschner 2012; Crane et al. 2009; Bally 2009).

C.1 Uptake of soil-applied nitrogen fertiliser into mango trees

Introduction

Mango trees obtain N from the soil and take it up into their root systems, mostly in ammonium (NH_4^+) and nitrate (NO_3^-) forms. Soil organic matter often contains much of the soil reserves of nutrients, in particular N. However, soils in the NT are low in organic matter because they are oxidised during the long annual dry periods. Carbon (C) levels in well-drained soils are less than 1 % on the surface horizon and the mineral fraction of most soils has very low cation exchange capacity. Low soil fertility and high permeability also increase the leaching and lateral movement of nitrates and phosphates. Therefore, most soils are highly erodible, have poor natural fertility, and have low water holding capacity (Smith and Hill 2011).

External application of N fertiliser from organic and inorganic sources is important to meet the crop demand. However, the response of mango to N fertiliser application is also influenced by its source, its rate, timing, and method of application, tree-growth stage, climate, edaphic conditions, soil moisture status, and cultivar vigour (Bally 2009). Improper or excess application of N may lead to nutrient deficiencies and toxicities which result in reduced tree growth, yields and fruit quality. Additionally, excessive application of N can negatively affect the natural environment. Thus, N

fertiliser management practices should continue to be refined and developed. This involves monitoring the dynamics of N at different growth stages of the plant.

Our research interest is geared towards understanding the uptake, dynamics and recycling of N during the different growth stages of mango. The partitioning and recycling of N can be determined by using stable isotope-labelled fertiliser. In this study, we will quantify the nutrient uptake and mobilisation of N. This information will help to improve the N fertiliser management and N use efficiency in mango production systems. Experimental work was conducted on young Kensington Pride (KP) mango trees at CPRF and on mature KP trees at a commercial orchard in the Katherine region of the NT.

Methods

The experiment was conducted from June 2015 to November 2019. Seeds of KP were collected from Jabiru Tropical Orchard (JTO) in Lambell's Lagoon. Embryos were extracted from the mango seeds and germinated in commercial potting soil, in plastic pots. Two months after emergence, individual seedlings were repotted into larger (1.5 L) pots, then grafted with KP budwood, also sourced from JTO. Three blocks of twelve grafted trees were transplanted into a field site at CPRF in September 2015. A row of buffer trees was planted around and between the blocks. In each block of twelve trees, three replicates of four levels of N (0, 20, 40 or 60 g tree^{-1}) in the form of ammonium sulfate (NH_2SO_4) were applied according to a randomised block design. This was equivalent to 0, 5, 10 and 15 kg ha^{-1} at a planting density of 250 trees ha^{-1}. Foliar application of micronutrients was completed at 6 and 12 months after planting. Also applied were the commercially recommended quantities of P (35 kg ha^{-1}), K (76 kg ha^{-1}), S (20 kg ha^{-1}) and Ca (from lime, 6 kg ha^{-1}). In January 2016–2019, 300 g of MPK was applied to each tree. As a risk mitigation strategy, a duplicate set of three blocks of twelve trees was established immediately adjacent with the same management and fertiliser application, to be sampled in the event trees were lost due to weather (cyclones or storms) or destruction by *Mastotermes darwiniensis*. Fruit was harvested from the duplicate blocks of trees in 2018 and 2019.

To enable quantification of tree uptake of soil-applied NH_2SO_4, an enriched form with 2.10 atom% excess ^{15}N was applied at the various treatment levels to the soil under the canopy drip lines of all trees in January 2017 and January 2019. During the experimental period of 2015 until 2019, usual orchard management practices were followed for pest management and micronutrient application, with no potassium nitrate foliar sprays.

Data collected over time included tree height, canopy size, N content of tree components, fruit yield and N uptake from soil-applied N as trees grew from the juvenile stage in 2015 past the first harvest of commercial quantities of fruit in 2019. To quantify N uptake of soil-applied N, a single block of twelve trees was destructively sampled in 2017, 2018 and 2019 (Figure C.1).

Figure C.1: Preparation of tree material started with grafting of KP shoots onto KP rootstocks (a), growing the pots (b), leaves flushing on grafted KP trees (c), setting up the orchard irrigation prior to planting (d), planting grafted KP into the orchard (e), pruning for shape (f), measuring and lifting trees from the soil to measure N content and uptake through the trees (g and h).

Soils

Soil nitrate and ammonium content in soils at CPRF did not vary widely in response to the range in quantities of applied N fertiliser in January of each year 2017–2019 (Table C.1).

Table C.1: Orchard soil nitrate (a) and ammonium content (b) at CPRF did not vary over time or in response to annual soil-applied fertiliser. Mean, standard error of the mean (sem), n=3.

a. **Measured soil nitrate (µg g^{-1})**

Applied N							
g tree^{-1}	kg ha^{-1}	**2017**		**2018**		**2019**	
	@250 trees ha^{-1}		±sem		±sem		±sem
0	0	0.5	0.1	0.6	0.3	0.5	0.1
20	5	0.7	0.1	0.9	0.4	0.5	0.1
40	10	1.0	0.1	0.5	0.2	0.4	0.1
60	15	0.7	0.1	0.8	0.2	0.4	0.1

b. **Measured soil ammonium (µg g^{-1})**

Applied N							
g tree^{-1}	kg ha^{-1}						
	@250 trees ha^{-1}		±sem		±sem		±sem
0	0	11.6	0.7	5.3	0.6	13.3	1.5
20	5	13.0	0.4	5.1	0.3	18.3	4.9
40	10	13.5	1.1	5.0	0.8	12.4	0.6
60	15	15.9	0.1	5.0	0.9	15.0	1.5

Tree harvesting and biomass partitioning

A method of lifting whole trees was developed to enable quantification of tree components, N content, ^{15}N-labelled content and uptake of N applied to the soil into mango trees over time. Equipment used for creating fence post holes was modified to do this. A hollow metal probe of approximately 1.6 m length, with a perforated, tapered tip was connected to hoses, which were then attached to a firefighting water tank fitted with a pressure pump (Figure C.2a, b).

Figure C.2: A method was developed to deconstruct, then lift trees out of the soil so the component parts could be weighed and dried for analysis. A hollow steel probe with a perforated, tapered tip was attached to a fire tank with a pressure pump (a, b). Trees were de-branched with pruning saws and a chain saw (c). The probe injected large quantities of water into soil surrounding the trunk of the trees, sufficiently softening it to lift out with most roots remaining intact (d).

Testing showed that injecting large quantities of water into the ground, to the full depth of the probe, around a 3 m radius from the tree trunk, softened the soil into a slurry. A webbing 'snatch strap' was wrapped around the trunk, then attached to a tractor forklift to elevate the tree out of the ground (Figure C.2c, d).

The lifting of a tree out of the ground followed a sequence:

1. Trees were irrigated overnight.
2. One tree was lifted and processed at a time, and fresh weights (FW) of components recorded.
3. Large quantities of water were introduced into the soil around the stem of a tree to make the soil into a slurry (Figure C.2a, b).
4. Branches were cut from the canopy and transported in a tarpaulin into an open shed and leaves were immediately stripped from branches into large plastic bins.
5. The stem had a snatch strap wrapped at the top, at the point of branching.

6. Additional water was introduced into the soil if possible, then the tractor forks lifted the snatch strap and attached tree slowly from the soil as roots released from the soil.
7. Soil attached to the roots and fine roots was washed off.
8. Stem was cut from roots using a chain saw, and slices (biscuits) cut from the stem (three cuts: top, middle and base of stem) and taproot (one cut at 20 cm below the soil surface).
9. All components were deconstructed and FW recorded, starting with leaves, then branches, stem and roots.

Blocks of twelve trees (three replicates for each N treatment level) were measured then lifted in April 2017, April 2018 and post-harvest in November 2019. In 2017, all harvested materials of the juvenile trees were transported to Berrimah Farm and dried in ovens at 50 °C until stable weights were achieved. In 2018, most above-ground materials were dried and, in 2019, materials subsampled from trees were dried and standard curves constructed from FW:DW data in the preceding two years were used to calculate the DW of each component.

Representative material was subsampled from across each quadrant of the tree in a standardised fashion for each year. Leaves (approximately 500 g DW randomly selected), branches (10 x 5 cm lengths of current season green branch and 10 x 5 cm lengths for older brown bark branch), stem or trunk (3 x biscuits of wood were cut from the top, middle and base of stem) and roots (fine, < 2 cm diameter and 1 x biscuit from the taproot, subsampled for analysis in the ratio 1/3: 1/3: 1/3 on a DW basis).

The material was washed in tap water, rinsed in Millipore filtered water then dried in ovens at 50 °C until a constant dry weight was attained. All fruit on trees was harvested in October 2019. For analysis, three fruit from each tree were measured for dry matter content using a near infra-red meter calibrated for KP fruit (F-750, Felix Instruments, Camas WA, USA), FW recorded, then sliced and dried in ovens as described. All tree and fruit components were ground in a Rocklabs Standard Ringmill and analysed for total N, ^{15}N atom% excess and carbon by mass spectroscopy (EA-IRMS, Sercon Limited, UK) at the Institute for Future Environments at Queensland University of Technology (QUT). The % N derived from fertiliser (dff) was calculated using the method (IAEA 2001):

$$\%\ N\ dff = (atom\%\ ^{15}N\ excess_{plant} / atom\%\ ^{15}N\ excess_{fertiliser}) * 100$$

From % N dff, the amount of applied fertiliser in each tree and nitrogen uptake efficiency (NUE) were calculated from the tree N content and DW. The total N difference method was used to calculate the % N dff and NUE for 2018 (IAEA 2001). The fruit was harvested in August 2018 and October 2019 from duplicate blocks of trees (n=6). In 2019, three fruit were sampled from each replicate of each treatment level, dried, bulked, processed and subsampled as above (n=3).

Statistical analysis

Statistical analysis of the data was conducted using the International Rice Research Institute's free software Statistical Tool for Agricultural Research (STAR) version 2.0.1 for linear regression and ANOVA (IRRI 2019)The treatment means were separated using the least significant difference test (LSD) at 5 % level of significance. Alternatively, data were analysed using Prism 9.0 (Graphpad Prism®) for linear regression to assess the FW:DW relationships of tree components, one way ANOVA with Tukey's post-test for fruit yield, fruit % N dff, time series or two way ANOVA with Tukey's post-test for other characteristics measured-as indicated in the text or figure legends. The type 1 error or statistical significance level is p=0.05 for all tests.

Results

Fresh weight:dry weight relationships

Regression analyses showed strong relationships for FW:DW of tree material collected in 2017 and 2018: stem (r^2=0.997), leaves (r^2=0.999), branches (r^2=0.98) and roots (r^2=0.97), data not shown. These relationships were used to calculate the DW of tree components harvested in 2019. Also, the DW of the tree root mass was correlated with the DW of the total of above-ground tree components collected in 2017 and 2018: (regression analysis, r^2=0.954). This relationship was used to estimate the DW of tree roots of trees sampled in November 2019 when the tree root systems had become too difficult to lift with the method described above. Instead, total DW was estimated and root material sampled.

Tree growth and yield

Tree height and canopy volume increased over time (Figure C.3a, b). Fruit was harvested when mature in 2017 (scant, not quantified), 2018 and 2019. (Figure 3c). No significant differences were seen in yield response to N treatments for 2017 harvest (one way ANOVA, p=0.16) 37 months after planting, or for the 2019 harvest (one way ANOVA, p=0.14) 51 months after planting (Figure 3c)

Whole tree dry weight was measured over time and no significant differences were seen in response to N treatments (ANOVA, p=0.3, sem, n=3) (Figure C.4a). Whole tree N was also measured, with no significant differences in accumulation observed in response to the N treatments (ANOVA, p=0.24, sem, n=3) (Figure C.4b). No significant interactions between time and N treatments were found in tree DW or tree N content over time.

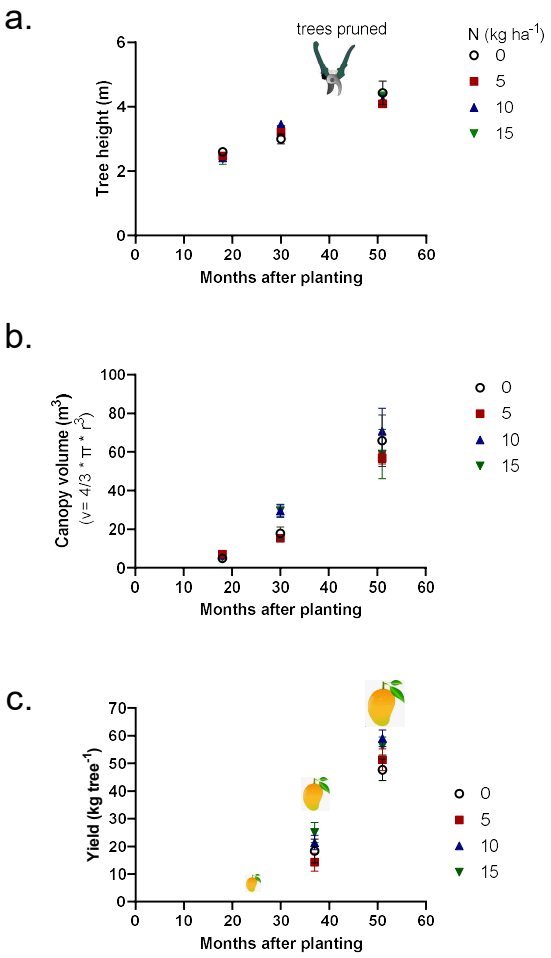


Figure C.3: Mango tree height (a) and canopy volume (b) were measured prior to a set of trees being lifted from the ground for N analysis and calculation of N uptake. No differences were seen in height in response to N treatments (time series ANOVA, p=0.81). However, note that trees were all hand and mechanically pruned to the same dimensions (4 m wide, 4 m deep and 3.8 m high) post-harvest in November 2018, 38 months after planting. No differences were observed in canopy volume over time in response to N treatments (time series ANOVA, p=0.37). Fruit was harvested when mature in 2017 (scant, not quantified), 2018 (one way ANOVA, p=0.16, sem, n=11–12) and 2019 (one way ANOVA, p=0.14, sem, n=5-6).

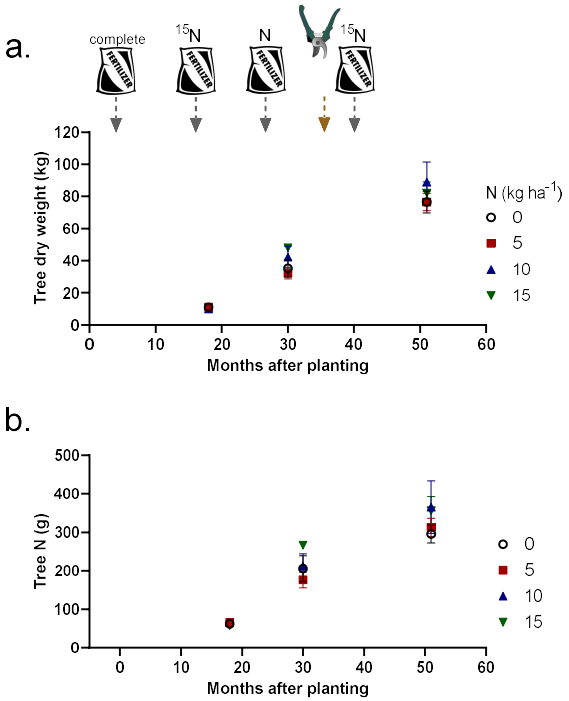


Figure C.4: Tree dry weight was measured over time and no significant differences were seen in response to N treatments (time series ANOVA, p=0.3, sem, n=3) (a). Whole tree N was also measured, with no significant differences in accumulation seen in response to the N treatments (time series ANOVA, p=0.24, sem, n=3) (b). Timing of initial complete fertiliser application at planting and subsequent N applications and pruning are indicated by icons in (a). No significant interactions between time and N treatments were found in tree weight or tree N over time.

The relative DW of each tree component was followed over time (Figure C.5a, c, e, g) along with the N content of each component (Figure C.5b, d, f, h). In juvenile trees less than two years old, leaves, branches and roots each make up approximately 30 % of tree DW (Figure C.5a, c, g), with the remaining 10 % being the stem (Figure C.5e). The proportion of tree N in leaves was initially ~65 % of the tree total N and reduced to just over ~40 % at 50 months after planting (Figure C.5b). In contrast, the branch proportion of whole tree DW increases from 30 % to 50 % as the trees matured, with the N % increasing from 20 % to ~40 % (Figure C.5c, d). The portion of the tree stem increases initially, but as the tree matures and branches increase in DW, it ranges between 10 % and 20 % of the total tree DW (Figure C.5e). Stem N is maintained at ~5 % over time, and root N at ~10 % (Figure C.5f, h). The root portion of tree weight is initially 30 % of the total, reducing to around 20 % as it matures (Figure C.5g). There were no significant differences in the tree component DW's or N % in response to any N treatment, nor were there any significant interactions between time and N treatment (ANOVA, mean, sem, n=3) (Figure C.5a–h).

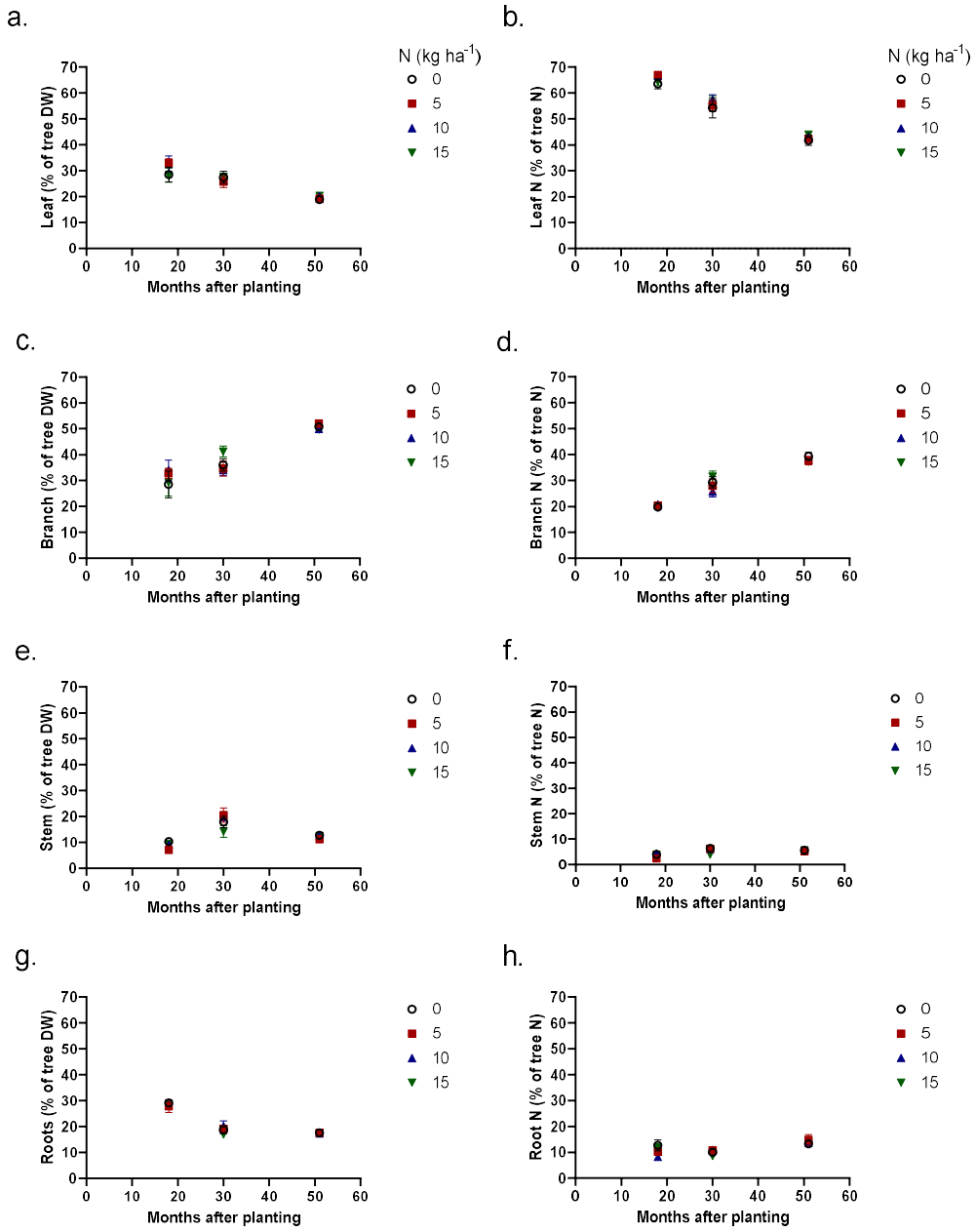


Figure C.5 The proportional DW of tree components were followed over time (a, c, e, g) along with the N proportion in each (b, d, f, h). Leaf % N and DW reduces as the tree ages (a, b) and branch % N and DW increases (c, d). No significant differences were observed in response to N levels applied to trees (time series ANOVA, p values not shown, sem, n=3).

The NUE of the trees increased annually, with the juvenile trees showing around 20 % NUE response to 5 kg ha^{-1} application, reducing as the applied N rate increased (Figure C.6a). The reduced uptake response to increasing application of N also occurred in 2018, with 40–50 % NUE. The NUE in 2019 was an impressive 75 % for trees with 5 kg N ha^{-1} applied but reduced significantly to 32 % and 16 % for 10 and 15 kg N ha^{-1}, respectively (Figure C.6a). Interestingly, the N dff within the harvested fruit reflected the amount of labelled fertiliser that was applied (Figure C.6b).

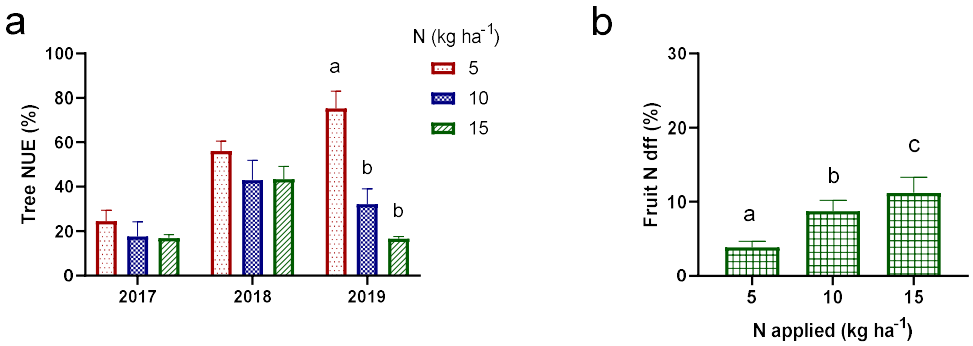


Figure C.6: Nitrogen uptake efficiency (NUE) of trees changed as trees matured and began commercial production in 2019. No significant differences were seen in fertiliser uptake in 2017 and 2018 in response to N treatments (a). In 2019, trees with the lowest quantity of N applied showed significantly higher uptake than the two larger N applications (letters indicate significant difference). It is acknowledged that the interaction of NUE with time is significant (21 % of variation) as expected with growing trees and repeated N applications (time series ANOVA, Tukey's post-test, p=<0.0001, n=3). The N content of fruit dff was, in round figures, 4, 9 and 11 %. These were significantly different, indicated by different letters (b) (one way ANOVA, p<0.0001, n=3)

Fruit production of the trees reached commercial levels in 2019, with variability and no significant links between the number of fruit tree^{-1}, yield on a weight basis, dry matter or the amount of N in the harvested fruit in response to the range of soil-applied fertiliser N levels (Figure C.7).

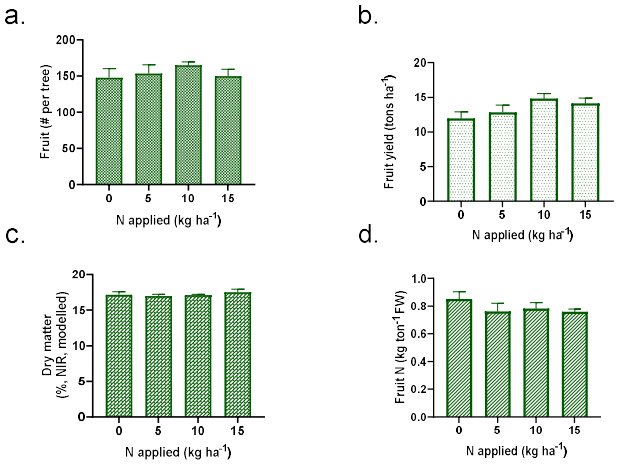


Figure C.7: At harvest the number of fruit from each tree was recorded (a) along with weight (b) the fruit % dry matter (c) and the amount of N leaving the orchard in fruit (d). No significant responses to the varying fertiliser levels were seen in the number of fruit tree^{-1} (p=0.66) (a), total yield (p=0.14) (b), fruit dry matter content (p=0.66) (c) or nitrogen content of the fruit harvested (p=0.47) (d). Data is standardised to 250 trees ha^{-1} (b and d). (one way ANOVA, mean, sem, n=6).

Discussion

It is well documented that nitrogen uptake efficiency in trees is highest when there is low availability (Marschner 2012). There is a range of methodologies for calculating nitrogen use efficiency in commercial crops with the simplest being profitability, i.e. the economic return relative to the cost of the inputs to generate the crop. For tree crops, nitrogen use efficiency is often considered in terms of nitrogen uptake related to the nut or fruit yield rather than biomass production (Antille and Moody 2021). In this work, the fruit yield was considered the indicator for efficient use, and no yield benefit was observed in response to increasing N levels.

The young mango trees with smaller root systems, fewer branches and smaller canopies showed lower NUE, with a maximum of ~20 % of applied fertiliser taken up in 2017. As the tree increased in size and root spread to maturity, NUE increased significantly to ~75 % at the 5 kg ha^{-1} application rate but remained lower at the 10 kg ha^{-1} and 15 kg ha^{-1} rates in 2019. The data suggest that tree uptake of the three rates were 3.9, 3.2 and 2.5 kg ha^{-1}, respectively – NUE reducing as application rates increase.

For NUE in terms of soil-applied N in mango orchards, efficiency is directly related to how much N is already available to the tree from sources such as litter decomposition, mineralised N and replenishment of N stored within the tree after harvesting of fruit. It is significant that 11–13 kg of N left the orchard in the harvested fruit. This shows that trees are acquiring a relatively small portion of their N requirements from soil-applied N.

Fruit N content appears to be quite consistent and, like leaf N content, does not seem closely linked to fertiliser N applied to trees. It appears to be relatively consistent between orchards and fertiliser management practices, implying that leaves and fruit are efficient sinks, taking resources they need for development and maturity from those stored within the tree or from the soil. Also interesting in this data is that the N content in harvested fruit dff does increase in proportion to soil-applied N (Figure C.6b), while fruit total N stays similar. If NUE was the same for all treatments this would be expected, as we know that labelled N infused into tree stems was thoroughly distributed around the entire tree over several weeks (Section C.3), but it doesn't reflect the tree NUE of 2019 (Figure C.6a) which implies the opposite. Further work is required to understand the processes behind this.

This project shows that mango fruit crops generally remove 10–25 % of tree total N content, depending on seasonal conditions, biennial yield cycling, irrigation and other yield-limiting factors such as temperature during flowering and fruit set (estimated from data here and Section C.3). The remaining N in trees appears to act as a buffer to deficiencies, so any visible N deficiencies, such as pale yellow foliage is probably indicating a longer-term deficiency.

In cropping and orchard systems, N is frequently applied in excess to needs, as inexpensive insurance to ensure sufficient cash return for the crop. Increasingly there is an environmental premium on nitrogen use, and it is necessary to refine our understanding of the N cycle. In addition to N outputs in fruit, the N content of litter and pruned material cycling in orchards, along with soil N availability and N mineralisation will help estimate annual N requirements in mango orchards.

C.2 Is nitrogen taken up across the leaf cuticle?

Introduction

Leaves have evolved to intercept light energy and take up CO_2 for photosynthesis in an atmosphere that contains low concentrations of water. All vascular plant leaves have a cuticle layer that is hydrophobic and acts as a barrier, controlling water loss from the plant or tree (Kerstiens 1996). The cuticle of leaves (and fruit, flowers and stems) protects plants from biotic and abiotic stresses such as insects, pathogens (Serrano et al. 2014) and ultraviolet radiation (Krauss et al. 1997). The cuticle is also a barrier to any solution applied to leaves, and movement of a solution across the cuticle is passive, despite being called 'uptake' (Fernández and Eichert 2009). Recent work on a *Quercus* variety has shown that local climatic conditions can affect the composition of cuticular waxes, but

had no impact on leaf water loss via the cuticle (Bueno et al. 2019). The leaf cuticle also has stomatal openings through it. Stomatal density and diameter vary widely on both the upper and lower surfaces of leaves and penetration of any solute into leaves via stomata can be highly variable and unpredictable (Eichert et al. 2008; Eichert and Fernandez 2012). It would be interesting to know how these observations apply to mangoes growing in different climates, and also whether cuticular wax varies between varieties.

Any foliar uptake of agrochemicals is by diffusion according to Fick's Law (not stomatal uptake), where the solute moves down a concentration gradient into leaves, depending on the permeability of the cuticle (Fernández et al. 2017). If N can cross the cuticular barrier and cell membranes into the cytoplasm, it is generally stored as nitrate in the vacuole (Tegeder and Masclaux-Daubresse 2018). In fully expanded leaves, the stored N is used to maintain the metabolism of photosynthesis (Liu et al. 2018). In mangoes grown in the Darwin region, varietal differences in net photosynthesis and stomatal conductance have been measured. The differences were more noticeable in the dry season, with KP unable to maintain photosynthesis in hot, dry season conditions (Lu et al. 2012).

Foliar application of fertilisers to commercial cotton, fruit and vegetable crops is common (Huett and Vimpany 2006; Luo et al. 2015; Lovatt 2013). Potassium nitrate (KNO_3), in particular, is applied to mangoes at flowering and fruit set with the assumption that additional potassium (K) will enhance fruit set and, hopefully, maintain more fruit on the flowering panicles (Oosthuyse 1993a; Sudha 2012; Protacio 2000). At low concentrations in water, the K^+ cation dissociates completely from the NO_3^- anion. No research publications were found tracing K^+ movement across leaf cuticles; however, K^+ movement has been quantified using artificial membranes (He and Rezai 2020). While no direct measurements of foliar uptake of NO_3^- or NH_4^+ across mango leaf cuticles have been published, foliar applications have been found to increase yields of mangoes, oranges and avocadoes when sprayed onto trees at flowering and fruit set (Lovatt 1999; Oosthuyse 1993a; Sudha 2012). Also, varietal differences have been observed in the seasonal stomatal conductance of leaves (Lu et al. 2012). While it is still uncertain, solution components may be taken up via stomata depending on the stomatal structure, particle size and the time of application (Eichert and Fernandez 2012; Asis et al. 2020). Commercial mango orchards carry out multiple spray applications of 1–4 % KNO_3 solutions to promote flowering and fruit set. In terms of orchard management, two applications of a 2 % KNO_3 solution adds about 5.5 kg of N ha^{-1}.

The main mango varieties grown in the NT are KP and Calypso. Pending commercial release are three varieties developed in the Australian National Mango Breeding Program (NMBP). There is significant commercial interest in establishing orchards of these three varieties. This created an opportunity to see whether varietal differences exist in cuticular uptake of foliar-applied N.

As part of determining the annual nitrogen budget and efficient nitrogen use in mango orchards of the NT, the question is: how much N is diffused into leaves when trees are sprayed with water-based solutions of KNO_3? How much does it contribute to the N needs of the trees? Are there varietal differences in leaf N uptake in mango? To find the answer, we designed a controlled, pot-based experiment and quantified the uptake of foliar-applied N using ^{15}N-labelled KNO_3.

Methods

Potted KP seedlings were grafted with scions of B74 (Calypso), KP and NMBP varieties 1243, 1201 and 4069 in January 2019 and grown in the plant nursery at Berrimah Farm, Darwin, NT. Trees were fertilised and monitored for the next vegetative flush. Once flushing leaves were fully expanded, 20 similar-sized plants of each variety were selected and the plants moved into the full sun to harden the leaves to replicate orchard conditions.

An experiment was conducted on July 25–26, 2019 during the dry season, at the same time orchard grown trees were flowering and usually receiving foliar KNO_3. The treatments were time of foliar application (midday or dusk) of 2 % KNO_3 on five mango cultivars (B74 (Calypso), KP, NMBP 1243, NMBP 1201 and NMBP 4069) with five replicates and arranged in split-plot design. An untreated control was also included to determine the ^{15}N abundance level in experimental plants. The lists for the midday group (1–50, control and to be treated) and the dusk group (1–50, control and to be treated) were randomised using the Microsoft Excel 2016 randomisation function and arranged in randomised complete block design in pot racks placed on benches. The plants were dipped in the same randomised order (Figure C.8).

Figure C.8: In preparation for dipping leaves into ^{15}N-labelled KNO_3 solutions to assess leaf N uptake, mango varieties Kensington Pride, B74 (Calypso®) and National Mango Breeding Program varieties 1201, 1243 and 4069 were grafted onto Kensington Pride rootstocks. Plants were sun-hardened to replicate orchard growing conditions and set out in a randomised block, split-plot design.

Plant leaves were dipped at either midday (1100–1300 h) or dusk (1700–1900 h Australian Central Standard Time) to assess any temporal differences in uptake response (n=5, at two time points, five mango varieties). Pots were watered to field capacity on each morning of the experiment to ensure similar water potential conditions existed for each tree. The dipping sequence for pots at each time point was completed in the order of the randomised experimental design.

For each pot, 8–10 fully expanded, attached leaves at the top of each plant were very loosely bunched using a soft rubber band and the plant was inverted to submerge the leaves completely into either the control solution of 0.4 % LI 700® or a treatment solution of 2 % KNO_3, containing 2 atom% ^{15}N and 0.4 % LI 700®. LI 700® is a commonly used adjuvant in KNO_3 foliar applications. It is a surfactant, penetrant, and acidifier of solutions that claims to temporarily loosen waxy components

of leaf cuticles to facilitate movement of various ions into leaves (Nutrien 2018; Nufarm 2006). Both solutions were prepared at room temperature (25 °C) with Millipore filtered water with pH of 3.18 (control solution) and 3.20 (treatment solution). On leaf removal, excess solution was allowed to run back into the container until dripping ceased, leaves were gently released to avoid splashing and the seedlings were replaced outside on benches to air dry, then returned to their designed position (Figures C.8, C.9). The amount of solution transferring to leaves was measured by weighing the solutions before and after each dipping event. Dipping was repeated 24 hours later and the dipped leaves sampled 46 hours after the initial dipping.

To remove dipping solution residues from leaf cuticles, leaf samples were washed individually and thoroughly by rubbing both sides of the leaf blade and along all venation under running tap water, then rinsed three times in Millipore filtered water. Leaves were scanned to measure leaf area then dried in ovens at 50 °C and weighed. Samples were ground in a Rocklabs Standard Ringmill and analysed using mass spectroscopy (EA-IRMS, Sercon Limited, UK) at the Institute for Future Environments at QUT. The percentage of N derived from the fertiliser (% N dff) was calculated (IAEA, 2001).

Figure C.9: On each seedling, the top 8–10 fully expanded, mature leaves were loosely secured with a rubber band, then the leaf blades were fully immersed in a solution of either ^{15}N-labelled KNO_3 or water, 24 hours apart (a, b). The loose banding minimised damage to leaves as they were dipped, and stopped 'flicking' of droplets of solution as excess drained from the bunched leaves. Dipped plants were placed outside to air dry, and were replaced in their design positions. Both dipping solutions contained surfactant to replicate commercial practices and facilitate even coverage of leaf surfaces. Twenty-two hours after the second dip, treated leaves were sampled and washed thoroughly before processing.

To assess reproducibility and consistency of the dipping method, leaf area and the measured quantity of solution left on the leaves after two dipping events were plotted (Figure C.9a). To test the efficiency of the washing method in removing the labelled dipping solution from leaves, an experiment was conducted with five additional B74 plants grafted onto KP rootstocks using the same labelled KNO_3-LI 700 solution as the principal experiment. Eight to 10 leaves from each plant were dipped, removed from the plants, washed and processed as described (Figure C.9b). To validate the method, data were analysed using linear regression (Graphpad Prism®).

The amount of N in leaves derived from the labelled NO_3^- in the dipping solutions, or N derived from the fertiliser (N dff) was calculated using the formula:

$$N\ dff(\%) = \left(\frac{measured\ ^{15}N(atom\%) - naturally\ occuring\ ^{15}N(atom\%)}{solution\ ^{15}N(atom\%) - naturally\ occuring\ ^{15}N(atom\%)}\right) * 100$$

Nitrogen uptake efficiency (NUE) was calculated:

$$NUE\ (\%)\ =\ (quantity\ of\ N\ taken\ up\ into\ leaf\ (g)\ /\ quantity\ of\ N\ adhered\ to\ the\ leaf\ (g))\ *100$$

The N derived from foliar fertiliser and leaf N uptake results were statistically analysed based on analysis of variance (ANOVA) with mean separation at 5 % LSD using STAR statistical software version 2.0.1 (IRRI 2019). Normality and homogeneity of variances were checked based on Bartlett's test and Shapiro-Wilk's test, respectively. If required, data were normalised using square transformation to meet the assumptions of ANOVA. Data were back-transformed for interpretation and visualisation.

Results

The relationship between leaf area and the quantity of solution left on leaves after dipping them twice was found to be robust. Linear regression showed no significant difference between the lines of best fit for control and treatment solutions held on the leaf surfaces, p=0.012, with r^2 values of 0.78 and 0.86 respectively, n=50 (Figure C.10). An alternative method of remnant solution removal from leaves using cellulose acetate in acetone was trialled, but discarded as unsuitable for the scale of this experiment (Silcox and Holloway 1986). Instead, the efficiency of the leaf washing method was tested. Washing the leaf surfaces was highly efficient (0.99 +/- 0.006, mean, sem, n=5, data not shown) indicating that leaf washing has effectively removed ions on the external surface of the leaves prior to grinding and nutrient analysis.

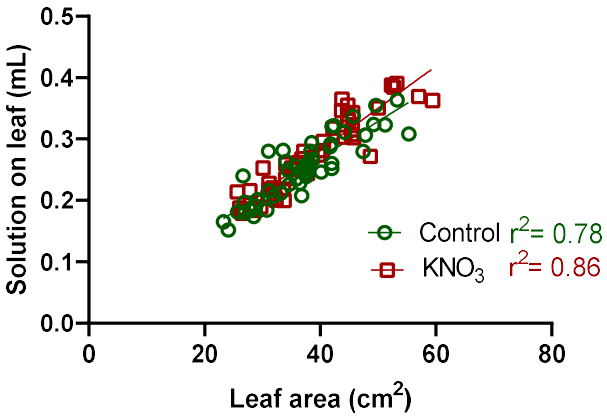


Figure C.10: The dipping method was found to be valid. The relationship between leaf area and the volume of solution held on the leaves after two dipping events was examined. Linear regression analysis indicated there were no significant differences between the slopes (p=0.12). The r^2 values were 0.78 and 0.86 for leaves dipped in control and KNO_3 solutions respectively, n=50.

For leaves sampled from each variety, the mean leaf N content of control plants ranged between 0.98 and 1.25 %, and 0.98 to 1.30 % for the treated plants. Also, the leaf ^{15}N content of the control plants was comparable with that of the natural ^{15}N abundance indicating that plants did not receive any external N during treatment application (data not shown). All varieties dipped in the ^{15}N-labelled KNO_3 solution showed a significant increase in ^{15}N-labelled nitrogen in their leaves compared to the natural abundance of ^{15}N measured in leaves dipped in the control solution (p=0.0001). However, no significant differences between leaf N uptake at midday or dusk were seen (data not shown, ANOVA, p=0.76).

Leaf analysis showed that 4–6 % of the total N in leaf content was derived from the dipping solution (Figure C.11a). Varietal differences were seen with leaves from NMBP varieties 1201 and 1243 having 3.8–3.9 % of their leaf N derived from the solution which is significantly less than the 5.6–6.1 % leaf N content in KP, B74 and 4069, (p=0.0009, ANOVA, mean, sem, n=10, LSD post-test). Leaf NUE was also calculated to assess what portion of the N was taken up from the KNO_3 dipping solution that dried on leaves. Again, significant differences were seen between varieties (ANOVA, p=0.033, LSD post-test, mean, sem, n=10) with NMBP 1201 taking up the least with 27 % mean uptake (Figure C.11b). The highest N uptake across the cuticle occurred in leaves of NMBP 4069, with a mean uptake of 44 %.

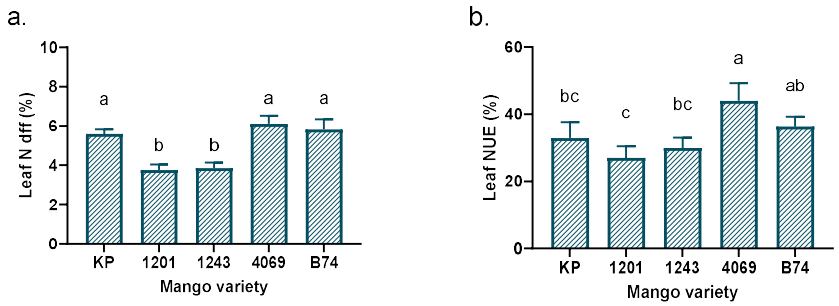


Figure C.11: The amount of N derived from the KNO_3 dipping solution showed significant varietal differences with letters indicating similarities and differences (a) (ANOVA, p=0.0009, mean, sem, n=10, LSD post-test). Varietal differences were also significant when nitrogen uptake efficiency was calculated (b). Means with different letters are significantly different at 5 % LSD (p=0.033, mean, sem, n=10).

Discussion

Foliar spraying of a low concentration (1–4 %) foliar KNO_3 onto mangoes during inflorescence development is known to increase yields by increasing fruit retention (Oosthuyse 1993a). It is also known that an excess of N in mango trees can result in excess foliage growth at the expense of fruit yield (Stassen et al. 1999) and reduced post-harvest fruit quality due to skin that stays green when the fruit is ripe (Nguyen et al. 2004). It is still not clear whether the N, K, or both elements potentially enhance the yield when applied around the inflorescence. N transport from leaves into flowers, fruit, and seeds may be via the phloem and released into the symplast, or the xylem and released into the apoplast, with either route unloading into adjacent parenchyma or transfer cells of fruit tissues (Tegeder and Masclaux-Daubresse 2018; Patrick et al. 2001).

This work provides direct, quantitative evidence of N uptake into mango leaves from foliar application of a dilute KNO_3 plus adjuvant solution (Figure C.11). It is known that leaf N content can reduce from the recommended 1.0–1.5 % N to around 0.7 % during flowering, fruit set and growth on mango trees (Catchpoole and Bally 1999) as the reproductive sink becomes stronger than the vegetative sinks (Tegeder and Masclaux-Daubresse 2018). The processes are complex, however, as there is evidence that leaves close to floral terminals have lower photosynthetic capacity due to a reduction in the light saturated rates of the photosynthetic electron chain because of a reducing leaf N content (Léchaudel et al. 2005a; Urban et al. 2008). It is possible that the foliar application of N at flowering increases leaf N content sufficiently to increase leaf photosynthetic capacity and contribute to the delivery of sufficient solutes to maintain more fruit on a tree. Perhaps flowers can take up N applied this way too, another potential avenue of fruit retention. However, there are some limits

with foliar applications of nitrogen, as concentrations of 3 % KNO_3 and higher have been reported to cause necrosis around leaf margins, which could reduce the photosynthetic capacity of the tree.

Significant differences in NUE with foliar N applications were seen among the tested varieties, with NMBP 1201 having the lowest and NMBP 4069 the highest. NUE ranged from 25 % to 43 % efficiency. Further work is needed to understand the differences in uptake, whether it is related to cuticle or leaf structure or some other physiological differences between varieties. In this group, the varieties with the lowest NUE in these terms are both Irwin x KP progeny. The variety with the highest NUE is the progeny of Van Dyke x KP. This could be a starting point for further investigation.

It is acknowledged that these results reflect the fact that both surfaces of the leaf were covered with the KNO_3 solution, whereas in-orchard spraying is likely to result in partial coverage. In addition to potential localised leaf toxicity, the effect of repeated or frequent spray applications over time is not known, but as N is highly mobile within plants, it is likely to be utilised if there are deficiencies. The cumulative amounts should be kept account of, as excess inputs will recycle in orchards in litter and soils. Experimental work carried out in the KP orchard at Katherine Research Station in 1995–2000 indicated that while no significant differences were found annually, collated data from the five-year period did show that foliar application of 140 grams tree^{-1} of N as KNO_3 when fruit was golf ball size, or at the post-harvest leaf flush, gave significantly higher marketable yields and number of fruit compared to untreated trees (unpublished). This supports the use of foliar KNO_3 as an efficient method to counter N deficiencies in an orchard and maximise yields.

The foliar NUE values are comparable to soil-applied NUE (refer to Section C.1), albeit on the low end of the scale in terms of application rate. Along with other commercial fruit crops, we confirm foliar N application in mango orchards is efficient, targeted and more environmentally friendly when compared to the uncontrolled losses of N from runoff and leaching that is associated with soil application (Tanou et al. 2017). They suggest that foliar application is comparatively efficient for applications of small amounts of N, likely to be rapidly available for use in the tree. This is highly useful knowledge for mango producers and forms part of the crop N budget. It should be incorporated into annual fertiliser assessments and planning.

C.3 Nitrogen movement within the tree

Introduction

A nitrogen content of 1–1.5 % in a mature dormant or quiescent mango leaf is the accepted industry standard for quality fruit production, although Catchpoole and Bally (1996) recommend a range of 0.8–1.9 %. Small varietal differences in leaf N content are seen; however, they are insignificant compared to N variation between phenological stages over a year (de Almeida et al. 2014). The dynamics of N uptake, movement and storage in mango trees over time has not been investigated in tropically grown mango. It may be useful to quantify how much N is stored in trees and to understand how it moves within the system in flowers, leaves, fruit, pruning litter and the environment at key phenological stages. This will provide information that may be used to reassess N application and N use efficiency in commercial orchards, and thus identify potential cost efficiencies and reduction in N loss to the environment.

Mango growers in the NT use a variety of orchard management practices across a range of soils and climates, which culminate in a wide variety of nutritional states. Leaf N content is commonly measured in commercial orchards as an indicator of fertiliser requirements. However, leaf N changes throughout the year and is not directly related to fruit yields.

Nitrogen is highly mobile in mango trees, and it would be useful to know if N cycles within the system at key developmental time points in response to tree N demand. Tissues other than leaves may be more useful as indicators of tree N sufficiency. The questions are, does N content in tree tissues vary seasonally, and if so, how can that movement be characterised? In this context, fertiliser application timing and volumes can be reassessed in mango orchard systems.

Methods

Labelling technique using ^{15}N

A rapid labelling technique was modified from one used in spruce (Nair et al. 2014) and another in macadamia (Fletcher et al. 2009) and successfully trialled to generate ^{15}N-labelled leaf litter. The litter was used to generate quantitative data for litter decomposition and movement of N in soils of mango orchards in the wet-dry tropics (Section C.5) Tree labelling was also used to assess soil-applied N uptake in two orchards using N dilution *in planta* to estimate uptake and the movement of N over a year (Section C.1). The technique takes advantage of the soil-plant-atmosphere continuum, where water moves passively along a water potential gradient that is slightly negative in the soil, becoming more negative across root cell membranes, across the cortex and into the apoplastic pathway. The pressure gradient becomes progressively more negative as it moves up stem xylem vessels and tracheids into branches and the canopy where leaves transpire and water is lost into the atmosphere (Taiz and Zeiger 1991).

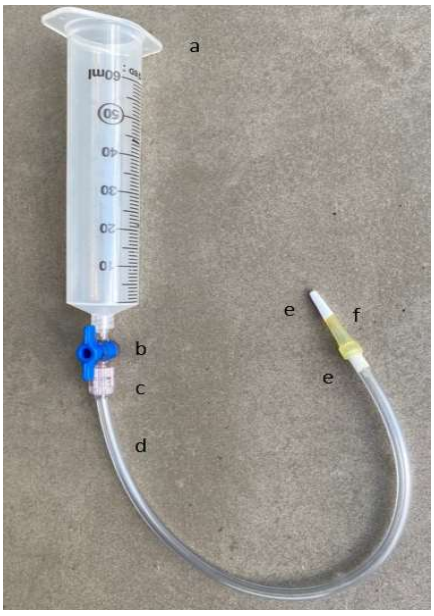


Figure C.12: A tree infusion device was created using a 60 mL syringe with fittings (a, b, c) and tubing. (d). A disposable plastic 200 µL tip was secured with plumbing tape (e, f).

A 60 mL syringe with a female ‘slip tip’, also known as Luer lock (Figure C.12a) was attached to a male ‘slip tip’ (Figure C.12b). A 3 mm internal diameter vinyl tubing was softened in just boiled water (Figure C.12d) then attached to the other end of the slip tip and the collar tightened to seal firmly

(Figure C.12c). Teflon thread seal tape was stretched and wound around the other end of the tubing (Figure C.12e) and a 200 µL disposable pipette tip softened in just boiled water then attached firmly over the tape (Figure C.12f). The tip was trimmed to less than 30 mm length and seal tape was applied generously at the end to be inserted into the xylem of the tree.

Millipore filtered water was loaded into the infusion equipment and all air bubbles aspirated or run through to remove. A 4 mm diameter, 30 mm deep hole was drilled into the stem at a 45-degree angle to the surface. Flushing of the bit and hole with Millipore filtered water was continuous during drilling and continued until the infusion apparatus was inserted and sealed. The aim was to maintain a continuous uptake of water or solution with minimal embolism in xylem vessels – essentially a closed system without the elasticity of air bubbles or leaks to enable efficient uptake of the labelled solution. Once the syringe was secured to the branch above the tip entry into the xylem, most of the remaining water in the barrel was aspirated and replaced with the labelled $(NH_4)_2SO_4$. The open top of the syringe barrel was covered to reduce evaporation and contamination, then pierced with a needle to stop any vacuum development or resistance to solution uptake. The pipette tip/seal tape and tree phloem junction was sealed with silicone sealant. This method of solution uptake was found to be fast and efficient in the dry season when there was high evaporative demand on leaves during the day and low air humidity. Depending on the infusion solution concentration and density, the complete uptake of 50 mL could be complete in an hour or two. In the wet season, where air humidity was high and there was very little difference between the leaf mesophyll and air water potential, stem water potential appeared to be high (less negative) and uptake time was generally between 8 and 24 hours. At the completion of the infusions, drill holes were filled with silicone sealant-no evidence of disease or significant damage was visible at infusion sites when inspected a year later. Generally, drilled holes were closed and the sealant avulsed.

At CPRF, four sets of three trees were selected and infused with two 50 mL aliquots of a 0.757 M $(NH_4)_2SO_4$ solution prepared with Millipore filtered water (10 g of 60 atom% $(NH_4)_2SO_4$). The infusion commenced 11 December, and uptake was confirmed as complete (Figure C.13a). Litter traps under four replicate trees were also established, slung high to avoid the irrigation system (Figure C.13b), with weights of several kilograms in the base to stabilise the nets and prevent lifting and litter loss in windy conditions. Litter was collected weekly or fortnightly depending on the season and weather. It was sorted, washed, rinsed in Millipore filtered water, oven-dried at 50 °C and bulked into two monthly collections for January–February, March–April, May–June, September–October, and monthly in July, August and September over the flowering and fruiting period. Litter traps, n=4 until July when the tree was lifted as part of a replicate set, so n=3 for the remainder of season and trees harvested for fruit % N and % N derived from infusion (dfi). To provide baseline N %, leaves were sampled from adjacent trees of the same age and history, receiving the same management but no infusion during the active growth period and post-harvest, then processed with the same methods. Dried materials were ground in a Rocklabs Standard Ringmill, subsampled and analysed using mass spectroscopy (EA-IRMS, Sercon Limited, UK) at the Institute for Future Environments at QUT.

Figure C.13: A ^{15}N-labelled solution was infused into the xylem tissue of trees over several days in December 2018 (a). Litter traps to capture all material falling from the trees were placed under four trees and regular collections of the litter were made in the following year (b). The nets were weighted so that litter fell to the low point of the traps, and to prevent material loss from lifting or billowing in windy weather.

From tree FW:DW data collected in this orchard previously (see 'Methods' under Section C.1), established relationships were used to calculate the DW of all tree components harvested in 2019.

Three replicate trees were lifted from the ground at four time points, reflecting four distinct phenological stages: active growth (20 February 2019), quiescence (25 May 2019), flowering and fruit set (16 July 2019) and post-harvest (11 November 2019) (Figures C.14 and C.15). Trees were separated into the root, stem (trunk), branches and leaves, as described. Additionally, branch sections subsampled across the canopy were separated into xylem and phloem tissues and then dried. Sections taken from the top, middle and base of the stem were also separated into xylem and phloem tissues before drying. Analysis of the tree tissues enabled quantification of N and labelled N. The percentage of N within the tree material derived from the infused nitrogen (% N dfi) was calculated (IAEA 2001). The fruit was harvested on 8 October 2019. Further details of the tree-lifting method and the field site are found in Section C.1. It is acknowledged that a portion of fine roots was not collected during tree lift; however, the weight was considered insignificant in the context of root total dry weights ranging between 13.6 and 17.1 kg DW, and their N content 0.29–0.63 %.

Figure C.14: To characterise N content and N movement around mango trees at phenological time points of active growth, quiescence, flowering and fruiting and post-harvest, trees were lifted from soil saturated with water. The canopy was deconstructed by pruning all branches from the main stem (a, b, c). Branches were immediately stripped of leaves, any flowers or fruit and all components weighed when fresh (d). Subsamples were collected from across the canopy for washing, drying and processing. The stems were used to lift the roots out of the saturated soil.

Figure C.15: During the active growth period in February 2019, after branch removal, replicate trees were lifted from soil using a snatch strap attached to the stem and lifted by a forklift connected to a tractor. Dirt was washed from the tree roots (a) before subsampling root tissue and chainsawing stem and trunk biscuits for analysis (b).

Xylem sap extraction

Prior to each tree lift, twelve branch tips 6–8 mm diameter and 15 cm length were cut at chest height from around the entire canopy of each tree, placed in a cliplock bag and held on ice, then refrigerated at 4 °C overnight. Phloem tissue was separated from the branch tips, and the mid 6–8 cm of xylem tissue was cut and placed into new, 50 mL centrifuge tubes (Figure C.16). Filled tubes (6–8 cm branch xylem sections) were centrifuged at 4,000 x gravity for one hour to release xylem contents (Beckman Coulter Allegra 25R). Aliquots of sap were stored in sealed microfuge tubes at -20 °C. Samples were dehydrated, ground, then analysed using mass spectroscopy (EA-IRMS, Sercon Limited, UK) at the Institute for Future Environments at QUT.

Figure C.16: Branch tip sections were stripped of phloem and remaining xylem centrifuged to elute xylem sap. The sap was analysed for N to see how the N concentration varied over a season.

Results

Tree nitrogen at phenological timepoints

In the NT, the N content of mango tree components varies over time, with the highest measured N tree^{-1} at the end of February during active growth over the wet season (Figure C.17a, b). The first tree lift occurred at the end of February, 70 days post-infusion of ^{15}N. The N content of leaves, branch xylem and branch phloem started higher than other tissues, then followed a similar pattern, reducing over time (Figure C.17a, b). Stem xylem and phloem N quantities peaked during active growth and were relatively stable for the balance of the cycle and root N was lowest at flowering and fruit set but had increased post-harvest (Figure C.17b).

Some seasonal variation can be seen in N distribution across the components of mango trees over a season. Leaf N variation is well documented, with high N in preparation for translocation to flowers and fruit, then falling until after harvest (Figure C.17a). Branches follow a similar pattern with a lower portion of tree N over the season, with branch xylem holding or transporting more N than the branch phloem. Root N followed a parallel time course with branch phloem until flowering and fruit set, and increased post-harvest. Root tissues were the only component to show an increase at any time point (Figure C.17b). While acknowledging the experimental trees had received no additional N application since planting in 2015, the harvested fruit in October 2019 accounted for just 10 % of the tree N at the time of picking (Figure C.18). This is less than half of the harvested fruit N content per tonne FW measured on adjacent trees as part of the soil NUE experiment at CPRF in 2019 (refer to Section C.1).

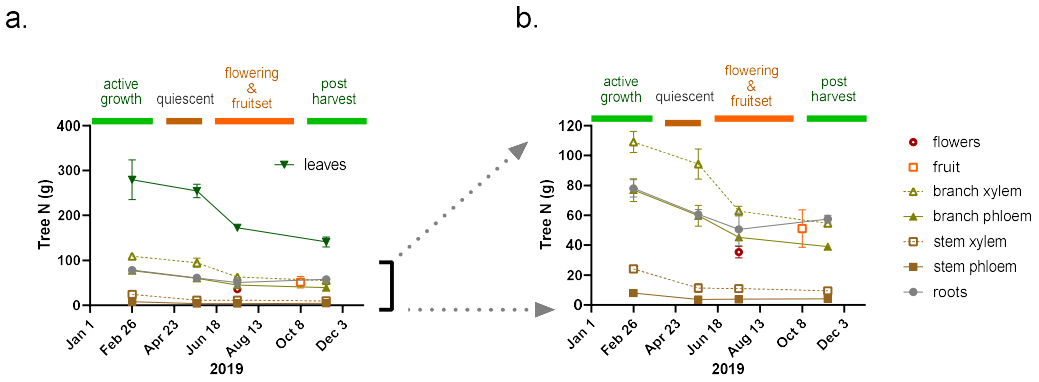


Figure C.17: All tree components had a maximum N content during the active growth phase over the wet season. Leaf N change over a season is well documented, with a reduction at flowering and fruit set in July (a). The N content of tree roots drops to a minimum at flowering (b, expanded from (a) above), and increases post-harvest in association with break of season rain events and associated soil activity (refer to Sections C.8 and C9). Branch xylem and phloem N content follow a similar pattern to leaves, with xylem N significantly higher than branch phloem at each time point (mean, sem, n=3).

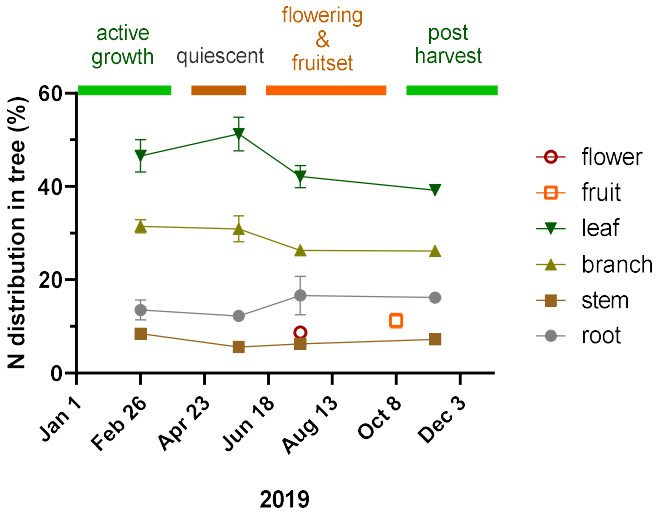


Figure C.18: There is variation in N distribution across mango trees over a season. Leaf N variation is well documented, with high N in preparation for flowering and fruit set, then drops until after harvest. Branches follow a similar pattern with a lower portion of tree N. Roots were the only component to finish the season with a higher proportion of N than it had during active growth (mean, sem, n=3).

During active growth, leaf % N was higher than all other tree components, except for flower N % in July (Figure C.19a). At 1.8 % N DW (Figure C.19a), leaves were at the higher end of the recommended 0.8–1.9 % N (Catchpoole and Bally 1999), and also have high 1 % N dfi values with high variability (Figure C.19 b). Considering the labelled N was infused directly into xylem vessels, it probably moved directly into leaves via the xylem transpiration stream before being redistributed around the tree. By mid-May, leaves had reduced from 1.8 % N to 1.1 % N, consistent with adjacent control trees, and 0.4 % N dfi. During flowering and fruit set in July, flowers had 1.8 % N, with 0.4 % N dfi and leaves had reduced to 0.4 % N dfi. This suggests that the leaf-labelled N had completely dispersed around the tree between February and May (Figure C.19a, b). The stem and the root had higher % N during the active growth period, dropping slightly to a consistent % N and % N dfi through the rest of the season (Figure C.19a, b). Fruit % N dfi was similar to leaves and flowers (Figure C.19b). N movement was further characterised by separating xylem and phloem tissues. The branch phloem had ~1.8 times more N compared to branch xylem while the stem phloem had ~2.8 times more N

compared to stem xylem across all time points. N content is higher during active growth, then reduces and stays relatively constant for the balance of the season. The % N dfi for branch xylem and phloem is consistent for the entire season, suggesting labelled N is thoroughly distributed in those tissues (Figure C.19c, d), and is similar for stem xylem and phloem (Figure C.19e, f).

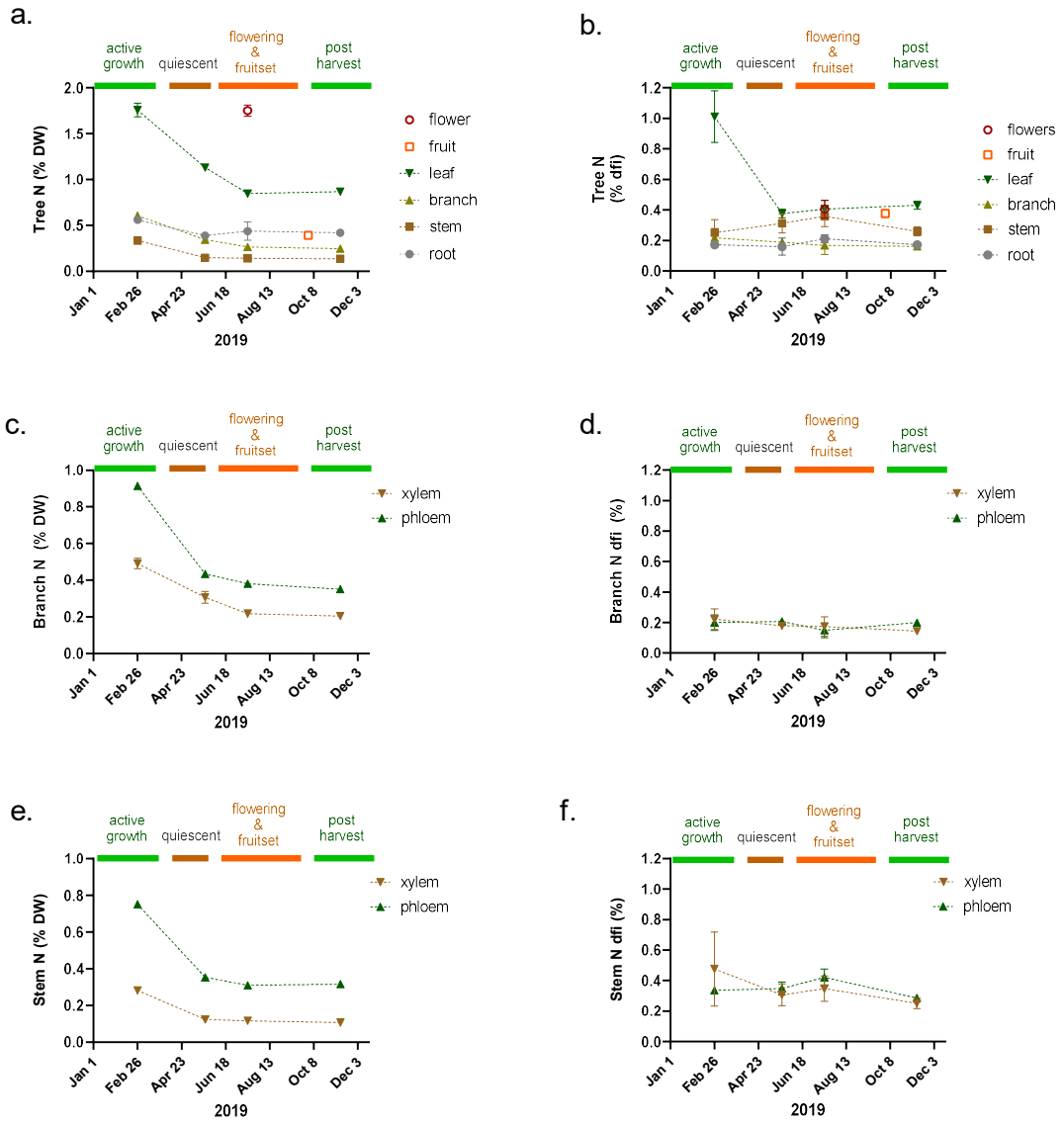


Figure C.19: Nitrogen (N) distribution in tree components over time are quantified (a), along with the amount of N in each component derived from the labelled N infusion (b). The high leaf % N and high variability in % N derived from infusion (dfi) is likely to reflect infused N moving unevenly to leaves via the transpiration stream (a, b). This appears to be redistributed prior to the tree lift in May 2019, where the % N dfi has dropped to values similar to the stem, flowers and fruit. Branch and stem xylem and phloem N contents are significantly different (c, e), but % N dfi values do not change over the season. Mean, sem, n=3 trees at each time point.

Litter and fruit

Most of the litter was collected between May and August 2019, during the dry season when there is no rainfall and flowering and fruiting occurs – consistent with results in Section C.5. All litter components collected over the season accounted for 51.4 g of N, and harvested fruit contained 51.2 g of N (Figure C.20a). Leaf and panicle litter % N was consistent over the season (Figure C.20a), with the previous season's remnant and senescing panicles showing low % N dfi as expected, until

those which developed and dropped in 2019 were collected from July onwards (Figure C.20b). Litter % N dfi was consistent across flowers, leaves and panicles.

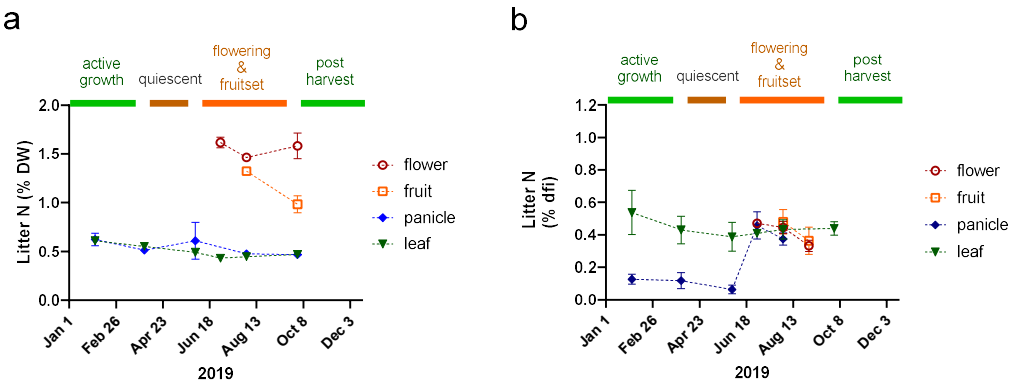


Figure C.20: Panicle and leaf litter % nitrogen content were similar and showed a slightly decreasing trend from 0.62 to 0.47 % across the season. Abscised flowers had a high N content of 1.5–1.6 % N, higher than abscised fruit (a). The remnant panicles on the trees from 2018 continued to abscise up until flowering commenced in 2019. These panicles are mostly senescent as reflected in the very low uptake of N derived from infusion (dfi), which increases as soon as flowering commences in July 2019 and litter is from material generated post-infusion from December 2018 (b). Leaf, fruit and flower litter N dfi is quite consistent over the year, with N potentially being resorbed from leaves before dropping. Litter traps: mean, sem, n=4 December 2018 to July 2019, n=3 for August, September and October 2019 (a, b).

Nitrogen recovery efficiency

Over time, total tree N content declined, from 576 g at the end of active growth to 306 g post-harvest (Figure C.17a). This is consistent with the post-harvest tree N contents measured in Section C.5 (data not shown, one way ANOVA, no significant difference between N treatments, p=0.65, mean=330 g) Mean tree DW ranged between 70.1 +/- 4.5 and 92.6 +/- 2.3 kg with trees lifted in February being the lightest and those lifted in May the heaviest. Tree N content is shown, along with the tree N:tree DW ratio to illustrate N loss during the season (Figure C.21a).

An assessment showed that 70 days post-infusion, ~96 % of infused N was recovered. However, it is noted this point represents a single replicate because there were two aberrant values for leaf atom% N excluded (Figure C.21b). The excluded values implied high quantities of infused N were in the selected leaf subsamples analysed, yet to be redistributed. The remaining data is complete. The portion of infused N that was accounted for in the trees, litter and fruit (combined, Figure C.21b) declined during flowering and fruit set to 73 %, then to 66 % in November, including infused N recovered from fruit harvested and litter collected. This is a tree N content reduction from ~ 576 g during active growth in February to 306 g post-harvest in November, a reduction of 270 g. Litter collected contained 51 g of N when combined, and the harvested fruit another 50 g N, leaving ~170 g or close to 30 % of tree N unaccounted for.

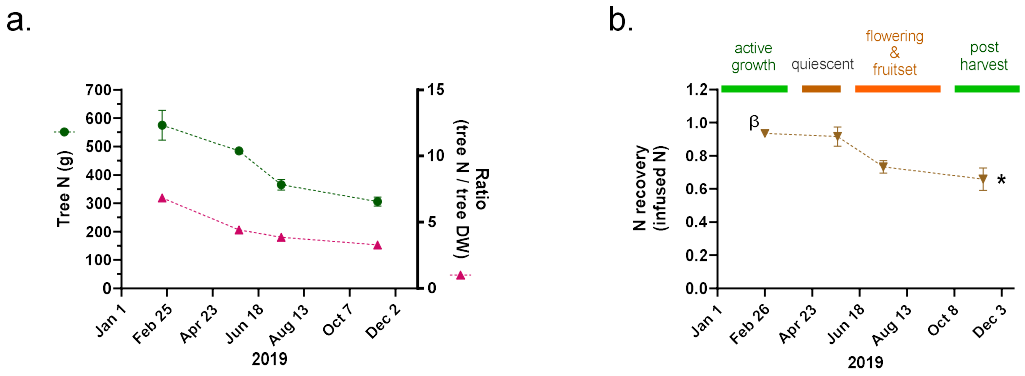


*Figure C.21: Tree N content declined over the season (a). The quantity of infused N that could be accounted for declined over the season also, reducing from 94.0 % to 66.6 % (b). The asterisk * at 66.6 % recovery in November 2019 includes N dfi from collected litter (n=3-4) and harvested fruit (n=3 trees) (b). Mean, sem, n=3. Note: ß indicates n=1 at this point as two replicates of leaf litter ^{15}N content were aberrant, excessive values.*

The leaf N content in adjacent untreated trees during active growth was 1.16 % and 0.84 % N post-harvest. The nitrogen infused into each tree, 2.1 g, was 0.36 % of the measured whole tree nitrogen content on a DW basis at the first tree lift in 2019 and increased to 0.67 % over the season as tree total N reduced (Figure C.21a, b). The calculated N dfi values across the year were consistent with these quantities (Figure C.19b, d, f, Figure C.20).

The N content of xylem sap varies between 0.04 % during active growth and less than 0.02 % during the quiescent period (Figure C.22). From there it increases during flowering and fruit set, and is increasing in November post-harvest. While the N content varied, the % N dfi was relatively stable, declining slightly over time. This implies that the infused and labelled N was distributed evenly in xylem tissue across the tree canopy in the first 70 days post-infusion.

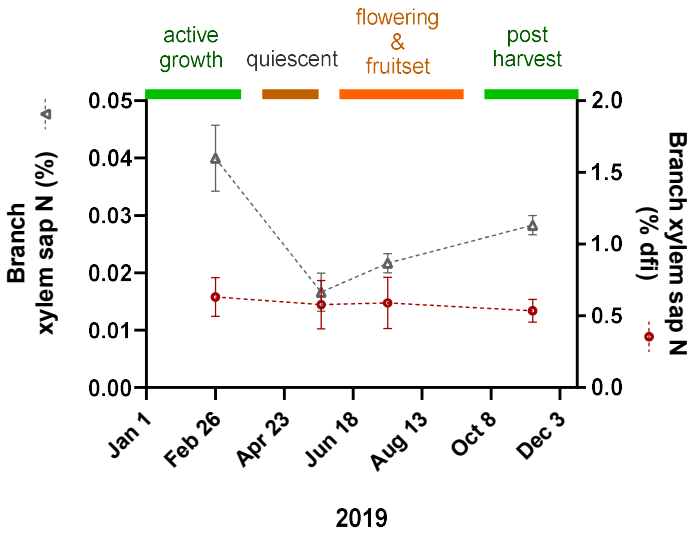


Figure C.22: The N content of xylem sap is highest during active tree growth and lowest during the quiescent period prior to flowering. The stable values for N content derived from the infusion (% dfi) over the period suggest that it was rapidly transported around the entire tree between mid-December 2018 and the first tree lift in February 2019. The declining trend reflects the reducing infused N recovery over time but variability is acknowledged (mean, sem, n=3).

Discussion

Plants can take up inorganic N in both nitrate and ammonium forms into roots, across the root endodermis or pericycle and into the apoplastic space or via parenchyma tissue to access xylem vessels and the transpiration stream. Passive and active transport mechanisms are involved in N transport to active sinks or storage pools in tree tissues (reviewed in Tegeder and Masclaux-Daubresse 2018). Also, N can be transferred from the xylem to the phloem of active sinks, or via parenchyma tissue and symplastic transport into phloem tissue (Van Bel 2003; Tegeder and Masclaux-Daubresse 2018). In this work, N content in both branch and stem xylem were higher than the phloem tissues (Figure C.19c, e). Movement of water and solutes in the xylem tissues is via mass flow, so while the infused N will have reached leaves on that path, all redistribution throughout the tree, including down to the roots, would be via parenchyma tissue or rays within the xylem into phloem tissues. Once out of the xylem and into the phloem, all transport would be active via the symplast, not passive. Recirculation of N from phloem tissue back into the passive xylem transpiration stream is also possible.

By loading labelled ammonium sulfate directly into the transpiration stream, it was anticipated that labelled N would accumulate within leaves at high levels, and this was observed (Figure C.19b). What was surprising, however, was the speed with which the labelled N was distributed to all tissues, including the roots. While leaf N was reduced to less than 1 % N during flowering and fruit set, the % N dfi remained stable for the rest of the season. As N content of tissues varied in xylem, phloem, flowers and fruit, the % N dfi stabilised in all, indicating that the labelled N had been completely redistributed around the tree tissues. The fact that while xylem sap N content varied seasonally, the % N dfi remained steady supports this. It is expected that tree N content would again accumulate during the active growth stage following fruit harvest. The work shows that N transport is rapid and highly dynamic in both xylem and phloem tissue. It was so rapid that it was unable to identify and track the seasonal source to sink N demand as hypothesised.

The question remains, where is the ~150 g N that is unaccounted for post-harvest? As litter collected over the eleven months contained only 50 g N, and the traps were large, high, and deep with litter frequently collected (Figure C.20b), it is unlikely that two thirds of it was lost into the orchard. Also, when extrapolated to annual litter N collected at a tree planting density of 250 trees ha^{-1}, it is 12.5 kg N ha^{-1}, approaching that collected in an older, mature KP commercial orchard in the Darwin region over 2017 (Section C.5).

Seasonal decline in tree N content has also been documented in pot culture of the South African mango variety, Sensation (Stassen and Janse van Vuuren 1997). However, this was attributed to the annual N loss to litter and harvested fruit. In this current work, harvested fruit N content was low, as trees were five years old and had received no additional N since planting. Litter and fruit N have been accounted for, and there is strong evidence of rapid N distribution throughout the entire tree. The below ground processes such as root exudation or root-mycorrhizal interactions are unaccounted for and may be responsible for the unexplained losses. For example, it is known that trees improve their drought tolerance by exuding solutes or amino acids (which are N rich) from their roots to lower the water potential in the surrounding soil surrounding, thus increasing soil water availability (Williams and de Vries 2020). Other recent work suggests that root exudates deposited in the soil can assist plants to recover and rapidly take up N as soon as soil microbial activity is reactivated by break of season rain events (Karlowsky et al. 2018), consistent with our findings (Pandeya et al. 2020). The

trees in this experiment were irrigated throughout, with more frequent irrigation over flowering and fruit growth until harvest. However, the sprinkler throw in this and most other orchards is limited and most of the root zone under the canopies do not receive water directly. The proportion of tree N in root tissues increased from the quiescent period to flowering and fruit set. This could be from root growth, N uptake from the soil or a root focus on processes involving nutrient uptake. Increased root activity at this time has been suggested, but the information source wasn't specified (Winston undated). Our Katherine-based work has shown a sudden increase in leaf N with a break of season rainfall and no applied N (Section C.4). Together, these observations indirectly support a working hypothesis that a portion of tree N may be released as exudate from roots into the soil after active growth, during the quiescent period, and available for increasing drought tolerance or future uptake and tree N replenishment to balance orchard losses in harvested fruit (Coskun et al. 2017).

In trees, N can be stored, sequestered, used in metabolic processes or growth. It can also be lost as litter, pruned material, harvested fruit or from roots. From stored resources *in planta* or the soil, N is remobilised in response to phenological or environmental demand (Millard and Grelet 2010). The seasonal N loss appears to be a significant and dynamic element in the annual N cycling in mangoes.

C.4 Nitrogen impacts on fruit yield and quality

Introduction

Nitrogen fertilisers are relatively cheap and are often applied as insurance against potential deficiency rather than an indicated crop need. The results of 'insurance' nitrogen inputs are not always positive in orchard crops. High levels of N applied to fruit trees are linked to high variability in the maturity of fruit on trees, changes in fruit skin colour and higher disease susceptibility (Weinbaum et al. 1992). These factors have an impact on harvest decisions, picking costs and, importantly, the financial premiums associated with high-quality fruit.

In the mango industry, it is known that higher levels of applied N have negative effects on post-harvest fruit quality, in particular the skin of the fruit staying green as the fruit ripens (Nguyen et al. 2004; Oosthuyse 1993b). The fresh fruit market demands fruit with skin that shows warm blush or golden tones when ripe, depending on variety, and premium prices are paid for this. Fruit is harvested when physically mature, but still green and firm. The ripening process continues slowly, the rate largely dependent on the temperature the fruit is being held in. Frequently, the ripening process is managed or accelerated prior to sale by exposing fruit to ethylene gas which enhances flesh softening and changes in skin colour (Barry and Giovannoni 2007), creating a visually attractive product for buyers.

There are no recommended guidelines for N application in commercial mango orchards in the NT. The question is, at what level of N application is there a risk of harvesting fruit with 'stay green' skin characteristics or other post-harvest quality defects? Also, can post-harvest ethylene treatment overcome the 'stay green' skin on ripening fruit?

Methods

Work was conducted over 2018–20 at a commercial orchard in the Katherine region on KP trees planted in 2000. Trees were selected to be of consistent size, age and health within the allocated rows. A randomised complete block design with four replicates was used. Within each block, four

sets of three trees were randomly assigned an N treatment (Microsoft Excel randomisation function). Recent N application history for the trees was by post-harvest soil application in early January 2017 and two 2 % KNO_3 spray applications at flowering, in July 2017.

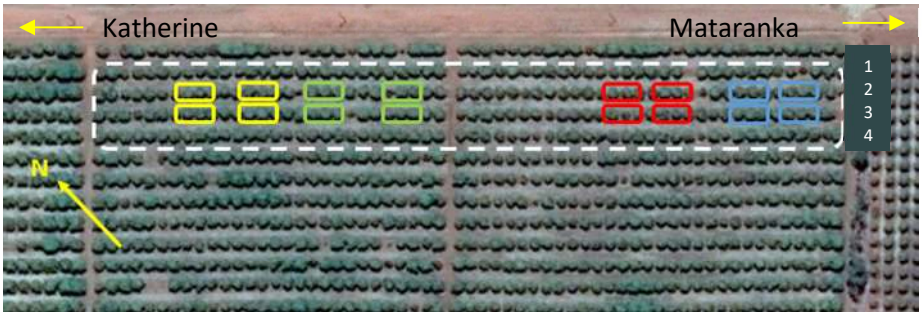


Figure C.23: Aerial view of the experimental site south-east of Katherine. Each treatment (0, 12.5, 25 or 50 kg ha^{-1} of N) was randomised within the four blocks, (blue, red, green and yellow outlines). The experimental site was buffered with a row on either side receiving no N fertiliser (white dashed line above) and treatments were separated by a minimum of one tree. Blocks of trees were selected for uniformity of age and canopy. Yellow arrow with adjacent N indicates the direction north.

Nitrogen application

Buffer rows and trees were defined around the experimental trees and no N treatments were applied to these trees (Figure C.23). A range of N levels was applied to the soil in January 2018 and 2019 according to the design; 0, 50, 100 and 200 g N tree^{-1} (equivalent to 0, 12.5, 25 and 50 kg ha^{-1} at a planting density of 250 trees ha^{-1}) in the form of ammonium sulfate.

Pest management and associated chemical controls by the commercial orchard operators continued as scheduled. Flowering commenced mid-June 2018 and 2 % KNO_3 foliar sprays were applied on 25 and 28 June 2018 during fruit set. There were a number of inflorescence flushes that contributed to several 'select picking' events at harvest because not all fruit matured at the same time. Leaves were sampled periodically from the central tree of each replicate set in each block, then washed, rinsed in Millipore water.

The foliar KNO_3 applied was equivalent to ~22 g N tree^{-1} or up to ~6 kg N ha^{-1} at planting densities of 250 trees ha^{-1}. This is conventional commercial practice although the frequency and concentration of solutions applied can vary.

Soils

Soil is classified as red Kandosol (Isbell and NCST 2021). Soil samples to a depth of 60 cm were collected within the drip line from each side of the central tree in each treatment level of each block. For the initial analysis, they were mixed well, bulked, dried at 50 °C, sieved (2 mm screen) and subsampled for analysis (Table C.2). For the post-experiment soil analysis, three samples to 60 cm depth were collected from the drip line, and 50 cm and 100 cm within the drip line of each central tree and mixed thoroughly, (but not bulked) then processed for analysis using the same techniques (Table C.3). Soil analyses were completed by CSBP Soil and Plant Analysis Laboratory in Perth, Western Australia.

Table C.2: Soil nitrogen content, pH and cation exchange were measured in the commercial Kensington Pride orchard in the Katherine region prior to the commencement of experiments in January 2018. Samples in each quadrant of the central tree in each treatment were collected to a depth of 60 cm, bulked for each block and subsampled. Mean, sem, n=4.

		Mean	±
NO_3	mg kg^{-1}	1.00	0.58
NH_4	mg kg^{-1}	0.03	0.00
N	%	0.45	0.04
C	%	0.38	0.04
pH		7.88	0.11
EC	dS m^{-1}	0.04	0.00

Table C.3: Soil nitrogen content, pH and cation exchange properties were sampled in a commercial Kensington Pride orchard in the Katherine region after experimental work in January 2020. Soil was sampled to 60 cm in each quadrant of the central tree in each treatment in each block. Mean, sem, n=4.

		0 kg ha^{-1}		12.5 kg ha^{-1}		25 kg ha^{-1}		50 kg ha^{-1}	
		Mean	±	Mean	±	Mean	±	Mean	±
NO_3	mg kg^{-1}	<1		<1		<1		<1	
NH_4	mg kg^{-1}	4.00	0.41	4.25	0.48	4.50	0.29	4.50	0.96
N	%	0.04	0.00	0.04	0.00	0.04	0.00	0.04	0.00
C	%	0.19	0.02	0.19	0.02	0.25	0.04	0.23	0.03
pH		8.48	0.09	8.73	0.12	8.50	0.07	8.60	0.11
EC	dS m^{-1}	0.04	0.00	0.04	0.00	0.04	0.00	0.04	0.00

Harvest and post-harvest assessment 2018

Flowering continued over several weeks in 2018 which resulted in a staged harvest. The first select harvest at the Katherine region KP commercial orchard was conducted on 2 October, 104 days after 50 % flowering. The fruit was selected and picked at the mature, green stage 0 Soft on the softness scale ((Holmes et al. 2009) but minimal fruit was harvested. The second select harvest commenced on 9 October and a final strip pick was completed on 18 October 2018. 'Mature green' for KP fruit means having full shoulders that rise slightly above the pedicel connection and well-filled cheeks and tip, with a minimum dry matter (DM) content of 15 % (AMIA 2016). All fruit was harvested from the tree at the pedicel and washed to remove sap in a harvest aid vehicle containing Mango Wash® solution prepared and replenished according to label instructions. The fruit was counted, sorted and weighed as 'commercial' grade in terms of size, shape, colour and any other defects, or 'other', suitable for processing, pulping or juice, then transported to a commercial packhouse.

In each block, mango fruits from three trees were harvested for each treatment level and the mean yield was calculated. This was repeated in the four replicate blocks within the orchard (Figure C.23). The yield benefit ratio was calculated by dividing fruit yield (tonnes ha^{-1}) by the quantity of N applied (kg ha^{-1}), noting that data for fruit picked from 0 N treatment trees (see 'Nitrogen application', this section) are included because they received N in foliar sprays of KNO_3 at flowering and fruit set.

To estimate how much N left the orchard in harvested fruit, a separate sample of four fruit from each treatment and block were collected and dried in ovens at 50 °C. Fresh and dry weights were recorded. The dried fruit material from each treatment was bulked, processed and subsampled (n=4

for each N treatment) for N and C analysis at QUT as previously described (refer to 'Methods' under Section C.1). N content in the fruit yield was estimated from these analyses.

From fruit picked on 9 October 2018, 24 (two trays) from each treatment and replicate block were sampled for post-harvest assessment, 12 with all green skin and 12 fruit with a degree of blush. This was to ensure that representative fruits were sampled across the entire canopy as green skinned fruit tends to develop within the canopy and blushed fruit may have more light and sun exposure. The % DM of fruit was measured using a near infra-red spectroscope (F750, Felix Instruments) calibrated locally for KP (Anderson 2017) prior to the harvest. Fruit was stored overnight in a cold room at 13 °C at Katherine Research Station (KRS). It was transported to Darwin on 10 October 2018 and ripened in the post-harvest laboratory at Berrimah Farm at a room temperature of 22–24 °C with regular manual venting to standardise room air CO_2 content. (Note, in October in tropical Darwin the lowest temperature attainable was 22–24 °C in the 24-hour airconditioned laboratory. Temperature-controlled ripening rooms were unavailable in 2018.

Fruit was imaged on alternate days post-harvest until fruit reached stage 4 soft, ripe and ready to eat according to the industry rating scale (Table C.4) (Holmes et al. 2009). Skin colour was measured on the same points on each fruit (marked at an equatorial point on each full cheek) with a colorimeter using the CIE L*a*b System D65 calibrated daily (CR-A43 white) according to operating instructions (Konica-Minolta CR400). The system measured absolute reflected colour values for Lightness on a scale from black (0) to white (100), *a from green (-) to red (+) and *b from blue (-) to yellow (+). To account for variability in colour of a single fruit, both cheeks were measured, and the mean value was calculated for each treatment in each randomised block (n=48, cheeks=24 whole fruit) in the four blocks. Data were analysed over time using ANOVA, n=4, mean, sem, and second-order polynomial curves fitted.

Destructive sampling of the fruit was carried out once stage 4 Soft was reached for each replicate set of fruit (Table C.4), which, depending on N treatment, was not always consistent with the skin colour guide (Figure C.24). For consistency, one person, with extensive experience related to mango quality, assessed all fruits.

The flesh colour components L, *a and *b of both cheeks of each fruit (n=24) were measured using the colorimeter and the mean for each fruit was calculated. Sugar content (°Brix) of juice was measured (Atago, temperature compensated digital refractometer). A disc of the flesh of a cheek of each mango was cut and the skin sliced from the surface with a sharp knife leaving a 10 mm deep section. The texture was measured on the section, skin side up (Stable Micro Systems Texture Analyser fitted with a conical Perspex tip P/60C). The force (Newtons) required for the instrument tip to travel 5 mm into the flesh at a speed of 2 mm s^{-1} was recorded (Figure C.25a, b). Data were analysed using ANOVA on the split-plot design and mean separation based on 5 % LSD or Tukey's post-test using STAR version 2.0.2 (IRRI 2019) and Graphpad Prism® as described in figure legends. Normality and homogeneity of variances were checked based on Bartlett's test and Shapiro-Wilk's test, respectively. If required, data were normalised using square transformation to meet the assumptions of ANOVA. Data were back-transformed for interpretation and visualisation.

Table C.4: Fruit was assessed according to the industry standard for softness (ripeness scale) (Holmes et al. 2009). When fruit achieved '4' on the scale, which is considered ripe for eating Kensington Pride (KP), destructive sampling occurred to measure °Brix, flesh colour and texture.

Stage	Description
0	Hard – no 'give' in the fruit
1	Rubbery – slight 'give' in the fruit
2	Sprung-flesh deforms by 2–3 mm with extreme thumb pressure
3	Firm soft – whole fruit deforms with moderate hand pressure
4	Soft – whole fruit deforms with slight hand pressure

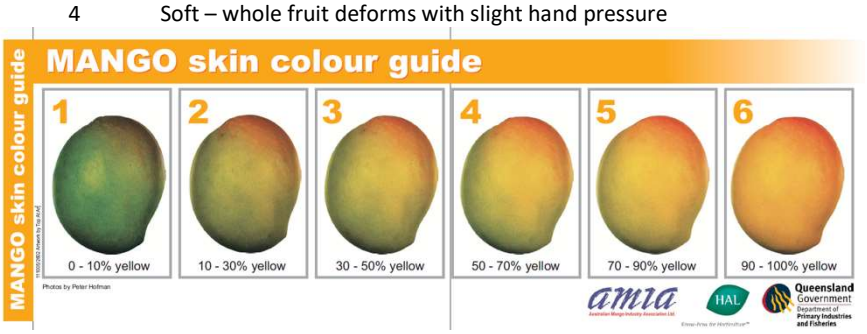


Figure C.24: Ripening of mangoes can also be tracked using a guide where the proportion of yellow skin colour is estimated on each fruit. Over six stages, yellow skin colour changes from 0–10 % to 90–100 % of the fruit (Ledger et al. 2012). Kensington Pride (KP) are considered to be 'eating ripe' at stage 5-6 or 70–100 % yellow.

Figure C.25: To assess any post-harvest quality effects of the in-orchard nitrogen application or ripening +/- ethylene, the flesh was examined for differences in texture in 2018 and 2019. Discs of fruit being prepared for texture analysis (a). A Stable Micro Systems Texture Analyser fitted with a conical P/60C measured the force required (Newtons) for the tip of the instrument to travel 5 mm into a 10 mm deep disc of mango flesh at the rate of 2 mm s^{-1} (b).

Harvest and post-harvest assessment 2019

The harvest and post-harvest assessments were repeated in 2019. An additional experiment was added to assess whether green skin on ripe fruit from trees fertilised to excess N can be reversed with post-harvest ethylene treatment. An extended flowering period was again observed, with 50 % full bloom occurring on 24 June. A 2 % KNO_3 foliar spray was applied that week and the following

week during fruit set. At 114 days after 50 % bloom, a series of four select picking events began; 16 October, 21 October, 29 October and a final strip pick on 7 November 2019. As in 2018, 24 mature green and blushed fruits from each treatment and replicate block were subsampled from the 21 October harvest. % DM was measured using the NIR spectroscope and fruits were stored overnight in a cold room at 13 °C at KRS. On day one post-harvest, fruits were transported to CPRF and 12 fruits from each treatment (six with green and six with blushed skin) were randomly selected and placed in a vented ripening room set at 21 °C. The remaining 12 fruits in the replicate were placed in an adjacent vented ripening room at 21 °C with ethylene gas set and maintained at 15 ppm for 30 hours, until fruit skin colour in fruit from trees receiving 0 soil-applied N changed from 0 % yellow to 10–30 % yellow, consistent with moving from stage 1 to 2 in the mango skin colour guide (Figure C.24). To maintain the same conditions for all ripening fruit over time, (with ethylene treatment as the only variable), all fruit was transported to the post-harvest laboratory at Berrimah Farm. Measurements and ripening continued at room temperature between 22–24 °C, with regular manual venting to remove excess CO_2.

Skin colour was measured on each fruit at the same location (n=12 fruit – ethylene, n=12 fruit + ethylene treatment), commencing 2 days post-harvest and repeated every two days until fruit reached stage 4 Soft (Table C.4). Measured values for L, *a and *b were taken for each level of N application to trees, +/- post-harvest ethylene treatment (n=12 whole fruit for each treatment and ethylene combination). Destructive sampling and analysis for flesh colour, sugar content and texture followed and data were analysed as in 2018. Data were analysed using the mean of each set of measured values using ANOVA. Means in figures with different letters above are significantly different at 5 % LSD. Normality and homogeneity of variances were checked based on Bartlett's test and Shapiro-Wilk's test, respectively. If required, data were normalised using square transformation to meet the assumption of ANOVA. Data were back-transformed for interpretation and visualisation.

To estimate how much N left the orchard in harvested fruit, a separate sample of four fruits from each treatment and blocks were collected and separated into skin, seed and flesh components and dried in ovens at 50 °C. Fresh and dry weights were recorded, samples were processed for analysis as described for 2018.

Results

Leaf N content was confirmed as sufficient at > 1 % dry weight in January of 2018 and 2019 and at or above the recommended minimum of 0.8 % during the quiescent or dormant periods prior to flowering (Catchpoole and Bally 1999). Monitoring of leaf N continued over the experimental period of January 2018 to January 2020 (Figure C.26). Soil nitrate reduced over that time to less than 1 mg kg^{-1}, while soil ammonium content increased from less than 1 to 4 or more mg kg^{-1} across the experimental blocks (Tables C.2, C.3).

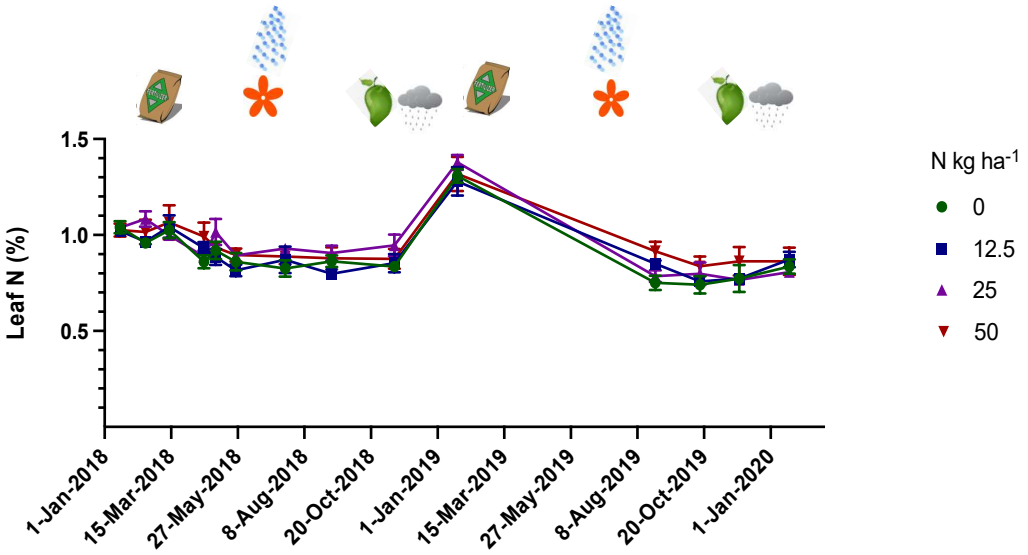


Figure C.26: Leaf N was monitored over two years in a commercial Kensington Pride orchard in the Katherine region. Management of the orchard over time is shown by images in the timeline: sack indicates soil-applied fertiliser, orange flower and droplets indicate foliar application of 2 % potassium nitrate, green mango indicates fruit harvested at the mature green stage, grey clouds indicate break of season rainfall events that mark the beginning of the wet season. Note the rapid increase in leaf N in January 2019, prior to additional N application. Leaf N increase in all treatments to ~1.4 % N is probably the result of locally decomposing litter and soil microflora and microfauna activity in response to the indicated rain events. Mean, sem, n=4.

Harvest yields 2018 and 2019

'Commercial yield' is defined as a fruit that has symmetry, correct size and shape, and no blemishes. 'Other yield' may be small, misshapen, have a skin blemish or physical defect but are suitable for pulp or juice. In 2018, mean yields from each block ranged between 18.6 and 22.6 tonnes ha^{-1} (Figure C.27a). In contrast, the 2019 mean yields from the same trees ranged between 37.2 and 38.5 tonnes ha^{-1} (Figure C.27b). There was a significant difference in yield over the two years (ANOVA, $p<0.0001$, with no significant interaction between year and N applied, $p=0.67$, $n=12$). No significant differences were seen in total yield in response to soil-applied N (ANOVA, $p=0.8$, $n=12$). The ratio of harvested yield/N applied follows the same pattern in 2018 and 2019, with sharply reducing benefits as N application increased (Figures C.27c, d). Note that the yield benefit calculation includes 0 kg of soil-applied N because all trees received just under 6 kg N ha^{-1} via foliar sprays of KNO_3 at flowering and fruit set as part of conventional orchard management practices.

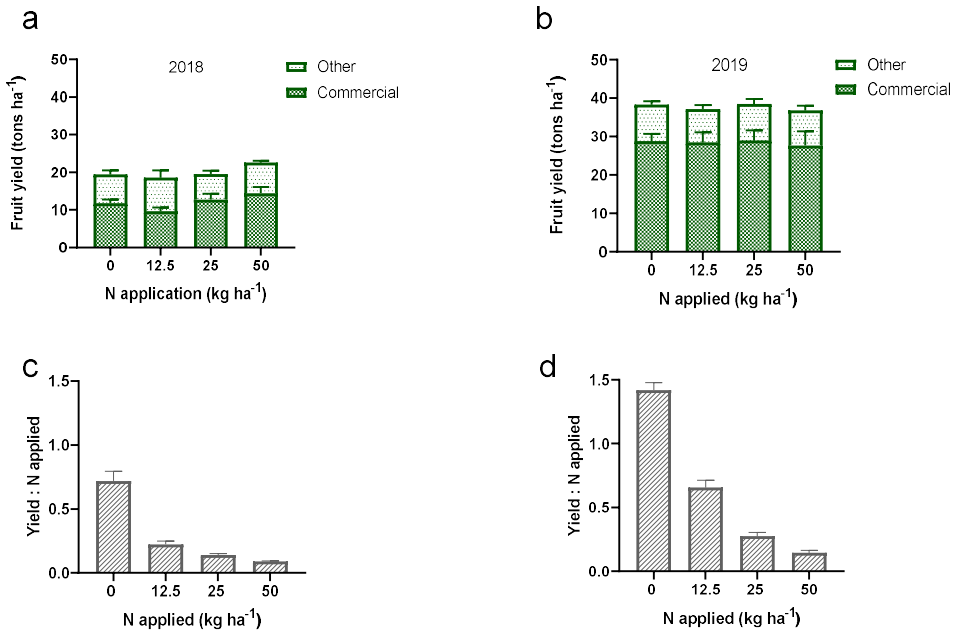


Figure C.27: Total yield for Kensington Pride (KP) mangoes in a Katherine region orchard varied over 2018 (a) and 2019 (b), illustrating the recognised KP varietal habit of alternate year bearing. There was a significant difference in yield over the two years (ANOVA, p<0.0001, with no significant interaction between year and N applied, p=0.67, n=12). No significant differences were seen in total yield in response to soil-applied N (ANOVA, p=0.8, n=12). The yield benefit ratio of yield (tonnes ha^{-1}) / N applied (kg ha^{-1}) is shown for 2018 (c) and 2019 (d). Data were standardised to 250 trees ha^{-1}.

For the 2018 harvest, the mean N content in a tonne of fruit ranged between 0.84 and 0.93 kg tonne^{-1} with the concentration in fruit from trees treated with 12.5 kg N ha^{-1} being highest (Figure C.28a). In 2019, the quantity of fruit harvested from the 25 and 50 kg ha^{-1} N treatments contained higher N content of ~1.1 kg per tonne compared to 0.96–0.98 kg N per tonne for the two lower N treatments (Figure C.28b). When applied to the total fruit yields, in 2018 it is estimated that 16.3–21.0 kg N ha^{-1} left the orchard in fruit (Figure C.28c), and in 2019 the exceptional harvest resulted in 36.7–42.7 kg N ha^{-1} leaving the orchard (Figure C.28d) across all treatment levels. These results are extrapolated from analysis of fruit subsampled from each treatment during the harvest (n=4), thus are estimates only, mean and sem are shown.

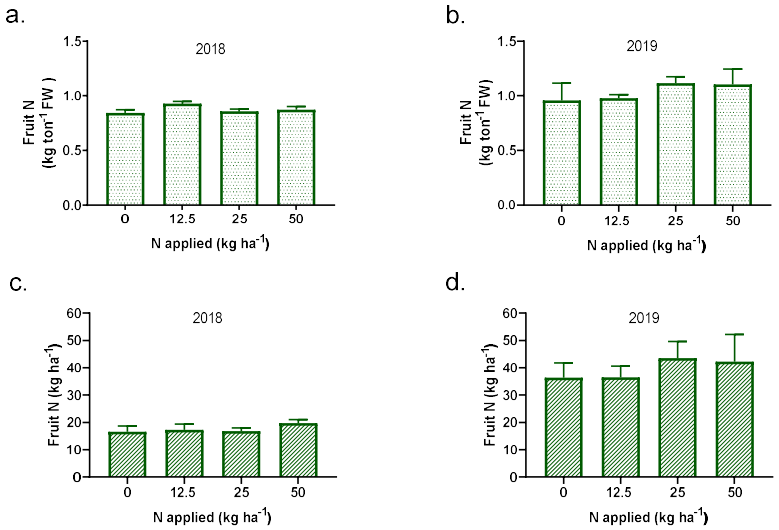


Figure C.28: Nitrogen content in harvested fruit was estimated on a fresh weight basis for comparison between years with extreme variation in yield (a, b). In 2018, it is estimated that 16.3–21.0 kg N ha^{-1} left the orchard in fruit (c), and in 2019 it was around 36.7–42.7 kg N ha^{-1} (d). These are indicative quantities of nitrogen leaving the orchard in the crop, estimated from a relatively small subsample of fruit. Therefore, no statistical analyses were performed, n=4, mean and sem.

Post-harvest quality assessment 2018

There were no significant differences in % DM in response to N levels applied to trees (p=0.26). Fruit harvested from trees treated with 25 kg N ha^{-1} had the lowest mean with 17.9 % DM and fruit harvested from trees treated with 0 kg N ha^{-1} had the highest with 18.5 % (Figure C.29).

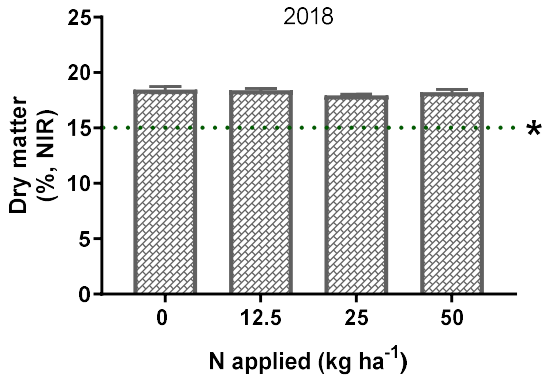


*Figure C.29: Dry matter in harvested fruit was measured on the day of harvest with a near infra-red gun calibrated for Kensington Pride (KP) in the 2018 season (Anderson 2017; Subedi et al. 2007). No significant differences were found between blushed and green harvested fruit (ANOVA, p=0.36, mean, sem, n=24). * denotes the Australian mango industry recommended standard for a minimum dry matter of harvested KP fruit (AMIA 2016).*

Imaging of fruit over the ripening period showed visual differences in colour between the sets of fruit across the range of N treatment levels over time. Fruit harvested from trees receiving N at 50 kg N ha^{-1} exhibited 'stay green' skin, a post-harvest quality fault that occurs when trees are supplied with excessive N and becomes evident as fruit ripen. Fruit from trees receiving 25 kg N ha^{-1} appeared green, remaining at stage 1 Colour (Figure C.24) while stage 3 Firm (Table C.4), taking another two days before achieving stage 4 Soft and ready to eat. Fruit from trees receiving 25 kg N ha^{-1} achieved stage 2–3 Colour on day 10 post-harvest, while fruit from trees receiving 0 and 12.5 kg N ha^{-1} N while stage 3 Firm had fruit with skin ranging from stage 3–4 Colour.

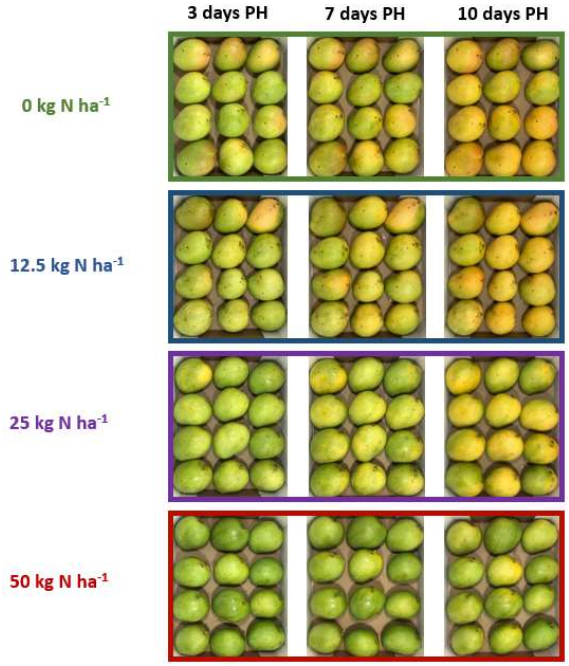


Figure C.30: Fruit from trees with a range of levels of nitrogen were harvested on the same day and imaged every second day to visually track ripening progress. At each level of nitrogen application, 0, 12.5, 25, and 50 kg N ha^{-1} (above, top to left bottom), the same tray of replicate fruit from a single tree is shown 3 days post-harvest (left column of images above), 7 days post-harvest (centre column of images) and 10 days post-harvest (right column of images above).

Quantification of skin colour changes over the ripening period showed that fruit from trees receiving 50 kg ha^{-1} of soil-applied N had significantly darker skin on the L (lightness) scale (Figure C.31a, b) compared to those applied with 0, 12.5 or 25 kg N ha^{-1} (p=0.007). This difference remained relatively consistent from harvest until fully ripe. L changed significantly over time as expected. There was a significant interaction between N and time (ANOVA, p=0.05), and the LSD post-test confirmed that fruit from trees receiving the highest N treatment started the time series with significantly lower mean L relative to the others, then maintained that curve through the series (see asterisk in Figure C.31b). Immediately post-harvest, the skin of all treatments had similar *a values. Overall, there was no significant difference between *a measured at each N treatment level (p=0.052), but with significant interaction between N levels and time (p=0.0). The LSD post-test confirmed a noticeable trend, with the fitted curves suggesting that over time, ripening skin of the fruit from trees receiving higher N treatments had higher *a values (Figure C.31a, c). The curves had similar relationships over days 2–6 post-harvest, then diverged in a consistent pattern over days 8–12 post-harvest. The skin of the fruit from trees receiving 0 and 12.5 kg N ha^{-1} crossed from the green -*a values into +*a red values when fully ripe, while the other levels sustained negative values (Figure C.31c). On the *b blue-yellow scale, skin of the fruit from trees receiving the highest N

treatment of 50 kg ha^{-1} maintained significantly lower values (p=0.008) or reflected less yellow through the ripening process. No significant interactions were found for *b (Figure C.31c, d).

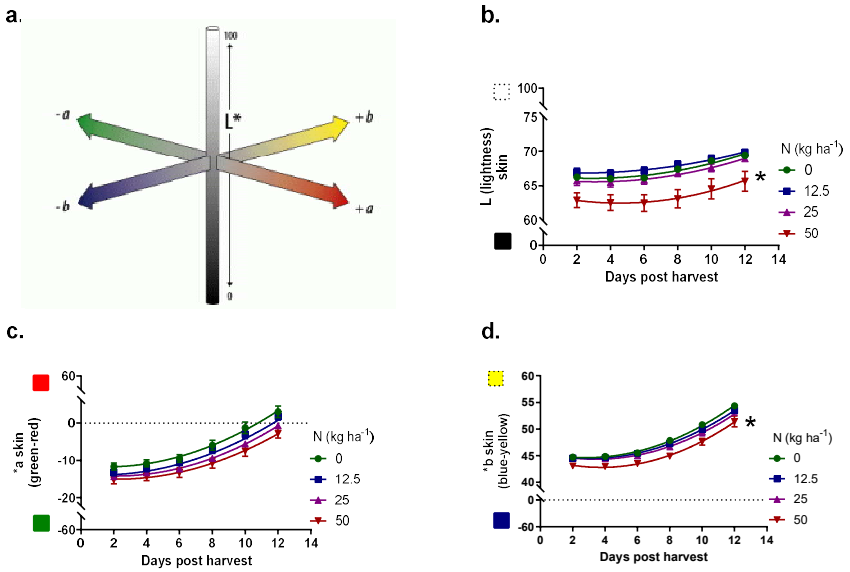


*Figure C.31: Colour components of skin colour were measured on ripening mangoes with a Konica-Minolta colorimeter using the CIEL*a*b system (a). Skin Lightness showed significant variability in response to tree-applied N levels (p=0.007), Significant interaction between N level and time occurred, and LSD post-tests indicate that fruit skin from trees with 50 kg ha^{-1} N applied was significantly darker over the period compared to fruit from trees with lower N treatment (* in b above). Measurements of skin *a (green-red) showed no significant differences in response to N treatments (p=0.052); however, there was a strong trend implying that as nitrogen application to trees increases, fruit skin colour tends to be greener as it ripens (c). Values for *b (blue-yellow) show significant differences in response to N levels applied and no significant interactions (p=0.008), LSD post-test indicates that skin of the fruit from trees with 50 kg N ha^{-1} is less yellow than from trees with lower levels of applied N (* in d above). ANOVA, mean, sem, n=4.*

Flesh attributes of ripe mangoes were assessed when fruit reached ripening stage 4 Soft. Small but significant differences were noted in the sugar content of fruit when ripe (p=0.04). Mean values ranged between 14.6 and 15.62 °Brix, with fruit from trees treated with 0 kg N ha^{-1} having the highest sugar content and those receiving 25 kg N ha^{-1} the lowest (Figure C.32a). Texture analysis of the flesh showed no differences in response to the nitrogen treatments (p=0.99) (Figure C.32b).

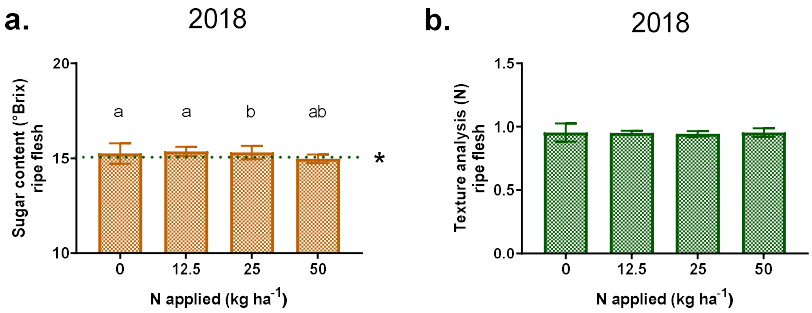


Figure C.32: Sugar content (°Brix) of mango fruit when soft and ready to eat was found to be small but significantly different between treatments, with letters indicating the differences (a) (p=0.04, ANOVA, LSD post-test, n=24). The dotted line indicates the industry recommended sugar content of fruit when ripe for eating (a). The texture of the flesh of mango cheeks was measured at stage 4 Soft and no differences were found between all treatments (b), ANOVA, p=0.99, sem, n=24.*

Flesh colour was measured with a colorimeter and no significant differences were seen in L (p=0.48) or *a (green-red) of flesh (p=0.34) (Figure C.33a, b). For *b (blue-yellow), mean values were between 59.9 and 62.1, with flesh from fruit harvested from trees treated with 12.5 kg N ha^{-1} having a significantly higher value (p=0.04). (Figure C.33c). There was a noticeable incidence of the post-harvest fruit quality defect 'soft nose', with a trend suggesting that as applied N increased, the incidence of the defect increased; however, it was not significant (data not shown).

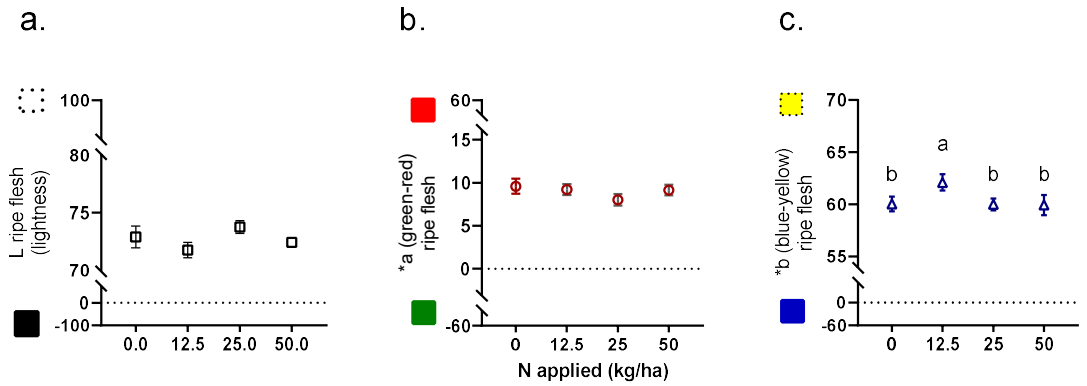


*Figure C.33: Colour components of stage 4 Soft flesh were measured. The range of nitrogen treatments did not impact significantly on L (p=0.46) or *a (p=0.58) (a and b). However, the *b in ripe flesh of fruit from trees receiving 12.5 kg N ha^{-1} had significantly higher values (p=0.04) (c). Letters indicate significant differences (c). ANOVA, Tukey's post-test, mean, sem, n=24.*

Post-harvest quality assessment 2019

Fruit was harvested at a lower % DM in 2019 compared to 2018. Mean values for % DM ranged between 16.35 % +/- 0.43 and 17. 05 % +/- 0.42 in the replicate sets of 24 fruit, with no significant differences between N treatments (p=0.28). No trends were noticeable (Figure C.34).

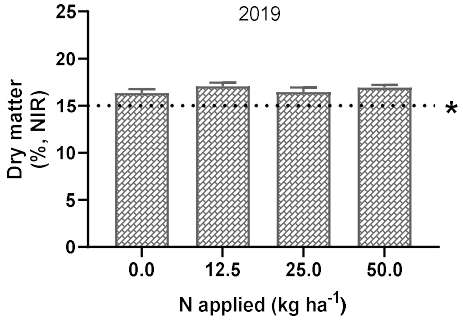


*Figure C.34: In 2019, dry matter in harvested fruit was measured on the day of harvest with a near infra-red instrument calibrated for local KP (Anderson 2017). * denotes the Australian mango industry minimum standard of 15 % for dry matter of harvested fruit (AMIA 2016).*

Ethylene-treated fruit achieved stage 4 Soft (Table C.4) and stage 5 skin Colour (Figure C.24) five days earlier than control fruit (Figure C.35). Skin appeared more consistently yellow in ethylene-treated fruit compared to control fruit.

Figure C.35: Post-harvest ripening of fruit with (15 ppm) and without (control) exogenous ethylene for 3 days was assessed, with the skin colour of fruit measured at the same location with a colorimeter over time. The fruit shown is from trees with nitrogen applied at 25 kg ha^{-1}. Above left, control fruit and above right, ethylene-treated fruit at 9 days after harvest. Control fruit continued to ripen for another four days, whereas the ethylene-treated fruit were stage 4 Soft and ready for destructive sampling. Fruit skins were marked to ensure that repeated measurements taken with the colorimeter were at the same location on each fruit at every time point.

The L skin colour component responded with significant differences to N treatment applied to trees. Fruit from trees receiving 50 kg N ha^{-1} maintained darker skin colour over days 1–9 post-harvest than the skin of the fruit from other treatments. However, the final mean L for skin of the fruit from trees receiving all N levels were the same (Figure C.36a). Ethylene impacted significantly on the time course of L measured on the skin of ripening fruit days 1–9 post-harvest (Figure C.36b), with ethylene-treated fruit skin having a mean L value of 68.0 when ripe compared to 70.5 for untreated fruit. For *a, small but significant differences were noted between mean N values over time, with fruit from trees receiving 50 kg N ha^{-1} having the lowest mean value of –6.47, compared to those from trees receiving 12.5 kg N ha^{-1}, with the highest mean value of –4.08 (Figure C.36c). Over time, the differences reduced, and all fruit had similar *a values when soft-ripe. Ethylene had significant effects over the time course of fruit ripening for *a, but was close to the same at the ripening endpoint with 5.1 and 5.3 despite being measured 5 days apart (Figure C.36c, d).

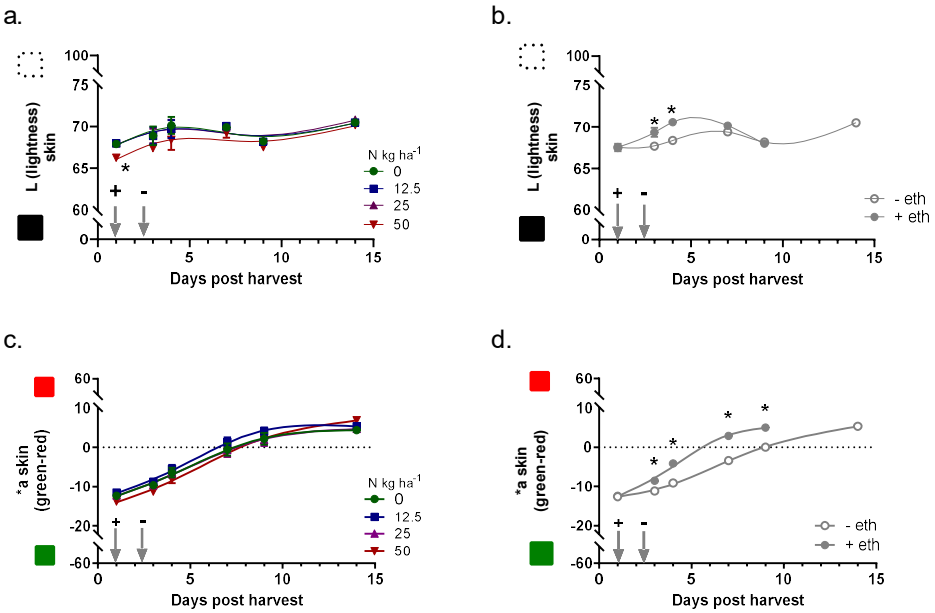


*Figure C.36: Skin colour components were measured over time as the fruit ripened. Fruit was initially held in controlled environment ripening rooms with, or without, ethylene for 30 hours (refer to arrows adjacent to the x-axes). For L, significant differences were measured between fruit from trees with different applied N levels. Fruit from trees receiving 50 kg N ha^{-1} had significantly darker skin over days 1-9 post-harvest (a) (p=0.0). Interaction between ethylene and ripening time for L was significant, with asterisks indicating differences (b) (p=0.0). When soft-ripe, mean L was 68.0 for ethylene treated fruit, and 70.5 for control fruit. For skin *a, nitrogen had a small but significant impact, with fruit from trees receiving 12.5 kg N ha^{-1} having the highest mean value for *a over days 1–9, and fruit from trees receiving 50 kg N ha^{-1} the lowest (b) (p=0.0). The ripening response of *a to ethylene was significant over time, with differences indicated by asterisks (c) (p=0.003). The ripening end-point of *a for all fruit was 5.1–5.3 (d). ANOVA, LSD, mean, sem, n=8.*

Colour component *b of ripening skin showed significant differences between fruit from the range of N treatments, ethylene response and significant interactions between the two (Figure C.37a, b, Figure C.38a-d). The *b mean value at the ripening endpoint for fruit that received no ethylene was between 53.9 and 57.3, compared to that of treated fruit with *b ranging from 49.3 to 50.8. The *b skin colour for soft ripe fruit was inconsistent with 2018 measurements, with fruit from trees receiving 12.5 and 50 kg N ha^{-1} having the highest values.

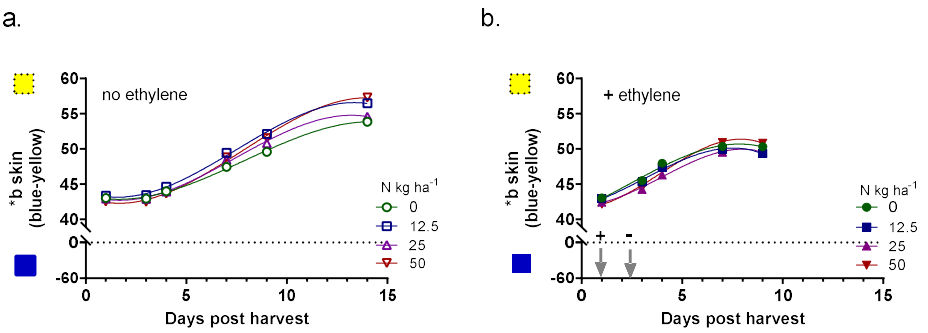


*Figure C.37: The blue-yellow colour component *b of the skin of ripening fruit was significantly different over days 1–9 post-harvest in response to N levels applied to the trees (p=0.04) and significant interaction between received N levels and ethylene treatment was noted (p=0.002) (a) and (b). The *b values for fruit ripened to soft-ripe without ethylene over 14 days had means between 53.9 (0 kg N ha^{-1}) and 57.3 (50 kg N ha^{-1}), which were significantly higher than *b measured for fruit from trees treated with 25 and lower than 12.5 kg N ha^{-1} respectively (a). In contrast, ethylene-treated fruit was soft ripe 9 days post-harvest and had mean *b values for all N levels of 49.3–50.8 (b). ANOVA, LSD post-tests, mean, sem, n=8.*

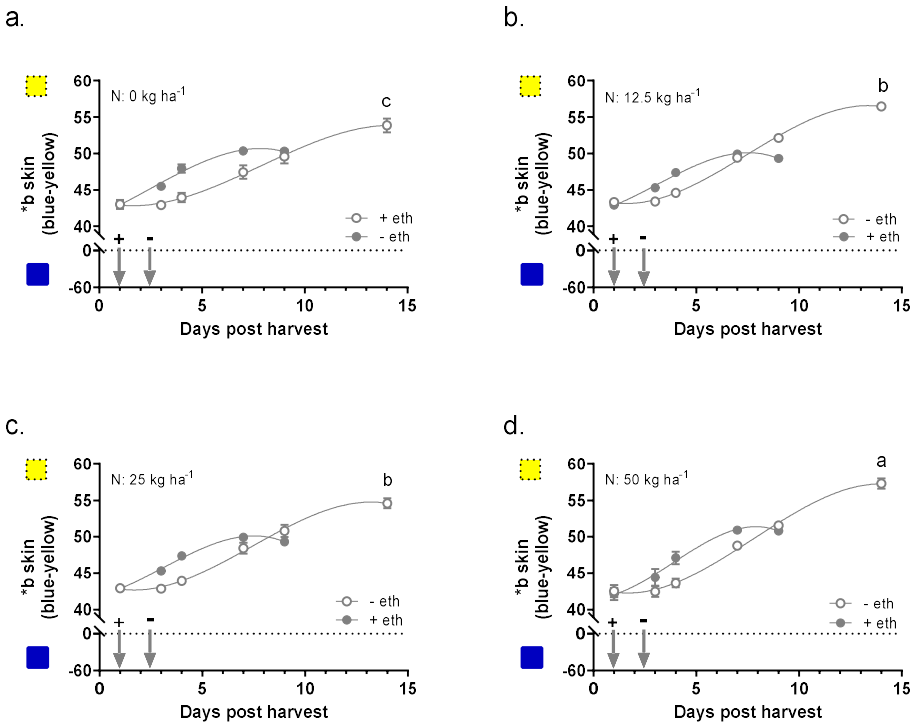


*Figure C.38: Colour component *b of ripening fruit skin varied significantly between +/- ethylene-ripened fruit and fruit applied N levels at each time point days 3–9 post-harvest after ripening started at the same *b value for all conditions (p=0.01). Significant differences were noted in ripening endpoints for *b of fruit from all N treatment levels, with letters denoting differences at day 14 (a-d). Fruit from trees receiving no ethylene treatment had higher *b values, with fruit from trees receiving 50 kg N ha^{-1} the highest at 57.3 and those from trees receiving 0 kg N ha^{-1} the least with *b of 54.0. Fruit treated with ethylene had *b values ranging from 49.3–50.8 at their ripening endpoint (ANOVA, LSD post-tests, mean, sem, n=8).*

The fruit ripening colour time course was significantly different at all levels of N treatment, with the *b measurements of ethylene-treated fruit diverging immediately, reaching peak values on day 7, then dropping by day 9 post-harvest (Figure C.38a-d). In contrast, *b values for skin of untreated fruit were initially static, with small increases at four days post-harvest. All ethylene-treated fruit had *b rising continuously to a maximum value when soft-ripe 14 days post-harvest, with a mean range of 53.9–57.3. When ripe, ethylene-treated fruit had lower *b values, with mean values between 49.3 and 50.9, so the skin was less yellow and past peak *b. Fruit from untreated fruit from trees receiving 50 kg N ha^{-1} developed the highest *b and those from trees receiving 0 kg N ha^{-1}, the lowest.

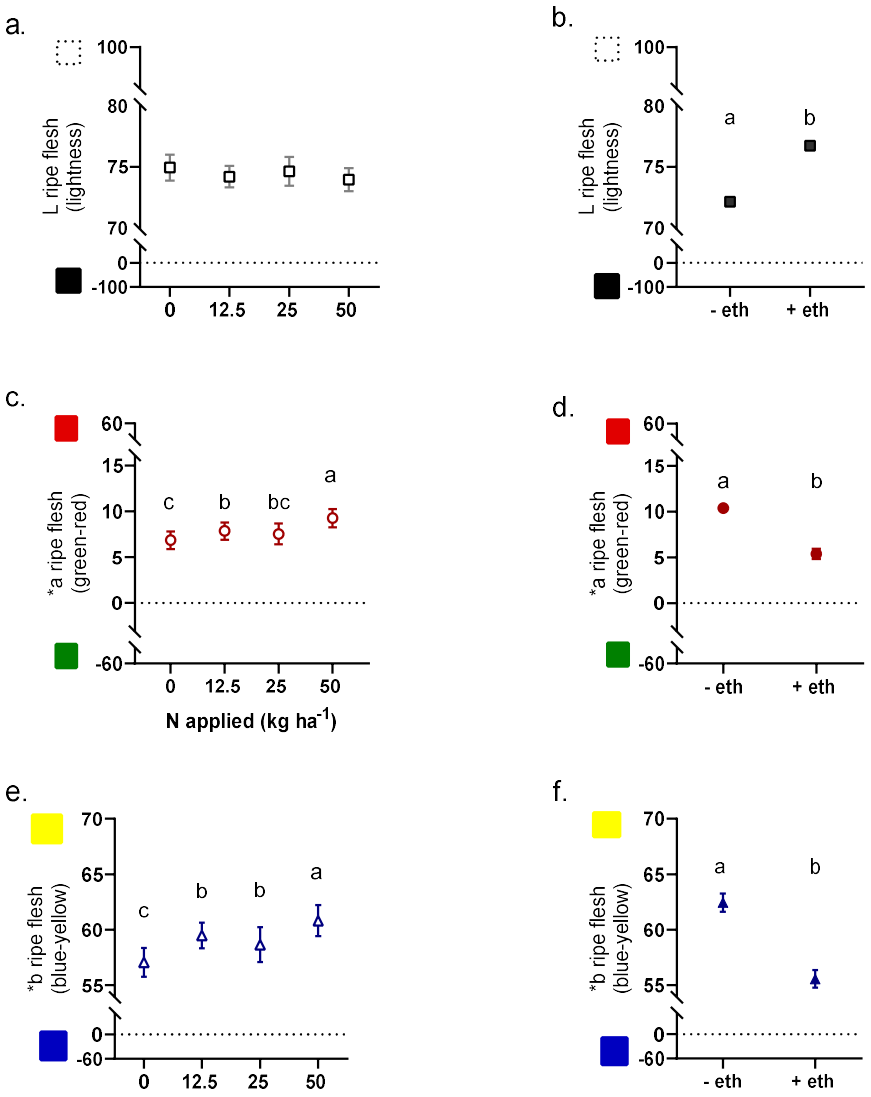


*Figure C.39: Flesh colour of fruit was measured when ripe and ready to eat according to the softness scale (Table C.4). The L colour component of ripe mango flesh was not impacted by different levels of N application to trees. However, both *a (p=0.001) and *b (p=0.0) showed significant differences in response to N applications, as designated by letters in (c) and (e) above. Fruit from trees receiving 50 kg N ha^{-1} had flesh that was redder and more yellow than other treatments, with fruit from trees receiving 0 kg N ha^{-1} reflecting the lowest *a and *b. Mean, sem of all (+/- ethylene) measured colour values of fruit skin in response to N treatments (a, c and e). All skin colour components responded significantly to ethylene treatment (L, p=0.09 , *a, p=0.004, *b, p=0.003), shown above (b, d and f) with letters indicating differences (ANOVA, LSD, mean, sem, n=8).*

Measurements of colour in ripe flesh found no response in varying N levels for L (Figure C.39a). In both *a and *b, fruit from trees receiving 50 kg N ha^{-1} had the highest mean colour values, and those receiving 0 kg N ha^{-1} the lowest (Figure C.39c, e). Ethylene treatment impacted all colour component values measured on ripe flesh, with treated fruit having higher or lighter L, and lower *a and *b values than naturally ripened fruit (Figure C.39b, d, f).

Varying N applications had no impact on the sugar content of ripe fruit in 2019 (Figure C.40a). However, fruit ripened without ethylene had a higher mean of 1.1 °Brix more than fruit treated with ethylene (Figure C.40b). The texture, measured at the end-point of ripening for all fruit showed small but significant differences, with fruit from trees receiving 0 kg N ha^{-1} having more resistance when

soft-ripe than the other N levels (Figure C.40c). There were no differences in texture between the ethylene-treated and untreated fruit (Figure C.40d).

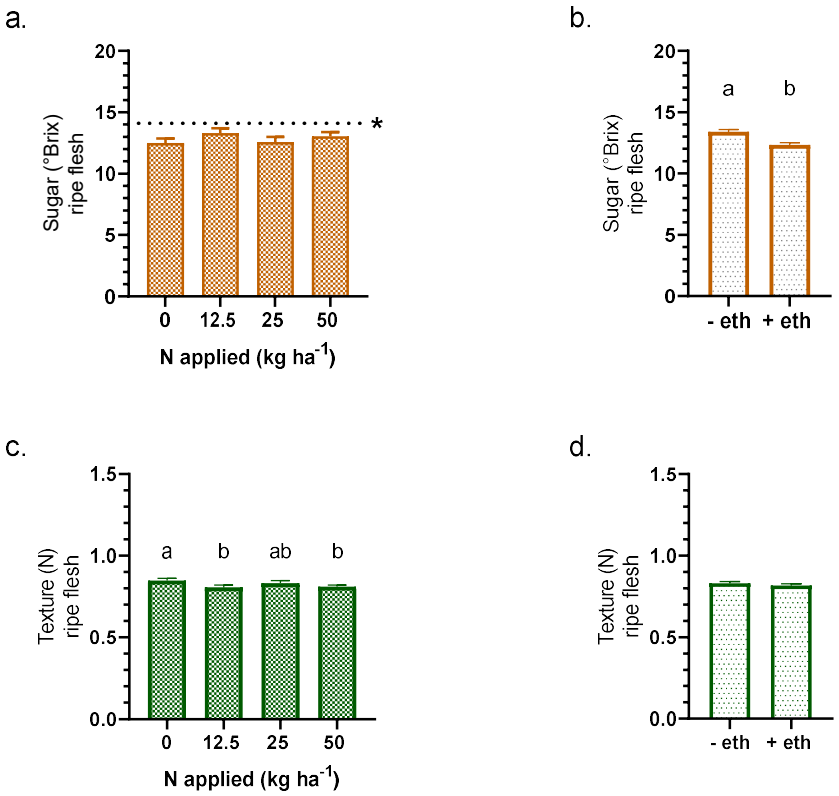


Figure C.40: The sugar content (°Brix) in ripe flesh was not influenced by N application (p=0.09) (a) but was significantly lower as a result of ethylene treatment (p=0.006) (b). Mean °Brix for fruit ripened without ethylene was 13.4 compared to 12.3 °Brix for fruit ripening with ethylene (b). The texture of ripe fruit showed small but significant differences in response to N applied to trees, with fruit from trees treated with 0 kg N ha^{-1} having the highest flesh resistance. Differences are indicated by letters (c). No significant ethylene response or other interactions were observed. Dotted line at 15 °Brix (a) indicates the industry recommended minimum sugar content for ripe fruit (ANOVA, LSD, mean, sem, n=8).

Discussion

Yield

The nitrogen application levels ranging between 0 and 50 kg ha^{-1} reflect the general range of mango management practices in the NT. While conservative compared to other environments, they were chosen to induce stay green skin at the highest level applied, but do not leave ongoing quality problems in the commercial orchard. There were no significant impacts on yield in both the 2018 or 2019 harvests (Figure C.27a, b) and a rapidly reducing yield-benefit for the nitrogen inputs in each year (Figure C.27c, d). Interestingly, despite the large differences in yields for 2018 and 2019, the nitrogen content was relatively similar on a fresh weight basis, ranging between 0.84 and 1.11 kg tonne^{-1} of fruit harvested across all treatment levels. When expressed in terms of N leaving the orchard in the fruit, in 2018, 16.3–21.0 kg N ha^{-1} left the property compared to 36.7–42.7 kg N ha^{-1} in 2019. Growers need to factor in these quantities of N when considering how much to apply the following year to ensure that soil fertility is maintained and nutrient mining of the soil is avoided (Majumdar et al. 2016). It is notable that in January 2018, at the beginning of the experiments, the commercial orchard soil N content was 0.45 %. In January 2020, soil N was down to 0.04 %, which may reflect high 2019 yields in conjunction with fruit N content maintained around 1 % FW. Lower

soil N could also be due to heavy break of season rains in December 2019 and January 2021 (Figure C.26), potentially flushing nutrients down the soil profile and out of reach of tree feeder roots.

The post-harvest defect of 'soft nose' in fruit was noted across all N treatment levels in 2018, with an increasing incidence as tree-applied N increased; however, it was a trend and not significant (data not shown). 'Soft nose' occurs in the mesocarp of mangoes, distal to the seed tip where cells appear translucent or senescent while the rest has cells with intact membranes and solid colour. The cause has been linked to calcium deficiency and excess supply of nitrogen in fruit tissues, but this is not certain (Raymond et al. 1998). In this instance, the in-orchard soil calcium level was higher in 2018, the year 'soft nose' was observed, compared to the 2019 harvest with no incidence of the post-harvest defect. Soil pH was within the generally accepted range for calcium being available for uptake, with the pH ranging between 7.9 and 8.7 over the two seasons (Tables C2, C3). Work on the physiological defects associated with fruit ripening is ongoing, with the phytohormones abscisic acid and ethylene thought to dominate the control processes (Forlani et al. 2019).

Dry matter measurements are part of the decision-making process for the timing of the mango harvest. The general industry recommendation to reflect sufficient fruit maturity in KP is 15 % DM (AMIA 2016). However, a minimum of 14 % is often the decision point to commence harvesting, as fruit can be transported while still firm and green, for example from the NT to the east coast of Australia. This has the potential to reduce fruit damage and increases the time between harvest and ripening, depending on travel storage conditions, particularly temperature. Dry matter in fruit is related to carbohydrate and starch content when harvested, and the sugar or soluble solids content when the fruit is ripe. It follows that harvesting fruit at a lower % DM can have negative impacts on sugar content and eating quality when ripe. In B74 (Calypso), for example, % DM increases approximately 0.07–0.12 % daily, or 0.49–0.84 % per week (Anderson et al. 2017). This suggests that harvesting at 14 % rather than 15 % DM means between one and two weeks less time on the tree, potentially accumulating fewer carbohydrates and ultimately fruit with lower °Brix when ripe. In this work, there was no link or variability in dry matter accumulated in fruit with any level of applied N in commercial orchards.

Fruit quality

Mango fruit maintaining green skin while ripening is recognised as a problem associated with excess N application in orchards (Nguyen et al. 2004; Oosthuyse 1993b), and is particularly common in KP (Nguyen et al. 2004). Anecdotally, commercial growers of mangoes on the nutrient-poor soils in the NT apply less N on their orchards than orchardists in other parts of Australia, but 'stay green' skin can still be a quality problem and this work indicates the N level where it becomes a consideration. The colour component, L, of mango skin of the fruit from trees receiving up to 25 kg soil-applied N (plus 5.5 kg foliar-applied N ha^{-1} for all treatment levels) in 2018 followed a similar ripening time course. Fruit from trees receiving a total of 50 kg N ha^{-1} had darker skin, and stayed darker when ripe compared to that from lesser N levels. The *a colour component showed no significant differences, but a strong trend indicated that as N application increased, the greener the *a component of skin colour. For *b, skin colour was less yellow on ripening fruit from trees receiving the high-level N treatment. Also, the ripe flesh *b colour component of fruit from trees receiving 12.5 kg ha^{-1} developed higher yellow values. In terms of the colour and softness scales used in the mango industry (Table C.4 and Figure C.24), they appear to be designed as a pair, with mangoes being ready

to eat when both scales reach stage 4 as described. This work indicates that excess application of N results in a mismatch between colour and softness during ripening, where the excess N prevents skin colour change and softening processes continue in the mango flesh.

Overall, in the Katherine commercial orchard in 2018, with a yield of close to 20 tonnes ha^{-1}, minor differences in ripe skin colour were seen at N application levels of less than 25 kg N ha^{-1}, and major differences at 50 kg N ha^{-1}.

The colour components of the skin of ripening fruit from trees receiving a range of N levels, with and without a brief ethylene gas application in 2019, were different compared to those collected in 2018. The obvious 'stay green' phenotype of ripening skin was not replicated in fruit from trees receiving higher N levels. This is not surprising, considering the seasonal yield difference. There were measurable differences, however, with the L colour component of ripening skin again beginning the ripening process significantly darker at the 50 kg N ha^{-1} treatment level but all fruit skin had similar L values by the soft-ripe stage.

For *a, there were no clear trends in response to N levels, and similar ripening endpoint values with and without ethylene. The *b component of fruit from trees receiving the highest levels of N developed higher, more yellow values than fruit from trees receiving 0 kg N ha^{-1}. This could be a reflection of optimum fruit development for the higher applied N and the high yield of the 2019 season, rather than an excess supply response observed in 2018. The *b values for ethylene-treated fruit peaked at 7 days post-harvest, then fell slightly at the ripening endpoint. All fruit ripened without ethylene achieved higher *b values than ethylene-treated fruit, the difference ranging between 4 and 7 units. These measured differences can be analysed using other systems such as Hunter or CIELCh$_{ab}$, (McGuire 1992), but the human eye mimicking CIEL*a*b system was preferred. Interestingly, Hunter b component data was found to be a useful measure of mango flesh maturity and correlated with % DM (Subedi et al. 2007). It is likely that *b results in our data are also a flesh maturity measure of KP mangoes.

Applied N impacted on ripe flesh colour of fruit from trees receiving 50 kg N ha^{-1}, with higher *a and *b values implying more colour development. Small differences in flesh texture were measured in response to applied N levels but there were no textural changes in response to ethylene treatment. Overall, the application of ethylene to obtain even, golden or blushed skin colour shortened the time for fruit to reach stage 4 Soft-ripe by 5 days. The data suggest that exogenous ethylene skin colour change and accelerated softening, or perhaps senescence, of the mango pericarp are processes separate from flesh colour development and the conversion of carbohydrates to simple sugars. This is likely to reduce the saleable life index of the mangoes (Leger et al. 2003). It would be more desirable to have only skin colour affected by exogenous ethylene and not the tissues of the exocarp, leaving those tissues under control of endogenous processes. It is known that abscisic acid probably triggers the genes controlling ethylene biosynthesis. Also, the chemical genetics of signalling cascades and interactions associated with cell wall softening in dry and fleshy fruits indicate these two hormones dominate ripening processes (Forlani et al. 2019).

Fruit that ripened naturally had darker flesh with more red and yellow colour development. They also had ~1 °Brix more than ethylene-treated fruit. This may sound insignificant, but it is equivalent to a difference of ~10 g of sugar per litre of juice. Although the % DM of fruit harvested in 2019 was 16–17 %, well above the recommended minimum, the °Brix developed in the fruit when ripe, 12.5–13.3,

was below the desirable ≥ 14 °Brix for KP fruit (AMIA 2016). This may be related again to the high yield of 2019, although canopy and leaf cover was dense due to minimal pruning after the 2018 harvest, so the source:sink relationship between leaves and fruit should not have been limiting. It has been shown that high leaf:fruit ratios often result in fruit with high structural and other dry matter, but there is usually a concurrent increase in sugar content (Léchaudel et al. 2005b). The yield difference between the two years was mostly due to the higher number of fruit on each tree rather than the size of fruit (mean individual fruit weight in 2018 was 0.37 kg and in 2019, 0.38 kg). This suggests the trees had better flowering, fruit set or fruit retention than in 2017, but photosynthesis may have been inhibited and carbohydrate production interrupted. Lu et al. (2012) found that the period of maximum environmental stress for KP in the Darwin and Katherine mango growing regions occurred during fruit development. The result was reduced leaf stomatal conductance and inhibition of photosynthesis. This means lower rates of carbohydrate production and lower accumulation in the fruit. This could explain why a large volume mango crop did not achieve the desired sugar content despite having a larger canopy than in other, lower-yielding years.

In summary, while skin colour appears even and softening is uniform in fruit treated with ethylene, there is a quality penalty: a reduction in flesh colour and sugar accumulation. While 'stay green' skin was not reproduced in the harvest of 2019, the response of skin colour to a tree N application of 50 kg ha^{-1} was measurable and significant over the ripening time course, and sugar content of fruit when ripe was low and below the industry quality standards (AMIA 2016). Ethylene shortened the time taken to reach stage 4 Soft, but differences in flesh colour components L and *b and lower °Brix were significantly different at the ripening end-point.

An awareness of the potential loss of quality in fruit because of ethylene treatment is needed, otherwise an illusion of top quality is being sold and, if the buyer is disappointed, there is a risk of extending the time to the next mango purchase.

Research in the Burdekin region of North Queensland found that more than 150 g $tree^{-1}$ of soil-applied N risked producing fruit with 'stay green' skin (Nguyen et al. 2004). Foliar application of 50 g N $tree^{-1}$ was also linked with the 'stay green' skin of the fruit from orchards with a history of this post-harvest defect. This is equivalent to trees receiving 37.5 kg soil-applied N ha^{-1}, and 12.5 kg ha^{-1} of foliar-applied N (total N inputs of 50 kg N ha^{-1}) compared to this current, NT-based work. At these northern Queensland recommended N application levels, fruit with the 'stay green' post-harvest defect were produced in an NT commercial orchard yielding just under 20 tonnes ha^{-1}, at a planting density of 250 trees ha^{-1}. This is equivalent to a yield of ~80 kg of fruit $tree^{-1}$, similar to the 70–85 kg fruit $tree^{-1}$ produced in response to a range of N treatments in Nguyen et al. (2004).

Over the season, leaf N content on the experimental trees generally followed the expected time course, reducing as the tree entered the dormancy period in April and May (Figure C.26). The leaf N content did not significantly change in response to soil or spray application of nitrogen, although the 12.5 and 25 kg ha^{-1} treated trees did appear to increase leaf % N slightly in response to the KNO_3 sprays in late June. There was no increase in leaf % N over the fruiting and harvest period as depicted in the longstanding work of Ted Winston and the Queensland Department of Agriculture and Fisheries (Winston undated). Interestingly, the data from material collected in January 2019 showed a large, rapid increase in leaf % N (Figure C.26). No N had been applied to the trees; therefore, it is the result of local, naturally occurring conditions. Soil pH can increase over the dry season as large

quantities of alkaline irrigation water are applied. It is possible that wet season rain, which can have a pH of 5–6, flushed and reduced the pH of the soil. This reduction in soil pH may increase micronutrient and macronutrient availability for root uptake, including N. Also, we now have direct evidence of rapid decomposition of litter and pruned material occurring from break of season rains over the wet season, which, along with microflora and microfauna activity increased N mineralisation and nitrification of soil (see Sections C.7, C.8, C.9).

The N application history of the commercial orchard in the Katherine region varies from year to year according to advice received from service providers, but generally cycles between 140–220 g N tree $^{-1}$ from all applied sources. This is equivalent to 35–55 kg N ha^{-1} for a tree planting density of 250 trees ha^{-1}.

This work supports the hypothesis that in mango, unlike many other fruit crops, there is no yield advantage in applying N in excess. Any yield gap or possible yield increase for mangoes is likely to be resolved using ionomics and balancing nutrients required by crops in different environments (Mueller et al. 2012; Huang and Salt 2016). Therefore, the recommended starting point for N application (soil-applied or fertigation) in a commercial orchard would be 12.5–25 kg N ha^{-1} (80–160 g N tree^{-1}), adjusted according to N content in the soil, N cycling in litter on the orchard floor, how much N left the orchard in the most recent crop and any other known or potential deficiencies.

C.5 Nutrient cycling in mango orchards

Introduction

Nutrient cycling in agricultural and natural ecosystems, particularly nitrogen, phosphorous and carbon is of increasing interest as climate change impinges on crop productivity (Wright et al. 2004). and the need for sustainable practices to balance the demand for maximum yield (Thorburn et al. 2011). The Haber-Bosch process has been used over the past ~100 years to manufacture synthetic N fertilisers. This has supported massive increases in agricultural production and human population, with close to 40 % of the world population relying on food grown using these synthetic products (Erisman et al. 2008). Their manufacture and use mean that more reactive nitrogen compounds are being released, with negative impacts on air and water quality (White and Brown 2010; Lassaletta et al. 2014). However, there is a large body of evidence building around over-application and inefficient use of synthetic fertilisers (Liu et al. 2016). Thus, there is a need to develop N management strategies and technologies for widespread use at the farm-level to improve N use efficiency.

Mango (*Mangifera indica*) grow well in a wide range of climates and soils. In Australia, it is grown in the wet-dry tropics of Darwin, Pine Creek, Katherine and Mataranka in the NT and the Kununurra region of Western Australia (WA). Sub-tropical Carnarvon on the WA coast also has commercial plantings. On the east coast, extensive mango plantings exist from the wet tropics between Port Douglas to north of Townsville, and south to sub-tropical south-eastern Queensland and northern New South Wales. In 2019, the NT produced 52 % of the Australian 10 million tray mango crop. This equates to over 36,000 tonnes of a 52,000 tonne harvest yield (Brann 2020). Soils vary widely between regions, and while fertilising practices for east coast Australian orchards are well established, practices vary widely in the nutrient-poor soils of the NT. Successful harvest yields do not require high volumes of N; however, fruit quality defects can result from under or oversupply of N (Weinbaum et al. 1992; Nguyen et al. 2004). What amount of N constitutes ‘over’ or ‘under’ supply is not well understood and there are no current guidelines for N application in NT commercial mango orchards (AMIA 2018).

Nutrients leaving a mango orchard in harvested fruit can be estimated, but the timing, volume and nutrient content of fallen and pruned material, and the rates of decomposition of that material in local conditions have not been quantified for mango growing in the wet-dry tropics of the NT. Also, there is limited information on mobilisation and retention of nutrients taken up by mango trees. The resorption of leaf nutrients back into plants for storage or immediate transfer into new growth or other plant processes is relevant across ecology and horticulture in all environments. On a global basis, it is estimated the average percentage of N resorbed back into living plants is 62.1 % and for P the figure is 64.9 % (Vergutz et al. 2012). There are evolving views on how nutrient resorption is affected by climate, soils and plant types (Aerts 1997; Eckstein et al. 1999; Yuan and Chen 2009; Laliberté and Tylianakis 2012). Foliage nutrient concentrations were historically used to assess plant and environmental health (Chapin III 1980) but more recently the focus is reversed, instead examining how the environment influences foliage nutrient content (Brant and Chen 2015; Wang et al. 2018). This is a relevant consideration for mango, as ‘best management’ practices for this widely grown crop vary so widely depending on latitude, temperature, rainfall and soils. Also, it has long been acknowledged that mango leaf nutrient content is a poor indicator of over-fertilisation (Weinbaum et al. 1992) and fruit yield and quality (Catchpoole and Bally 1999).

The question is: what nutrients are cycling in mango orchards in the NT and what portion of the annual orchard nutrient budget do they form? A better understanding of these issues will contribute to the development of sustainable nutrient budgets and evidence-based guidelines for fertiliser application on commercial mango orchards in the wet-dry tropics.

In commercial mango orchards, substantial amounts of plant material senesce and drop onto the ground every year where it decomposes over time. The nutrients contained in the litter are mineralised, consumed, recycled or leached from the soil. Further, mango trees are annually pruned or hedged to maintain canopy shape and promote new growth to bear the next crop of fruit. The pruned material on the orchard floor is usually slashed into smaller pieces and left as mulch, joining litter as part of the cycling/recycling of N and other nutrients.

The timing, volume and nutrient content of this material has not been quantified in the wet-dry tropics of the NT. Also, the quantity of nutrients cycling within the orchard as plant residues move from biomass to necromass is not known. As part of quantifying the N budget in NT conditions, the questions posed were: 1) what amount of litter and pruned material is cycling annually, 2) when does it accumulate and in what form and 3) how much N and other nutrients from this material are cycling within orchards annually?

Methods

Annual litter drop and pruned material were collected at four commercial orchards in the wet-dry tropics of the top end of the NT, Australia. In 2017–18, the material was collected in two Darwin region orchards. Both orchards were mature, one growing Kensington Pride planted in 2002 and the other B74 planted in 2004 (Table C.6). During 2018–19, the material was collected in two Katherine region orchards. One had mature, Kensington Pride trees planted in 2000 that were minimally tip-pruned prior to litter collection commencing. In contrast, the other orchard had B74 trees planted in 2011 which were in the process of significant pruning and reshaping (Table C.6). All orchards were situated on Tippera, red Kandosol soils (ASRIS 2011; Isbell and NCST 2021).

Table C.6: Litter and pruned material were collected from four commercial orchards, growing either Kensington Pride or B74 mangoes. Planting densities, tree age, fertiliser application and management practices varied widely and are typical of the range of local practices. N application is for the year of data collection.

Region	Variety	Year planted	Tree density ha^{-1}	N applied kg ha^{-1}	Pruning	
					Type	Timing
Darwin	KP	2002	120	22.1	Mechanical top and side	Nov-Post harvest
Darwin	B74	2004	250	2.8	Mechanical top and side	Nov-Post harvest
Katherine	KP	2000	140	39.6	Mechanical top only	Feb 2019-minimal top prune
Katherine	B74	2011	300	58.6	Mechanical top and side	Apr 2019, end of wet season. Tree height reduction.

Litter traps were constructed using flexible fibreglass flyscreen mesh, metal droppers and twine, set up in an east-west orientation and extending from the trunk to just past the drip line of each tree, 0.5 m wide, n=10 randomly selected across orchard rows but including no perimeter trees. Litter collection area, or under-canopy area was measured for each tree. Litter fall was collected weekly or

fortnightly depending on the season to ensure minimal losses or decomposition within the traps. The collected material was sorted, washed and rinsed in Millipore filtered water then oven-dried at 50 °C. Dry weights were recorded and material ground using a Rocklabs Standard Ringmill for woody material and a Retsch SK1 mill fitted with a 1 mm screen for leaf material. Once processed, materials were subsampled for analysis.

The average crown or canopy spread of each tree was measured before pruning and canopy areas were calculated:

Average crown spread (m) = [longest spread (m) + longest cross spread (m)]/2 = tree canopy diameter (m)

Tree canopy area (m^2) = π [1/2 diameter (m)] ^{2}

Litter drop was calculated on a tree^{-1} basis:

*Tree litter (dry weight (kg)) = [(collected litter (dry weight)/trap area (m^2)]*tree canopy area (m^2)*

For pruned material, tarpaulins 1.2 m wide were placed on the ground at the trunk or stem of the tree and extended 0.5 m past the drip line to catch material as trees were machine pruned (n=10). The collected material was sorted, washed and rinsed in Millipore filtered water then oven-dried at 50 °C and weighed. Tree canopy spreads and tarpaulin collection area were measured prior to pruning and used to calculate the quantity of prunings on a tree^{-1} basis, as for litter.

Carbon and nitrogen content was measured for litter collected in each orchard over twelve months and the single pruning event for each orchard in 2017–2019. To estimate the complete nutrient content of the litter, samples of each litter component (leaves, flowers, panicles and branches, were collected at three consecutive time points from all orchards (n=4) during flowering and fruit set, BBCH (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie) stage 615–700 (Hernández Delgado et al. 2011) in August–September 2018, then processed as described and analysed. Pruned material was sorted, washed, dried and processed as described. Material from each orchard was bulked, subsampled and analysed.

Carbon and nitrogen analyses (CNS-2000, LECO Corporation, St Joseph, MI, USA) and nutrient analyses (Agilent 8800 LA ICPMS) were conducted at the Institute for Future Environments at QUT.

The orchard tree planting densities varied between 120 and 300 trees ha^{-1} (Table C.6).and data collected was standardised to 250 trees ha^{-1} for comparison.

Results

The majority of natural biomass abscission in all orchards occurred during tree floral induction, through fruit set to fruit maturity, a period of approximately 100 days. Litter components collected over time varied in quantity depending on the phenological stage of the trees and in-orchard management practices. The amount of flowers in litter peaked between late June and August in all orchards, contributing the surprisingly high ~150–200 kg ha^{-1} to the total litter DW over that period (Figure C.41). Also noticeable was the quantity of panicles in the litter, dropping mostly over fruit set- and peaking at 50 kg ha^{-1} in all orchards. Immature fruit drop was variable in orchards, with the Darwin orchards losing 25–50 kg ha^{-1} from trees over the fruit set period around August–September (Figure C.41a, b) and a significant quantity of mature fruit was left on trees to fall as they senesced (Figure C.41a, b). This post-harvest fruit drop into litter was not seen to the same degree in the Katherine region orchards. At the Katherine KP orchard, all healthy fruit was picked, regardless of commercial grade because there was access to a market for mango juice or pulp. The reworking of

the tree canopies over the 2018 year in the Katherine B74 orchard is reflected in the low rate of leaf litter drop, low volumes of fruit in litter and, as the trees recovered and regained productivity, the high volume of flowers (Figure C.41d).

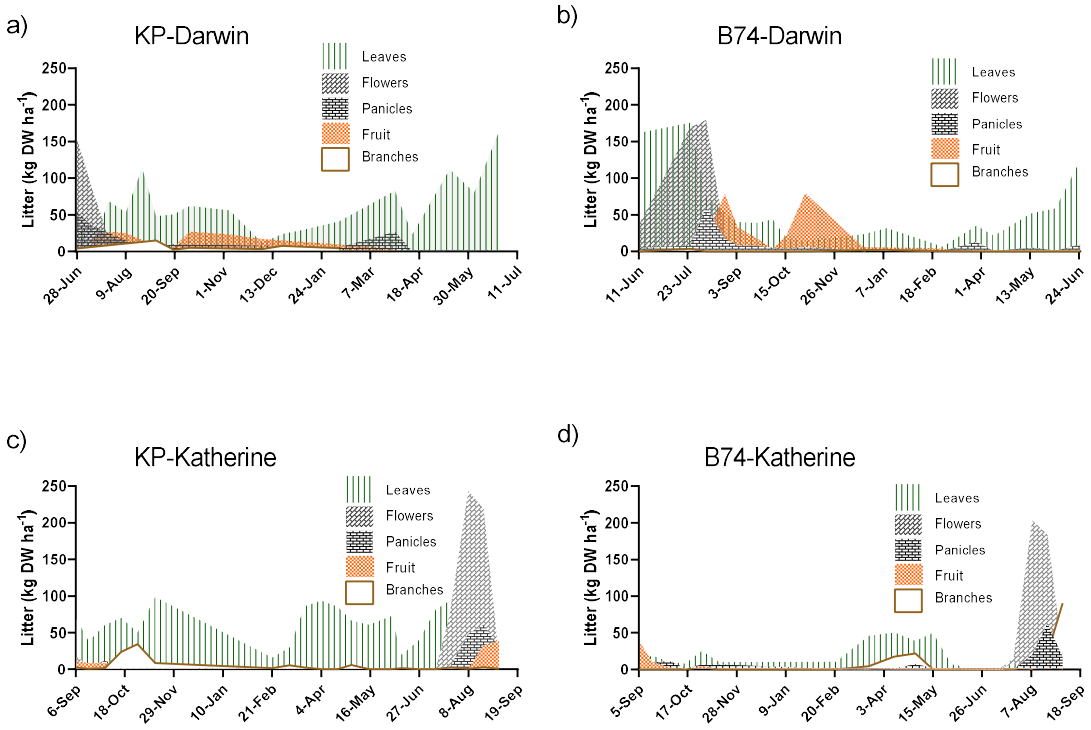


Figure C.41: Litter collected over a year in orchards in the Darwin region in 2017-–2018 (a, b), and the Katherine region in 2018–19 (c, d). The litter components vary significantly over time and reflect a range of management practices. N=10, Mean is shown. Data is standardised to a tree density of 250 trees ha^{-1}.

In the Darwin region, the KP orchard abscised 1.8 tonnes of litter and 2.2 tonnes of pruned material per hectare on a dry weight basis (Figure C.42a). The B74 orchard had a heavier litter drop of 2.9 tonnes collected per hectare but was balanced by a lower annual pruned material weight of 1.1 tonnes ha^{-1} (Figure C.42b). Overall, the quantity of material collected on orchard floors ranged from 4.0–4.2 tonnes ha^{-1} when standardised to a planting density of 250 trees ha^{-1}.

In the Katherine region KP orchard, management left the tree canopies minimally trimmed and removed 0.15 tonne ha^{-1} of material. The large quantities of leaf litter collected over the following months, 1.3 tonnes ha^{-1}, reflect the heavy tree canopy that remained (Figure C.42c). In contrast, the Katherine region B74 orchard trees were reshaped and the tree canopies pruned significantly, resulting in 3.2 tonnes ha^{-1} of pruned material. The annual litter quantity was low as a result, with 1.1 tonnes collected over the year (Figure C.42d). Three of four orchards cycled around 4 tonnes ha^{-1} of plant material over the year.

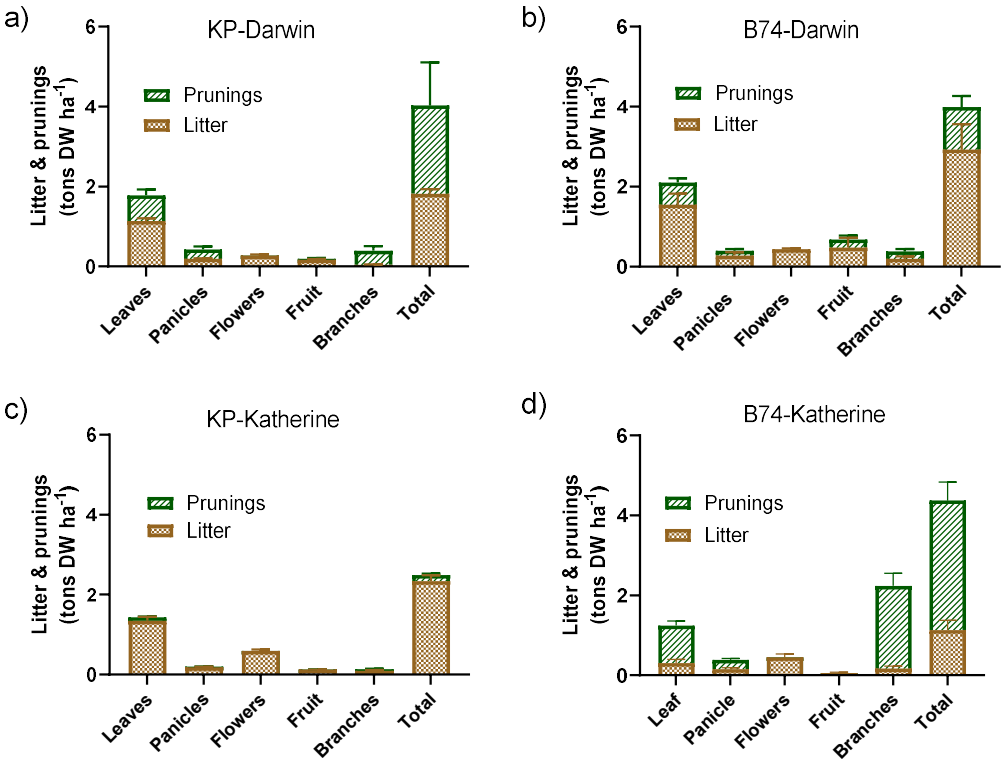


Figure C.42: Annual dry weights of litter and pruned material varied across the orchards, ranging from just over 2 tonnes at the KP Katherine site to around 4 tonnes at the other three sites. The litter and pruning data reflect the orchard management practices in the year material was collected. Mean, sem, n=10 collection trays at each site. Data is standardised to a tree density of 250 trees ha^{-1}.

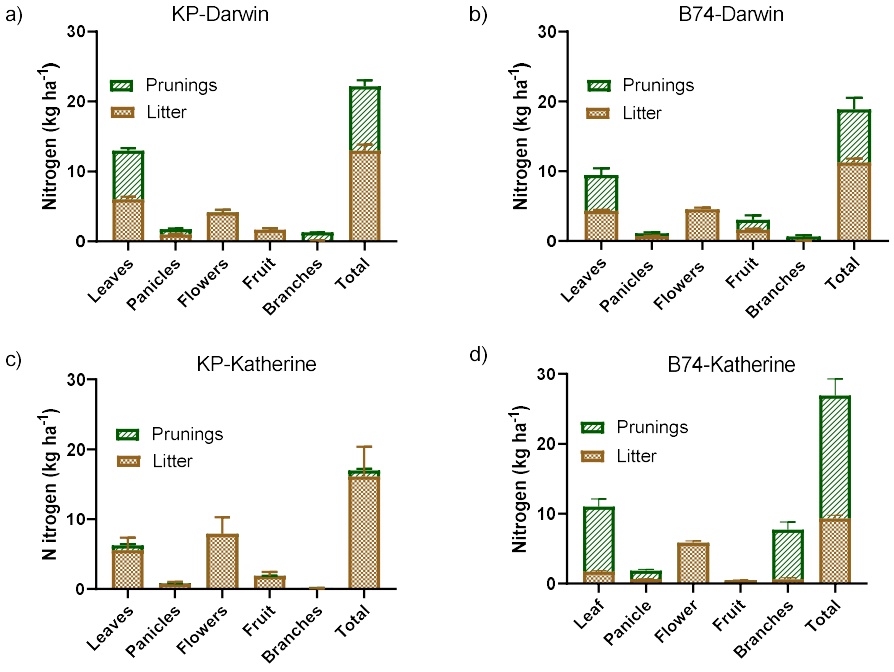


Figure C.43: The N content of in-orchard annual litter and pruned material in the Darwin region litter was similarly proportioned (a, b). In the Katherine region, the KP orchard with large, mature trees and minimal branch tip pruning, shed most nitrogen in litter over the year 2018–19 (c). In contrast, the B74 were pruned heavily and reshaped, with most N accumulated in the prunings (d). Mean, sem, n=10 collection trays at each site. Data is standardised to a tree density of 250 trees ha^{-1}.

Nutrient analysis of litter and pruned material indicated that Darwin region orchards have between 18.6 kg N ha^{-1} for B74 (Figure C.43b) and 22.2 kg N ha^{-1} for KP (Figure C.43a) falling onto the orchard floor annually. In the Katherine region, the N content of litter varied between 17 kg ha^{-1} for KP (Figure C.43c) and 26.9 kg ha^{-1} for B74 annually (Figure C.43d). Carbon content in the litter and prunings varied in a pattern similar to that of N. In the Darwin region orchards, the carbon content of litter and pruned material in the KP and B74 orchards were similar, being 1.4 tonnes ha^{-1} and 1.2 tonnes ha^{-1} respectively (Figure C.44a, b). In the Katherine region, the KP orchard material contained 1.1 tonnes ha^{-1} (Figure C.44c) and the B74, 1.0 tonne ha^{-1} of carbon (Figure C.44d).

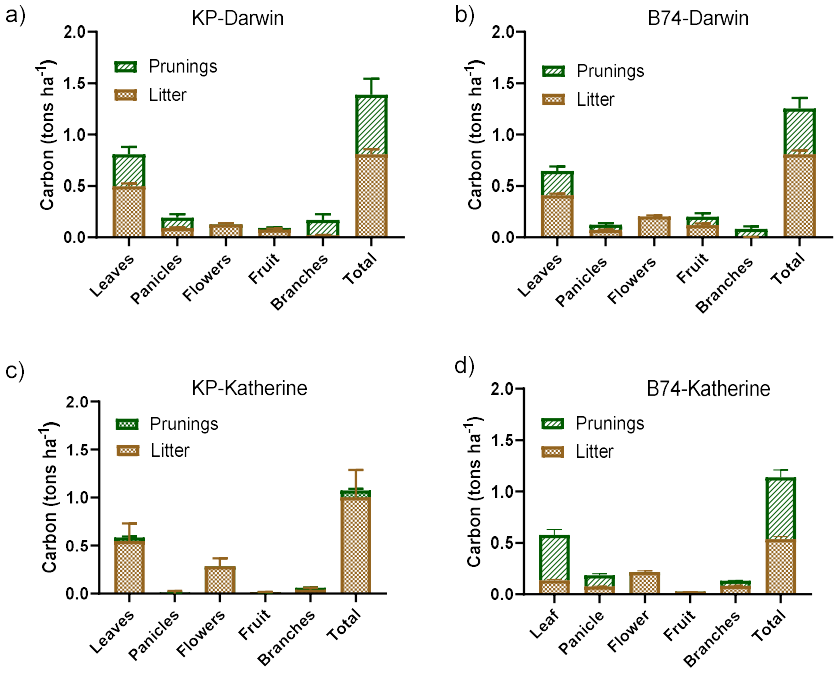


Figure C.44: In the Darwin region orchards, the carbon content of litter and pruned material in the KP orchard was 1.4 tonnes ha^{-1} (a) and in the B74 orchard 1.2 tonnes ha^{-1} (b). In the Katherine region, the KP orchard material contained 1.1 tonnes ha^{-1} of carbon (c) and the B74, 1.0 tonne ha^{-1} of carbon (d). Mean, sem, n=10 collection trays at each site. Data is standardised to a tree density of 250 trees ha^{-1}.

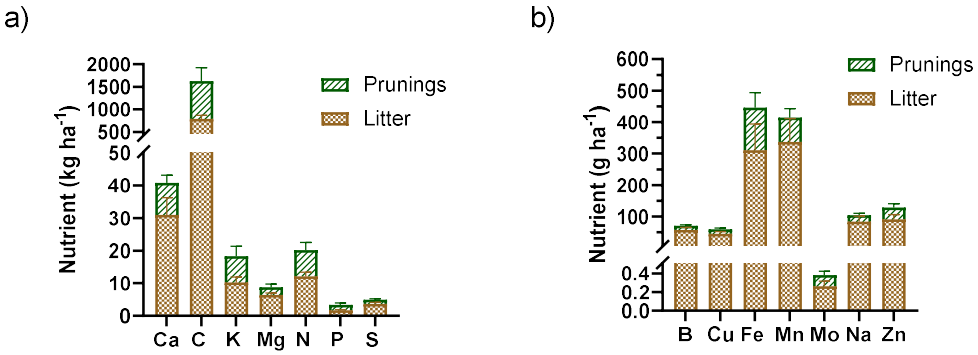


Figure C.45: Litter and pruned materials contained significant quantities of macro- (a) and micronutrients (b). Some will be taken up by roots, or mineralised and recycled within the orchard over time, while others will leach during the wet season or heavy rain events. Nutrient content of litter was estimated from a subset of samples of leaf, flower, panicle and branch litter from the four orchards during flowering and fruit set, n=3 time points for each component, from each orchard.

Other macro- and micronutrients were also cycling annually within the mango orchards (Figure C.45a, b). Over 40 kg ha^{-1} of calcium, 18 kg ha^{-1} of potassium and 8.5 kg ha^{-1} of magnesium are

dropped in tree material onto the orchard floor annually (Figure C.45a). Around 400 g ha^{-1} of iron and manganese and 50–100 g ha^{-1} of boron, copper and zinc were measured in the litter and pruned material (Figure C.45b).

The C/N ratio in leaf litter collected in abscission trays in the four commercial orchards ranged between 83.5 in the Darwin B74 orchard and 98.6 in the Darwin KP orchard (Table C.7). In contrast, the measured C/N ratio in leaves from pruned (living) material ranged from 40.6 at the Darwin region KP orchard to 51.6 at the Katherine region KP orchard (Table C.7). Panicle C/N ratios were generally higher than the C/N of leaves, with the exception of the Darwin KP orchard where they were similar. Abscised flowers had the lowest C/N ratio, with a range between 30.3 and 40.1. Abscised branch C/N ratios showed a wider range and standard error, noting that few woody branches abscised from trees outside of pruning events (Table C.7). Only abscised flowers and panicles were analysed for C and N, not attached material.

*Table C.7: The carbon/nitrogen ratio was calculated for litter components collected in the Darwin region 2017–2018 and the Katherine region 2018–2019, as shown in Figures C.44 and C.45. Mean, sem. *Mean only, material collected from 10 trees at each orchard was bulked and subsampled for analysis – refer to Methods.*

		Abscised litter C/N												Pruned litter C/N	
		Leaf	+/-	n	Flower	+/-	n	Panicle	+/-	n	Branch	+/-	n	Leaf*	Branch*
Darwin	KP	98.6	5.0	16	39.6	1.5	9	97.0	4.0	17	95.4	12.2	9	40.6	118.1
Darwin	B74	83.5	4.2	19	30.3	0.4	6	87.8	6.9	15	146.7	18.0	8	46.4	116.4
Katherine	KP	96.1	3.3	7	38.9	3.3	7	100.5	3.2	7	105.5	8.6	7	51.6	126.7
Katherine	B74	84.3	4.6	7	40.1	1.3	5	118.5	7.7	6	70.0	0.0	1	47.4	139.1

*mean only

Discussion

Litter and pruned tree material is biological yield rather than economic yield, but it is a resource that is not quantified and does contribute nutrients within an orchard. Most litter dropped to orchard floors between the flowering of the trees and fruit harvest. There was high variability in the quantity of each litter component during the year, reflecting the range and differences in management and harvest practices across commercial orchards over time. We know, for example, in the Darwin region B74 orchard a significant quantity of fruit dropped from trees after harvest (Figure C.41b). This is due to instructions given to pickers by orchard management about the quality of fruit required, which meant more fruit remained on the trees to fall naturally post-harvest. In other orchards, fruit with a wider range of quality were picked and sorted off orchards in the pack houses. Also, the decision to heavily prune the Katherine B74 trees between crops 2018–2019 was illustrated by minimal leaf litter collected as the trees re-grew and re-leafed from December to February (Figure C.41d). The minimal pruning in the Katherine KP orchard (Figure C.42c) meant that trees were impinging on vehicle access between the rows as fruit matured. It is estimated that the quantity of material pruned post-harvest was substantial, probably in excess of 2 tonnes ha^{-1}. The distribution of carbon between litter and prunings was also consistent with the differences in tree management at each orchard for the 2018–19 year. What is interesting overall, is the consistency in the total amount (DW) of material, containing significant quantities of N and other nutrients, that is cycling in orchards on a $tree^{-1}$ basis, regardless of management practices and stage of tree maintenance.

Resorption proficiency (RP) is the level a nutrient is reduced to in senescent leaves, usually expressed as % content on a DW basis. Calculation of the annual N content of the litter components and

prunings showed that % N in leaf litter ranged between 0.4 and 0.7 % on a dry weight basis, a reflection of RP of ~45 % for N in senescent mango leaves (refer to Section C.6). The leaf litter N content in the NT orchards is similar to the 0.7 % N in mango leaf litter collected under un-named varieties in Zimbabwe (Musvoto et al. 2000) and the Palmer variety in Brazil (de Almeida et al. 2014), but lower compared to the 1.5 % N measured in litter collected under an unspecified mango variety in India (Naik et al. 2017). Leaf litter deposited over a year contained 6.0–11 kg N ha^{-1}, with flowers adding a substantial 4.6–7.9 kg N ha^{-1}. Flowers collected in the litter of the orchards contained an average of 1.3 % N, +/- 0.07 (data not shown). It is well known that mangoes flower prolifically, but a very low percentage of those flowers set fruit, with most flowers senescing from panicles for a range of reasons (Pérez et al. 2019). What is surprising is the additive quantity of flowers in litter and its N contribution to nutrient recycling in orchards (Figure C.43). The combined leaf and flower litter N content is similar to that leaving an orchard in 10–15 tonnes of harvested fruit (refer to Section C.3).

Most litter is deposited on orchard floors during the dry season, May–September. It remains relatively inert until 'build-up', break of season rain events increase soil moisture to levels that facilitate the activity of the decomposer communities (García-Palacios et al. 2016; Naik et al. 2017). Despite tropical soils being nutrient-poor, there is evidence that meso- and macrofauna activity (soil fauna ranging in size from small beetles to large cockroaches) augment microfauna population, soil temperature and soil moisture to break down litter in these environments (Parsons et al. 2014; Peguero et al. 2019). Temperatures stay high in the tropics and rainfall, in particular, facilitates litter decomposition, mineralisation of organic matter, then either uptake of nutrients via roots or losses via leaching (Anaya et al. 2012).

Litter decomposes relatively quickly in the wet-dry tropics (Sections C.7, C.8, C.9), but this does not mean that the nutrients are immediately available (Murovhi and Materechera 2015; Musvoto et al. 2000). Work completed within this project by Pandeya et al (2020) confirms that break of season rainfall is the annual starting point for the release of N_2O and decomposition processes of litter on the orchard floor. Interestingly, the release of gaseous N_2O appears to be N-limited where there was no additional N fertiliser applied, but C-limited in the presence of N fertiliser but no litter on the soil. When litter and fertiliser were combined, N_2O losses were minor, with soil leaching and runoff being the likely major N loss pathway (Pandeya et al. 2020).

Resorption efficiency (RE) in leaves is a nutrient conserving mechanism, and is the percent reduction of a nutrient between green and senesced leaves as a percentage of leaf dry weight. The high C/N ratio in leaf litter is a reflection of the RE of N in mango leaves (Section C.6). Mango varieties in the NT, including KP and B74, resorbed between 53 and 62 % of leaf N, while C resorption was close to zero. Therefore the C/N ratios in senesced and abscised leaf litter of 83.5 to 98.6 compared to pruned, living leaves with C/N ratios between 40.6 and 51.6 are as expected (Table C.7). Mango leaf litter collected across the NT commercial orchards have a C/N ratio considered to be at the higher end in both tropical and other environments (Manzoni et al. 2010) which means it is less likely to increase soil organic C (Zhou et al. 2019).

Recent work shows that long-established mango orchards in both the Darwin and Katherine regions have almost identical total N and C pools compared to paired, adjacent, native savanna (Vickery 2019). This means that there has been no alteration in local soil N and C equilibrium in response to

long-term changes in land use, implying climate (temperature and rainfall) and perhaps biotic decomposers are the dominant factors (García-Palacios et al. 2016).

As litter has the potential to harbour mango pathogens, good orchard management and healthy, unstressed trees remain a priority. Orchard hygiene best practices should always be implemented, including the pruning or skirting of trees above the rainfall splash zone, and the cleaning and maintenance of equipment.

Litter and pruned material recycling in orchards enhance nutrient return to the orchard (Asigbaase et al. 2021; Blair 1988) with no additional monetary costs. The nutrients of litter, thus far, have not been included in the annual mango orchard nutrient budgets and can now be incorporated into higher precision planning of fertiliser application in commercial mango orchards.

C.6 Leaf nutrient economics

Introduction

Mangoes are evergreen and maintain their canopy during dry periods, unlike drought-deciduous trees in the wet-dry tropics (Ishida et al. 2006). Resource conservative evergreen plants tend to have long-lived leaves (Corte et al. 2009; Ishida et al. 2006) and, in India, leaf life span for mango trees growing in a range of environments and soils was found to be between 2.7 and 3.9 years. Longer leaf vitality was associated with varieties originating from regions with low soil nutrient availability (Ganeshamurthy and Reddy 2015). Leaf life span can vary widely between and within species, depending on growing conditions (Russo and Kitajima 2016; Eckstein et al. 1999). Similarly, there is wide variation in nutrient resorption from leaves back into trees as leaves senesce (Aerts 1997).

Nutrient resorption from senescing leaves back into trees is well studied in deciduous crops such as peach, cherry (Grassi et al. 2003; Ayala et al. 2014) and apples (Neilsen et al. 2001). It also occurs in evergreen trees, most efficiently in low nutrient environments (Eckstein et al. 1999; Tateno and Takeda 2010; Ishida et al. 2006). This mechanism allows nutrients to be recycled within the tree and stored or utilised according to seasonal demand.

Suggested optimum nitrogen content in mango leaves is variable depending on variety, usually 0.9–1.5 % despite there being no direct link between fruit yield and leaf N (Catchpoole and Bally 1999). As part of understanding nutrient cycling in mango orchard systems of the wet-dry tropics, we examined nutrient resorption in leaves of a range of mango varieties grown on Kensington Pride rootstocks. The questions posed are: 1) what nutrients are resorbed back into the tree as leaves senesce before abscission from the tree; and 2) are there varietal differences in leaf nutrient resorption?

Methods

Leaves were sampled from seven-year-old trees forming part of a randomised complete block trial of current commercial varieties and National Mango Breeding Program (NMBP) selections growing at Katherine Research Station, NT (Figure 46). Five varieties were selected: B74 (Kensington Pride x Sensation), Kensington Pride, 1201 (Irwin x Kensington Pride), 1245 (Irwin x Kensington Pride) and 4069 (Van Dyke x Kensington Pride). All trees were grafted onto Kensington Pride rootstocks and

received the same fertiliser and irrigation management since being planted in July 2012. Each tree in the NMBP trial received 18 g nitrogen, 35 g phosphorous and 44 g K over January and February 2019.

Orchard soils are Tippera, red Kandosol, thin, non-gravelly, clay loam (ASRIS 2011). Soil samples were collected across the orchard and analysed (Table C.8).

Figure C.46: The National Mango Breeding Program trial orchard at Katherine Research Station, 320 km south-east of Darwin, Northern Territory, in the wet-dry tropics, provided leaf material for an assessment of varietal variability in leaf nutrient resorption efficiency and proficiency. Photo from Google Earth 14 January 2021.

Table C.8: Soil samples from the NMBP trial orchard at Katherine Research Station were analysed at CSBP Soil and Plant Analysis Laboratory, Perth, Western Australia. Mean, sem, n=6.

	Units	Mean	± sem
Boron	mg kg^{-1}	90.5	2.5
Calcium	%	2.9	0.1
Chloride	%	0.1	0.0
Copper	mg kg^{-1}	11.1	1.0
Iron	mg kg^{-1}	80.9	5.8
Magnesium	%	0.3	0.0
Manganese	mg kg^{-1}	157.4	6.3
Molybdenum	µg kg^{-1}	585.1	134.8
Phosphorous	%	0.1	0.0
Potassium	%	1.0	0.1
Sulfur	%	0.1	0.0
Zinc	mg kg^{-1}	21.4	0.9
Nitrate	mg kg^{-1}	< 40	-
Total nitrogen	%	0.9	0.1

Leaf samples were collected on a single day in September 2019 during fruit development, from branches that were not fruiting. Vital (mature, green) and senesced (yellow and still attached to a branch) leaves were harvested from five replicate trees. Each sample consisted of 20 leaves collected

from the top and base of the canopy, on the eastern and western sides – five leaves from each aspect. Vital green and senesced leaves were collected from the same branches within the canopy. Leaves were washed in tap water, rinsed thoroughly in Millipore filtered water then oven-dried at 50 °C. Samples were milled using a Rocklabs Standard Ringmill. Soil and plant samples were analysed by CSBP Soil and Plant Analysis Laboratory in Perth, Western Australia for macro- and micronutrient content.

Leaf nutrient resorption efficiency (RE) was calculated for all macro- and micronutrients as the percent reduction of a nutrient between green and senesced leaves as a percentage of leaf dry weight:

*[(nutrient content in green leaves – nutrient content in attached, senesced leaves)/nutrient content in green leaves)] *100*

Leaf resorption proficiency (RP) is the leaf nutrient content of senesced leaves expressed as a percentage of leaf dry weight and was calculated for all nutrients.

Statistical analysis of RE and RP results were completed using one way ANOVA and Tukey's post-test, in Graphpad Prism ® version 9 (https://www.graphpad.com/scientific-software/prism/).

Results

Mean RE of macronutrients in mango leaves showed low variation between varieties. For example, leaf nitrogen RE ranged between 53 % for KP x KP and 67 % for NMBP 4069, a cross of Van Dyke and KP, but were not significantly different (Figure C.47a). Leaf RE mean values for carbon were not significantly different and ranged between 0.6 and 4.8 % (Figure C.47b). The RE of calcium was negative as expected (Figure C.47c), reflecting calcium immobility within the leaves (White and Broadley 2003; Vergutz et al. 2012) and its increasing proportion within the leaf material as other nutrients were resorbed back into the tree. Varietal differences were seen in RE of phosphorous and magnesium. B74 and 4069 showed complete, 100 % RE of phosphorous while the other varieties ranged between 61–65 % (Figure C.47d). The values for RP of phosphorous in B74 senescent leaves were lower than in NMBP 1201 (Figure C.47j), but variability was not as marked as in phosphorous RE. It should be noted that the green leaf phosphorous content was between 0.04 % and 0.08 %, which is below the sufficiency levels (Catchpoole and Bally 1999). Potassium RE showed little varietal difference (Figure C.47e); however, in terms of RP, B74, KP and 4069 resorbed significantly more K than 1201 and 1243 (Figure C.47k). Magnesium RE had varietal differences with 1201 and 1243 having higher efficiencies than the others (Figure C.47f). The RP of the leaf magnesium content of B74 was significantly higher than 1201, 1243 and 4069, but similar to B74 (Figure C.47l). Leaf sulfur RE ranged between 10 % for KP and 18 % for 1243, with no significant differences (data not shown).

Leaf micronutrients displayed less variability in RE and RP than macronutrients (Figures C.47, C.48). The only difference noted was that KP had a lower manganese RE than 4069 (Figure C.48d).

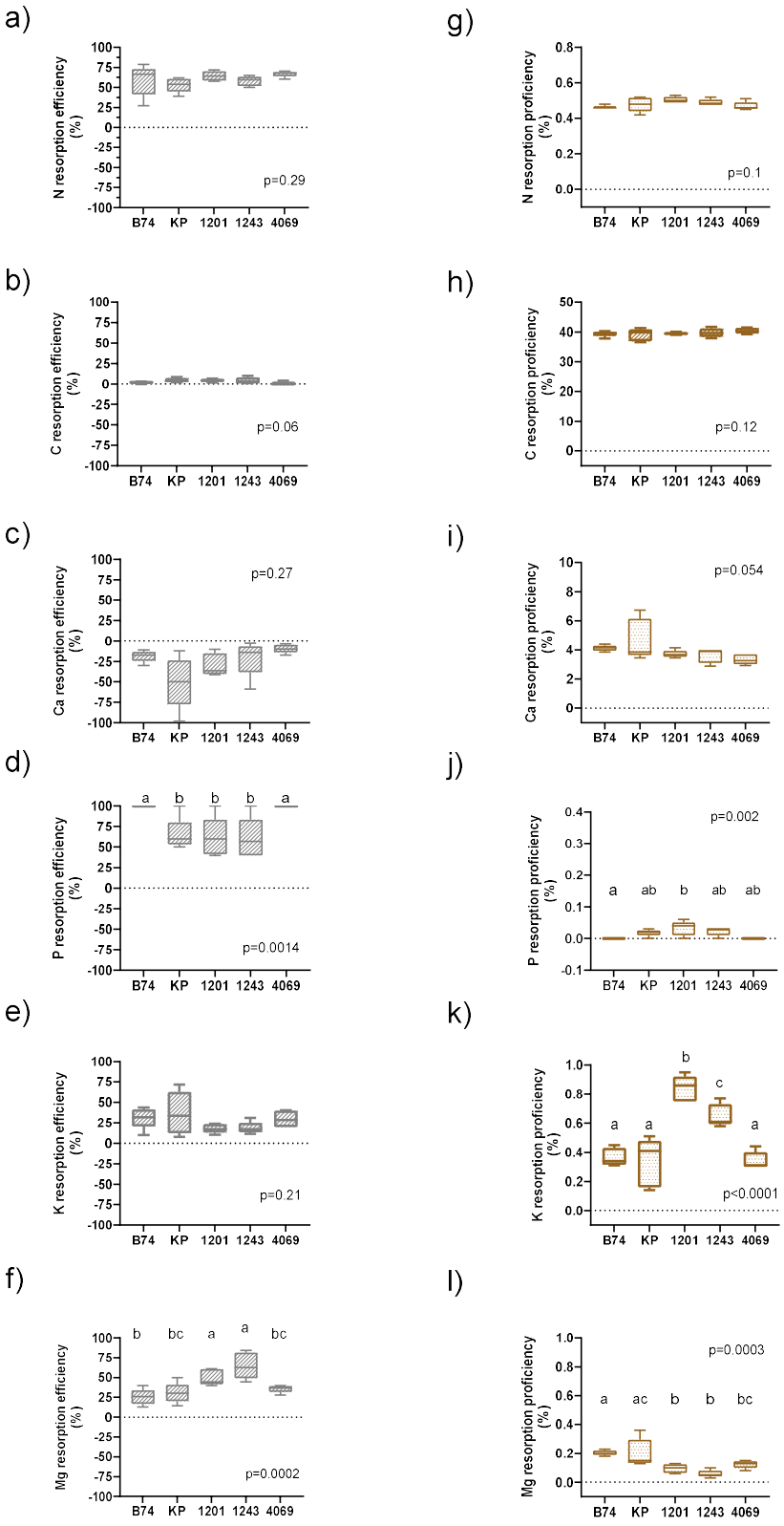


Figure C.47: Macronutrient resorption efficiency (a–f) and resorption proficiency (g–l) of green and senescent leaves from mango tree varieties. Significant differences between varieties were observed in phosphorous (d) and magnesium (f) resorption efficiencies. Also significant were phosphorous (j), potassium (k) and magnesium (l) resorption proficiencies. ANOVA, p<0.05, Tukey's post-test with letters indicating similarities and differences, n=5 trees sampled. Data are shown as boxplots with median, interquartile box and range (whiskers).

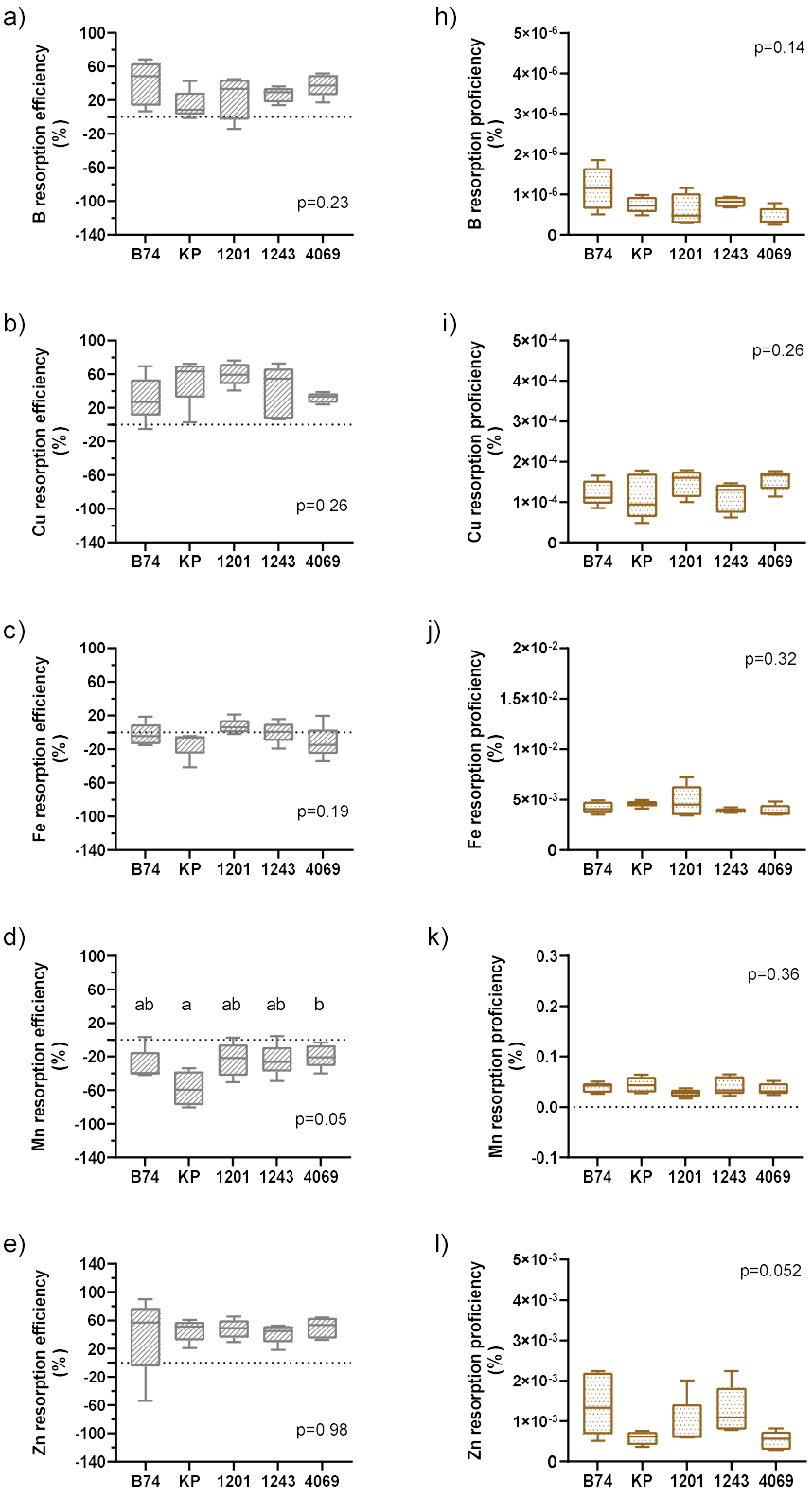


Figure C.48: The micronutrient resorption efficiency (a–e) and resorption proficiency (h–l) of green and senescent leaves were generally consistent between mango tree varieties. The exception was manganese (d) resorption efficiency, where KP was significantly lower than NMBP 4069. ANOVA, p<0.05, Tukey's post-test with letters indicating similarities and differences, n=5 trees sampled. Data are shown as boxplots with median, interquartile box and range (whiskers).

Discussion

Resorption of nutrients from leaves back into plants for re-use is highly efficient and a desirable characteristic for commercial fruit crops. It is an active process, but the energy costs of nutrient resorption compared to active uptake of nutrients from other sources, such as via the roots, are currently unknown (Brant and Chen 2015). Recent work on seven Indian mango varieties grown across a range of climates and soil types found N RE in leaves varied between 42.0 % and 46.5 % (Ganeshamurthy and Reddy 2015). All varieties analysed in this orchard had higher RE, between 53 % and 67 % with NMBP 4069 the highest. Possible reasons for a difference in RE could be an adaptive environmental response (Menge et al. 2011) or an inherited attribute.

All varieties in this work have KP as a parent, which is an early Australian variety identified in Queensland in the 1880s (Johnson 2000). KP is polyembryonic, unlike the monoembryonic Indian mango varieties, suggesting it is a cross between an Indian and a polyembryonic South East Asian type (Olano et al. 2005). The KP x KP cross had the lowest N RE, and NMBP 4069, a Van Dyke x KP cross, the highest. Van Dyke is a cultivar selected in the Florida, USA group of mango varieties prior to 1940. Early molecular work suggests a heritage including Haden, Terpentine 10 and Mulgoba which was imported into Florida from India (Olano et al. 2005). The first complete mango genome was recently published (Bally et al. 2021) and, while the focus so far has been on identifying quantitative trait loci (QTL) for fruit size, skin colour, canopy habit and other quality characteristics, there is now scope to use the mango mapping populations on hand to consider attributes such as RE, RP, water use efficiency, flowering at higher temperature and other abiotic stress tolerances, and identify the associated genes.

In terms of an adaptive environmental response, variability in RE from leaves back into plants can also be related to nutrient availability or constraint (Yuan and Chen 2015; Vergutz et al. 2012; Killingbeck 1996), although this is not certain (Aerts 1997; Yuan and Chen 2015; Brant and Chen 2015). Also, it has been noted that leaf RE of N (Kazakou et al. 2007) reduces as leaf nutrient content increases with the application of fertilisers, and nitrate resorption efficiency and uptake efficiency are higher when trees are growing under low nitrate conditions (Havé et al. 2016; Nacry et al. 2013). Leaf sampling in this instance occurred during fruit development, after green leaf N concentrations reach their annual minimum N content at flowering and fruit set, but were still at or above the recommended minimum levels of 0.8 % (Catchpoole and Bally 1999). The measured high values for N RE were likely connected to: a) fruit acting as a strong sink for N; b) the tree recycling N from readily available sources within the tree to the fruit; and c) low nutrient availability in soils with previously applied fertilisers leached out of range of active roots.

Soils in the wet-dry tropics of Australia have very low naturally occurring P, mainly due to the leaching effect of monsoon rainfall (Kooyman et al. 2017). Also, among Australian plant species it has been found that the older the soil they grow on, the concentration of leaf P is lower and RE of leaf P increases (Hayes et al. 2014). It is not known if this impacts on introduced tree species such as mango; however, it has been demonstrated that canola, wheat and white lupin grown in chronosequenced soils also have similar leaf P reduction and increased RE (Laliberté et al. 2013). The green leaves of all varieties had a P content below the recommended pre-flowering concentration of 0.12–1.30 % (Catchpoole and Bally 1999). Varieties B74 and NMBP 4069 resorbed 100 % of leaf P content back into the tree, significantly more than KP, NMBP 1201 and 1243 which reflects mean global estimates for evergreen leaf RE at 61–65 % (Vergutz et al. 2012). B74 and 4069 also had the

lowest green leaf P content, 0.04–0.05 %. Plants tend to resorb and recycle P more efficiently in low P environments, and the relatively high % RE for both N and P in mango leaves may reflect that growth is being limited by both N and P (Chen et al. 2010) despite applications of N and P during the preceding wet season.

Ca transport in plants is mostly apoplastic and connected to water uptake and transpiration. Symplastic Ca movement is thought to be minimal and, once translocated into cells, Ca functions as an osmoticum in vacuoles and cell signalling processes (Gilliham et al. 2011; McLaughlin and Wimmer 1999). It is well documented as an essential element in fruit development and cell wall stability (Hocking et al. 2016). The low mobility of Ca is reflected in the negative values for RE and less than 4 % RP in leaves.

K is essential for multiple plant processes such as protein, carbohydrate and ATP production, photosynthesis and enzyme activation (Marschner 2012) and is applied to soil and as a foliar spray in most commercial orchards. Compared to other evergreen trees growing in tropical environments which have a mean K RE of 52 %, mango leaf K RE was a low 18–37 % (Vergutz et al. 2012). NMBP 1201 and 1243 maintained higher K RP in senesced leaves, meaning more of their leaf K would become a component of litter on the orchard floor rather than be resorbed.

C.7 Soil N fixation and gaseous losses in mango orchard soils

Refer to:

Combined effect of nitrogen fertiliser and leaf litter carbon drive nitrous oxide emissions in tropical soils (Pandeya et al. 2020)

Summary

Intensification of agriculture in the tropics is likely to increase reactive N losses in the form of nitrous oxide (N_2O), but drivers of emissions from tropical soils remain poorly understood. This study investigated the effect of leaf litter and urea fertiliser on N_2O emissions from two Australian tropical mango orchards. Treatments included urea (25 g N m^{-2}), leaf litter (1,500 g m^{-2} dry matter), their combined application, and untreated control. Up to 80.5 ± 8.4 mg N_2O-N m^{-2} (N in the form of N_2O m^{-2}) were lost within two weeks of treatment application, accounting for more than 60 % of annual emissions m^{-2}. Indirect emissions of 30 mg N_2O-N m^{-2} per day were recorded at one site, potentially from groundwater. The highest annual N_2O emissions were observed from litter+urea with 130.4 mg N_2O-N m^{-2} per year, exceeding those from urea-only by a factor of two and those from litter-only by a factor of four. This resulted in residue and fertiliser emission factors (EF) of 0.01–0.37 %, well below the IPCC and Tier2 defaults, with whole orchard losses equivalent to 0.19–0.66 kg N_2O–N ha^{-1}. The findings suggest N_2O is N-limited when no N fertiliser is applied, even when applying large amounts of litter-N, and by carbon (C) in the absence of litter. The combined effect of litter and urea on N_2O emissions surpassed the effect of the sole application of litter and urea, demonstrating critical interaction between both substrates. This interaction effect needs to be considered when developing management strategies aimed at increasing soil C in tropical soils.

C.8 Leaf litter decomposition dynamics in tropical soils: the effect of N fertilisation and precipitation

Refer to:

Leaf litter decomposition dynamics in tropical soils: the effect of N fertilisation and precipitation (Pandeya et al. 2021, submitted).

Summary

Leaf litter in tropical orchards is a source of C and nutrients, potentially contributing to plant productivity, soil health and C storage in highly weathered tropical soils. However, the fate of leaf litter C and the interaction of its decomposition with fertiliser N in the high rainfall zone of the tropics remains largely unknown. This field study investigated litter decomposition and carbon dioxide (CO_2) emissions in two Australian tropical mango orchards established on tropical savannah soils across four treatments: Urea (250 kg N ha^{-1}), leaf litter (15 t ha^{-1} dry matter), their combined application, and an untreated control. Litter mass loss and CO_2 emissions showed strong seasonality, occurring mainly during the wet season. Litter+urea increased CO_2 emissions compared to the control, but litter decomposition and CO_2 emissions did not differ from litter-only. Intense rainfall probably washed fertiliser N out of the litter layer, where N remained limiting for decomposition. Litter decomposition differed between sites, demonstrating that increased rainfall at the coastal site promoted C substrate release and higher emissions of CO_2 as compared to the inland site with less rainfall. The overall litter C balance showed the effect of tropical conditions on decomposition rates, with up to 90 % of litter C emitted as CO_2. These findings are exemplary for the fate of litter C inputs to highly weathered savannah soils and show limited potential to build soil organic C by increasing C inputs to improve soil health in these tropical orchard soils.

C.9 Carbon and nitrogen flux dynamics in highly weathered tropical soils: interaction effect of leaf litter and fertiliser

Refer to:

Pandeya, H R. (2021) PhD thesis, submitted.

Summary

In recent decades, tropical land-use change by deforestation into agricultural systems has caused a rapid depletion of soil organic carbon (SOC), with the highly weathered, light-textured soils, such as those found in tropical savannahs particularly vulnerable to fertility loss. In tropical regions of Australia, mango fruit orchards are under expansion in savannah soils. Current practices during mango production in this region comprise non-evidence-based inorganic N fertiliser applications with little emphasis or thought on 'soil health'. Moreover, ecosystem function, such as nutrient cycling in mango orchards and the role that mango leaf litter plays as an added source of C and N on different pathways of nutrient supply and losses, remains unexplored in tropical Australia. Since northern Australia experiences environmental extremities and poorly structured soils, the lack of reliable information on reactive N (N_r) losses after inorganic N addition and the impact litter management

can have on loss minimisation further constrains our understanding of these highly weathered tropical soils.

This study, through the sole and combined use of leaf litter and inorganic N fertiliser, allowed evaluation of N and C release pathways, with research-outputs broadening our current understanding of seasonal net N transformations, N_r loss pathways (N_2O emissions, NO_3^--N leaching), decomposition dynamics and overall SOC balance associated with both inputs in highly weathered tropical orchard soils.

Findings demonstrate that net N mineralisation rates and N_r losses (N_2O emissions and potential NO_3^--N leaching) were substantially high early in the wet season following N application and during the dry-wet transition period due to high substrate availability in soil mineral N pool, with a subsequent reduction in the dry season. The application of litter and inorganic N fertiliser resulted in EF of <0.01–0.37 %, well below the IPCC (Tier 1) and Tier 2 defaults of 1.0 % and 0.85 %, respectively, with whole orchard losses equivalent to 0.19–0.66 kg N_2O-N ha^{-1} per year. The low N_2O EF reflects limitations of these highly weathered tropical soils by low cation exchange capacity (CEC) in general and both C and N since short residence time of labile C and N is likely to limit substrate for denitrifiers, even after a combined application of litter and inorganic N fertiliser. Importantly, comparing the drivers of N_2O production via denitrification from the sole application of inorganic N fertiliser suggests the emissions are limited by the lack of available C. However, the positive interaction between a combined application of litter and inorganic N fertiliser suggests that greater availability of litter C overcomes this limitation, promoting N_2O emissions directly by either providing an energy source for denitrifiers and/or indirectly by stimulating microbial activity to create high biological O_2 demand resulting in soil anaerobic conditions.

Leaf litter decomposition generally followed an exponential decay model and showed clear seasonal changes across sites corresponding to two distinct phases: an initial rapid phase where labile plant components were rapidly leached from the litter during the wet season, and a slower phase in the dry season where more recalcitrant litter components remain as they were more difficult to decompose. Of the annual total mass loss, >90 % of the dry matter loss occurred during the wet season, compared with the minimal mass loss in the dry season. This pattern of mass loss led to a seasonal release of C and N from decomposing mango litter over 12 months, with 52–74 % of initial N and 70–84 % of initial C at both sites released by the end of the wet season.

Climate was the dominant driver of litter and soil organic matter decomposition and associated CO_2 emissions from tropical mango orchard soils; however, soil application of inorganic N fertiliser did not influence either. Over 12 months, C input from the litter was sufficient to outweigh increased CO_2 fluxes, meaning an overall positive soil C balance (~40 % of the initial litter-C accrual for potential sequestration) at the site receiving lower rainfall (inland site) compared to the overall neutral C balance at the higher rainfall site (coastal site). While retaining litter is important to maintain soil quality in the long term, this study demonstrates the limited potential for C sequestration by increasing C inputs into highly weathered tropical orchard soils receiving high seasonal rainfall. This is due to a greater chance of CO_2 fluxes being released during microbial catabolism of fresh litter and native SOC. Abiotic factors (moisture and temperature) likely play a significant role in the initial period of litter decomposition, causing rapid leaching of soluble components from the litter. However, biotic factors (macro-faunal activity) govern the latter stages of litter decomposition

through fragmentation of litter. Current best N management practices must consider the judicious application of litter and inorganic N fertiliser to maintain the optimum plant-available mineral N in the orchard soils with a minimal adverse effect on the environment.

C.10 Preparing mango growers for precision agriculture technologies

Refer to:

Rahman M, Robson A, Bristow M (2018) Exploring the potential of high resolution WorldView-3 imagery for estimating yield of mango. *Remote Sensing* 10, 1866. https://doi.org/10.3390/rs10121866

Summary

Pre-harvest yield estimation of mango fruit is important for the optimisation of inputs and other resources on the farm. The current industry practice of visual counting of the fruit on a small number of trees for yield forecasting can be highly inaccurate due to the spatial variability, especially if the trees selected do not represent the entire crop. Therefore, this study evaluated the potential of high-resolution WorldView-3 (WV3) satellite imagery to estimate the yield of mango by integrating both geometric (tree crown area, TCA) and optical (spectral vegetation indices) data using an artificial neural network (ANN) model. WV3 images were acquired in 2016–17 and 2017–18 growing seasons at the early fruit stage from three orchards in the Acacia Hills region, NT, Australia. Stratified sampling technique (SST) was applied to select 18 trees from each orchard and subsequently ground-truthed for yield (kg tree^{-1}) and fruit number tree^{-1}. For each sampled tree, spectral reflectance data and TCA was extracted from WV3 imagery. The TCA was identified as the most important predictor of both fruit yield (kg tree^{-1}) and fruit number, followed by normalised difference vegetation index (NDVI) red-edge band when all trees from three orchards in two growing seasons were combined. The results of all sampled trees from three orchards in two growing seasons using the ANN model produced a strong correlation (r^2=0.70 and 0.68 for total fruit yield (kg tree^{-1}) and fruit number, respectively), which suggests that the model can predict yield on a regional level. On the orchard level also, the ANN model produced a high correlation when both growing seasons were combined. However, the model developed in one season could not be applied in another season due to the influence of seasonal variation and canopy conditions. Using the relationship derived from the measured yield parameters against combined vegetation indices and TCA data, the total fruit yield (t·ha^{-1}) and fruit number were estimated for each orchard. This produced 7 % underestimation to less than 1 % overestimation. The accuracy of the findings showed the potential of WV3 imagery to better predict the yield parameters than the current practice across the mango industry as well as to quantify lost yield as a result of the delayed harvest.

C.11 Conclusions

Precision agriculture

Remote sensing and image analysis is a broad research area, now being applied to agricultural crops for diverse reasons including plant health, precision application of fertilisers and crop yield estimation. An important aspect of this work is collecting ground-level data to establish real links

with the imaging data (ground-truthing). The work described has established the relationships between the TCA, yield and fruit number tree^{-1}, so modelling to predict yield is possible and has the potential to be improved for accuracy and industry use. This satellite image analysis collaboration is ongoing, with expanded real data collection underway.

Soils and litter

The savannah soil types of the NT where mangoes are grown include Kandosols, Rudosols, Chromosols and Hydrosols (Isbell and NCST 2021; Pandeya 2021). Due to soil age, temperatures and rainfall in the tropical region, all soil types have little structure, are highly leached and tend to be nutrient deficient.

A recent comparison of soils in natural bush sites with those in adjacent, long-established commercial orchards found that despite increasing organic matter and nutrients in orchard soils, there had been no increase in soil organic carbon content over time (Vickery 2019). The work of Pandeya et al (2020) found that litter decomposed rapidly in tropical conditions in the Darwin and Katherine mango growing regions, with over 90 % of litter mass lost each year over the wet season. Up to 74 % of N in litter and 84 % of C in litter was released over the wet season, with the climate being the dominant factor in decomposition rate (Pandeya 2021). About 90 % of the decomposing litter C was emitted as CO_2. The Darwin region orchard soils maintained a neutral C balance, while the Katherine region orchards had a positive C balance over a year with some potential to sequester C in the long term. Intense rainfall events leached available N from the soil litter layer, and the decomposition process was likely to be N limited

Mineralisation of litter N is also significant, with ~55 % of the N (~11 kg ha^{-1}) mineralised in the top 20 cm of soil in Darwin region orchards annually. In the lower rainfall Katherine region, this increases to 85 % of litter N (~17 kg ha^{-1}) mineralised in the top 20 cm of soil. Litter N cycling provides a short term 'bank' of nutrients available for uptake during the build-up, break of season rain events, which continues as litter decomposes over the wet season. It is, however, subject to leaching and erosion which is not quantified as yet, but indications are that most available N is lost annually during the wet season.

An investigation of N_2O gas emissions in response to applications of urea on soil and leaf litter combinations found that greenhouse gas emissions were well below Tier 2 recommendations of the IPCC guidelines for this form of agriculture (IPCC 2006).

Commercial mango orchards drop large quantities of litter and pruned material onto orchard floors annually, including ~20 kg N ha^{-1}. We now know that this is a major source of mineralised N in the top 20 cm of soil where tree feeder roots can access it, particularly associated with break of season rainfall. Litter is a biological yield, but it also has an economic value in providing up to half of the tree annual N requirements.

The resorption efficiency (RE) of N in leaves of the evergreen mango growing in the NT is high (53–67 %), but not as high as P (60–100 %). The high RE of P reflects the NT's low P soil environment. The other litter nutrient of interest is Ca, which is close to immobile once it has entered cells in leaves, and has a resorption proficiency (RP) of less than 4 %. It is estimated that the annual litter in orchards contains ~40 kg ha^{-1} of Ca that could potentially be released during litter decomposition (Blair 1988), but this has not been investigated.

A trend of reducing total tree N content, which paralleled a reduction in the recovery of infused ^{15}N over a season, posed interesting questions. How much N is lost in root exudates over a year, is it a stress response under dry season conditions, and does tree N content recover quickly once break of season rains initiate rapid uptake of soil available N? This aspect of N cycling and use in commercial orchards is ready for further research.

It is known that the currently available enhanced efficiency fertilisers in their various forms and coatings are unsuited for tropical application. This is because the coatings break down rapidly in the high temperatures and humidity, negating any potential for cost benefits of the slow-release coatings. The problem with prill coatings disintegrating into polluting microplastics is being addressed by the industry and eco-friendly coatings developed, but whether they are useful in tropical climates is untested so far.

Instead of enhanced efficiency fertilisers, alternative additions were investigated. Soils that have poor physiochemical properties can be amended to improve their nutrient retention capacity. Laboratory-based work using soil from the Darwin region and simulated rainfall events found that natural zeolites mixed into the topsoil of the sample had the highest potential to retain NO_3^- over 100 days compared to the other tested amendments which included biochar and leaf litter (refer to Appendix D). Soil with no surface amendments maintained the most NH_4^+, compared to the others tested. There is more work to be done to establish an affordable, efficient amendment in tropical commercial mango orchards, but these results are encouraging.

Foliar application of dilute solutions of KNO_3 is commonly applied at flowering and fruit set, with the assumption that the K supply maximises flowering and fruit retention. A 2 % KNO_3 solution applied across an orchard contributes around 6 kg N ha^{-1} to the N budget. Between 27 % and 44 % of the N applied to leaves can be taken up across the cuticle over 1–2 days depending on the variety. NMBP 1201 and NMBP 1243 have the least and NMBP 4069 the most efficient uptake. KP and B74 are mid-range. These N uptake efficiency (NUE) values compare favourably with soil NUE, but there is extra value with foliar application. It is usually applied in the dry season, and remnant solution is likely to stay on the leaves until washed off by rain, be diluted by dew and drip onto the litter below, or the leaves drop and become litter. In this way, there are two opportunities for the N to be taken up into the tree before wet season rains. It is additional N in the litter that contributes to rates of decomposition.

The rapidity of N uptake in leaves and how quickly infused N is thoroughly assimilated into all tree tissues is notable. Several weeks after being infused into the transpiration stream of trees, labelled N was passively and actively transported into all tissues via xylem and phloem in that time. Then, xylem sap maintained an even concentration of labelled N across the season while the actual N content varied as expected. This evidence suggests that foliar application of N can be an option in tropical mango orchards at any time of the year where no rain is anticipated for 48 hours.

Fruit yield and quality

There is not a strong link between applied N and yield. The disparate yields over two years in a Katherine region KP orchard highlighted this where the yield in 2019 was double that of 2018, with the same N application both years. Fruit stayed the same size but there was double the number, and double the amount of N left the orchard in harvested fruit. Apart from yield, the main difference was

that in 2018 with yields of ~20 tonnes ha^{-1}, fruit from trees that received 25 kg N ha^{-1} showed minor skin colour differences and fruit from trees receiving 50 kg N ha^{-1} had skin with major colour differences when ripening. In this orchard, with these N applications and yield, 'stay green' skin was identified as a quality problem. This quality defect, where skin stays green or has green blotches, is not visible when the fruit is harvested at the mature green stage. This was not reproducible with the same trees and double the yield in 2019.

Foliar N uptake and resorption

There may be a link between N applied and yield if a mango tree is deficient in N content, but this was not observed. The lowest fruit N content in this project was measured in fruit harvested from four-year-old trees that had received no N since initial establishment, apart from an infusion of N into the transpiration stream equivalent to 0.015 % of the tree total N of ~600 g. Fruit N was 0.4 % on a FW basis, compared to 0.8–1.0 % for fruit collected in orchards with an N application history.

It is estimated that across sites, harvested fruit removed ~10–25 % of tree total N content off the farm. This will vary according to year, yield and management practices, but it is a starting point in assessing what N inputs an orchard will need to prepare for the following season. On a harvested FW basis, mango contains a relatively low concentration of N, similar to that of apple, 0.9 kg $tonne^{-1}$ FW. This is significantly lower than peach, pear, orange and kiwi-fruit (Carranca et al. 2018).

The results from this project have quantified N uptake, N cycling, N inputs and losses in commercial mango orchards in the wet-dry tropics of the NT. From this work, N application recommendations can be reconsidered.

C.12 Development of 'best practice' nitrogen management in mango

Mango orchard systems are similar to other cropping systems in that 40–60 % of N inputs are lost to the environment. N is highly mobile and can be applied and taken up by trees at most times over a year in one form or another. Visible N deficiency in mangoes is likely to reflect a longer-term lack of N and a degree of soil nutrient mining. Thus, routine soil and leaf sampling assessments should continue as they indicate a range of nutrient deficiencies and excesses. They can also be used to assess whether the N application decisions being made are reasonable.

Times to be avoided are during the wet season to reduce losses of mineralised N and the period of quiescence or dormancy prior to flowering to avoid more foliage growth during the flowering induction period. Foliar application gives trees opportunities to take up the N directly into leaves and remnant N falls to the ground with rainfall or dew to the canopy drip line for feeder root uptake, or on litter where it aids decomposition and associated mineralisation of N in the orchard topsoil.

Taking the results from this project and other work into account, the following recommendations are made in the four R context.

Right time

Soil-applied N

- Apply to soil or fertigate immediately post-harvest (October to December) while the tree is actively growing. This takes advantage of break of season rains for immediate uptake, but before available N is flushed out of range of roots. Avoid applying during monsoon periods or when soil is waterlogged.
- Alternatively, consider the end of the wet season when growth is still active, soils are moist and before the last rains. This is difficult to time correctly, and risks late leaf flushes (perhaps at the expense of flowering) and a strong reliance on applications of paclobutrazol and foliar N to manage and mature the vegetative shoots.
- Split applications can be considered.

Post-harvest, tree N is significantly depleted with trees losing a significant portion of their N content in harvested fruit and litter (including flowers, which have a high N content). At this time, trees are entering an active growth phase which requires N.

The dry-to-wet-season transition period and the beginning of the wet season is when high levels of N mineralisation in orchard topsoils were measured, concurrent with break of season rainfall events.

N mineralised from leaf litter provides ~11 kg N ha^{-1} in the top 20 cm of soil in the Darwin region, and ~17 kg N ha^{-1} in the Katherine region. Feeder roots of trees can access this layer of soil and rain will facilitate uptake.

Soil-applied and litter-supplied N are available for rapid uptake at this time of the year, before nutrient leaching monsoon rains begin.

Fertigation

- This method can be used through most of the year, but should cease during the dormant or quiescent period to allow shoots to mature prior to flowering. Do not apply N to soils as the flowering induction period approaches.
- This is a convenient method to split N into smaller, more frequent applications to match tree needs and reduce N losses.
- Do not fertigate waterlogged soils.

Smaller applications of soil-applied fertiliser resulted in higher NUE. This implies that uptake of smaller applications of N via fertigation would follow the same trend; however, fertigation NUE was not trialled in this work.

Foliar application

- Conventionally prepared as a dilute solution of KNO_3 with a surfactant, it is usually applied before flowering induction and may continue periodically during the flowering and fruit set period. It needs to be prepared at a low concentration that will not damage leaves, usually 1-4 % KNO_3.
- This method can be used to apply N at any time of the year unless rain is expected in the following 24-48 hours.

Quantitative measurements of foliar uptake of N in mangoes show that between 27 and 44 % of the N applied to leaves is taken up across the cuticle over 1–2 days, depending on variety. NMBP 1201 and NMBP 1243 have the least efficient uptake and NMBP 4069 the most efficient uptake. KP and Calypso® are mid-range. These NUE compare favourably with soil NUE, but there is extra value with foliar application. It is usually applied in the dry season, and remnant solution is likely to stay on the leaves until washed off by rain, diluted by dew and falls onto the litter below, or the leaves drop and become litter. In this way there are two opportunities for the N to be taken up into the tree. Additional N in litter can contribute to increasing the rate of litter decomposition.

Right form

Commercially available fertilisers are recommended.

- In this work, ammonium sulfate [$(NH_4)2SO_4$] and urea [$(CO(NH_2)_2$] were used for soil, litter and infusion trials, and KNO3 for foliar application.
- Minimal N_2O emissions were measured with decomposing litter and/or $CO(NH_2)_2$ applied to soils.
- Preliminary work on soil amendments found that zeolite mixed with topsoil maintained water and nutrient content better than the other combinations tested. Biochar mixed with topsoil also performed well.
- There were no studies comparing the performance of different forms of enhanced efficiency fertilisers as they are considered unsuitable for the wet-dry tropical climate.

Soil amendments are likely to have a place in NT mango orchard management if an affordable, available form is found.

Right place

Placement or delivery of fertiliser will vary according to the method of application.

- Soil-applied fertiliser should be placed on soil under the drip line of the canopy, where tree feeder roots can easily access it. Avoid placing close to tree stems.
- Fertigation will depend on the orchard irrigation in place and the water/solution pressure within the system. Ideally, sprinklers would throw the solutions under canopies to deliver fertiliser evenly across tree feeder roots.
- Foliar applications should be made using spray equipment that is correctly calibrated to deliver the desired volume of N to each tree across the orchard.

Placement of N was not specifically researched in this project.

Right amount

This will vary according to location, soils, seasonal conditions and yield. It assumes that litter and pruned material are left on the orchard floor. Information to consider includes:

- Most available N is lost from soils annually between the break of season rains and the end of the wet season.
- Litter contains up to 20 kg N ha^{-1} in a commercial orchard annually. Decomposition of litter in response to rain events releases ~11 kg of available N ha^{-1} in the top 20 cm of soil in the

Darwin region each season and ~17 kg N ha^{-1} in the Katherine region. These amounts cycle each year. What is not taken up by trees is effectively lost.

- Mangoes in managed orchards contain ~0.8–1 kg N $tonne^{-1}$ of fruit harvested. This amount of N leaves the property and needs to be replaced. Trees receiving no N inputs over four years had fruit with 0.4 kg N $tonne^{-1}$ of fruit harvested.
- Growers need an understanding of the relationship between yield, excess N application and 'stay green' skin on ripe mangoes in their orchard.
    - For example, at a commercial KP orchard, with a yield of 20 tonnes fruit ha^{-1} and 250 trees ha^{-1}, fruit from trees receiving 25 kg N ha^{-1} had blotchy green skin when ripe, and at 50 kg N ha^{-1} the fruit stayed green when ripe. Fruit from trees receiving 12.5 kg N ha^{-1} or 50 g $tree^{-1}$ ripened normally.
    - There were no differences in yield, number of fruit, % dry matter (% DM), or fruit N content in response to 0, 12.5, 25 or 50 kg N applied ha^{-1}.
    - The same N rates applied after that harvest generated yields the following year that approached 40 tonnes ha^{-1}, and no fruit ripened with 'stay green' skin. The N content in fruit did not vary in response to applied N levels. Fruit numbers increased, fruit size was the same and there was no yield response to the range of N levels applied.
- How much N a maturing mango tree takes up from soil-applied fertiliser is linked to how much is applied.
    - In our work, ~75 % of 5 kg N ha^{-1} was taken up.
    - 35 % of 10 kg N ha^{-1} and 20 % of 15 kg N ha^{-1} was taken up.
    - The NUE reduced as more N was applied.
- Avoid applying 'insurance' N. Most of it will be lost and inaccessible to trees.
- Additional N can be applied via fertigation at most times, but avoid when soils are waterlogged, during monsoon rainfall and the period of quiescence or dormancy prior to flower induction.
- Foliar application of N can occur at any time in clear weather.

C.13 References

Aerts R (1997) Nitrogen partitioning between resorption and decomposition pathways: a trade-off between nitrogen use efficiency and litter decomposibility? Oikos 80 (3):603-606. doi:10.2307/3546636

AMIA (2016) Mango industry quality standards. Australian Mango Industry Association. https://www.industry.mangoes.net.au/cmsb/uploads/mg15002-2016-mango-industry-quality-standards.pdf. Accessed 11 May 2021

AMIA (2018) Understanding crop nutrition. A guide for Australian mango growers.

Anaya CA, Jaramillo VJ, Martínez-Yrízar A, García-Oliva F (2012) Large rainfall pulses control litter decomposition in a tropical dry forest: evidence from an 8-year study. Ecosystems 15 (4):652-663. doi:10.1007/s10021-012-9537-z

Anderson N (2017) Forecasting of the mango crop: quantity and quality. Masters thesis. Central Queensland University,

Anderson NT, Subedi PP, Walsh KB (2017) Manipulation of mango fruit dry matter content to improve eating quality. Scientia Horticulturae 226:316-321. doi:https://doi.org/10.1016/j.scienta.2017.09.001

Asigbaase M, Dawoe E, Sjogersten S, Lomax BH (2021) Decomposition and nutrient mineralisation of leaf litter in smallholder cocoa agroforests: a comparison of organic and conventional farms in Ghana. Journal of Soils and Sediments 21 (2):1010-1023. doi:10.1007/s11368-020-02844-4

Asis CA, Alexander T, Sarkhosh A, Umar M, McConchie C (2020) Optimising foliar nitrogen uptake of mango: effect of adjuvant, leaf position and time of potassium nitrate spray. ActaHortic 1299:269-274

ASRIS (2011) Soil investigation-Katherine Research Station 2011. Australian Soil Resource Information System. http://www.asris.csiro.au/mapping/hyperdocs/NatSoil/703%5EKRS_11%5E1.pdf. Accessed 14 January 2021

Ayala M, Bañados P, Thielemann M, Toro R (2014) Distribution and recycling of canopy nitrogen storage reserves in sweet cherry (*Prunus avium*) fruiting branches following ^{15}N-urea foliar applications after harvest. Ciencia e Investigación Agraria 41:71-80

Bally I (2009) Crop production: Mineral nutrition. . In: Litz RE (ed) The mango: botany, production and uses. CABI, Cambridge, UK,

Bally ISE, Bombarely A, Chambers AH, Cohen Y, Dillon NL, Innes DJ, Islas-Osuna MA, Kuhn DN, Mueller LA, Ophir R, Rambani A, Sherman A, Yan H, Mango Genome C (2021) The 'Tommy Atkins' mango genome reveals candidate genes for fruit quality. BMC Plant Biology 21 (1):108. doi:10.1186/s12870-021-02858-1

Barry CS, Giovannoni JJ (2007) Ethylene and fruit ripening. Journal of Plant Growth Regulation 26 (2):143. doi:10.1007/s00344-007-9002-y

Blaikie S, Cavenagh P (2003) Operation KP - a production guide for mango growers in northern Australia. TropHort, NTDBIRD, CSIRO, CDU and NTHA, Northern Territory

Blaikie SJ, Leonardi J, Chacko EK, Muller WJ, Scott NS (2000) Effect of cincturing and chemical treatments on growth, flowering and yield of mango, *Mangifera indica* cv. Kensington Pride. Australian Journal of Experimental Agriculture 39 (6):761-770. doi:https://doi.org/10.1071/EA98178

Blair JM (1988) Nutrient release from decomposing foliar litter of three tree species with special reference to calcium, magnesium and potassium dynamics. Plant and Soil 110 (1):49-55. doi:10.1007/BF02143538

BOM Available via Bureau of Meteorology, Australian Government. http://www.bom.gov.au/climate/data/?ref=ftr. 2020

Brann M (2020) Northern Territory confirmed as mango king, producing 52 percent of national crop. Australian Broadcasting Commission. https://www.abc.net.au/news/rural/2020-05-14/northern-territory-claims-title-of-biggest-mango-producer/12246604. Accessed 15 May 2020 2020

Brant AN, Chen HYH (2015) Patterns and mechanisms of nutrient resorption in plants. Critical Reviews in Plant Sciences 34 (5):471-486. doi:10.1080/07352689.2015.1078611

Bueno A, Sancho-Knapik D, Gil-Pelegrín E, Leide J, Peguero-Pina JJ, Burghardt M, Riederer M (2019) Cuticular wax coverage and its transpiration barrier properties in *Quercus coccifera* L. leaves: does the environment matter? Tree Physiology 40 (7):827-840. doi:10.1093/treephys/tpz110

Carranca C, Brunetto G, Tagliavini M (2018) Nitrogen nutrition of fruit trees to reconcile productivity and environmental concerns. Plants (Basel) 7 (1):4. doi:10.3390/plants7010004

Catchpoole D, Bally I (1999) Mango nutritional investigation. Horticulture Research and Development Corporation, New South Wales

Chacko EK, Reddy YTN, Ananthanarayanan TV (1982) Studies on the relationship between leaf number and area and fruit development in mango (*Mangifera indica* L.). Journal of Horticultural Science 57 (4):483-492. doi:10.1080/00221589.1982.11515082

Chapin III FS (1980) The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11 (1):233-260. doi:10.1146/annurev.es.11.110180.001313

Chen Y, Randerson JT, Van der Werf GR, Morton DC, Mu M, Kasibhatla PS (2010) Nitrogen deposition in tropical forests from savanna and deforestation fires. Global Change Biology 16 (7):2024-2038. doi:10.1111/j.1365-2486.2009.02156.x

Clonan M, McConchie C, Hall M, Hearnden M, Olesen T, Sarkhosh A (2021) Effects of ambient temperatures on floral initiation in Australian mango (*Mangifera indica* L.) selections. Scientia Horticulturae 276:109767. doi:https://doi.org/10.1016/j.scienta.2020.109767

Corte GN, Macchiaverni P, Dal Fabbro IM, Haddad CRB (2009) Nitrogen availability, leaf life span and nitrogen conservation mechanisms in leaves of tropical trees. Scientia Agricola 66:812-818

Coskun D, Britto DT, Shi W, Kronzucker HJ (2017) How plant root exudates shape the nitrogen cycle. Trends in Plant Science 22 (8):661-673. doi:https://doi.org/10.1016/j.tplants.2017.05.004

Crane JH, Salazar-Garcia S, Lin TS, de Queiros AC, Shu ZH (2009) Crop production: Management. In: Litz RE (ed) The mango: botany, production and uses. CABI Cambridge, UK,

de Almeida CX, Pita Junior JL, Rozane DE, de Souza HA, Hernandes A, Natale W, Farraudo A (2014) Nutrient cycling in mango trees. Ciências Agrárias 35 (1):259-256. doi:DOI: 10.5433/1679-0359.2014v35n1p259

de Freitas ST, Mitcham EJ (2012) Factors Involved in Fruit Calcium Deficiency Disorders. In: Horticultural Reviews. pp 107-146. doi:https://doi.org/10.1002/9781118351871.ch3

Diczbalis Y, Wicks C, Owens G (2006) Mango Irrigation Management Guidelines. Agnote No. 587. Northern Territory Government, Darwin

Eckstein RL, Karlsson PS, Weih M (1999) Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. New Phytologist 143 (1):177-189. doi:undefined

Eichert T, Fernandez V (2012) Uptake and release of mineral elements by leaves and other aerial plant parts. In: Marchner P (ed) Marschner's mineral nutrition of higher plants. 3rd edn. Elsevier, pp 71-84. doi:10.1016/B978-0-12-384905-2.00004-2

Eichert T, Kurtz A, Steiner U, Goldbach HE (2008) Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. Physiologia Plantarum 134 (1):151-160. doi:https://doi.org/10.1111/j.1399-3054.2008.01135.x

Erisman JW, Sutton MA, Galloway J, Klimont Z, Winiwarter W (2008) How a century of ammonia synthesis changed the world. Nature Geoscience 1 (10):636-639. doi:10.1038/ngeo325

Fernández V, Bahamonde HA, Javier Peguero-Pina J, Gil-Pelegrín E, Sancho-Knapik D, Gil L, Goldbach HE, Eichert T (2017) Physico-chemical properties of plant cuticles and their functional and ecological significance. Journal of Experimental Botany 68 (19):5293-5306. doi:10.1093/jxb/erx302

Fernández V, Eichert T (2009) Uptake of hydrophilic solutes through plant leaves: Current state of knowledge and perspectives of foliar fertilization. Critical Reviews in Plant Sciences 28 (1-2):36-68. doi:10.1080/07352680902743069

Fletcher A, Rennenberg H, Schmidt S (2009) Nitrogen partitioning in orchard-grown *Macadamia integrifolia*. Tree Physiology 30 (2):244-256. doi:10.1093/treephys/tpp107

Forlani S, Masiero S, Mizzotti C (2019) Fruit ripening: the role of hormones, cell wall modifications, and their relationship with pathogens. Journal of Experimental Botany 70 (11):2993-3006. doi:10.1093/jxb/erz112

Ganeshamurthy AN, Reddy YTN (2015) Fitness of mango for colonization in low fertility soils and dry lands: Examination of leaf life-span, leaf nutrient resorption, and nutrient use efficiency in elite mango varieties. Agricultural Research 4 (3):254-260. doi:10.1007/s40003-015-0164-8

García-Palacios P, McKie BG, Handa IT, Frainer A, Hättenschwiler S (2016) The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes. Functional Ecology 30 (5):819-829. doi:https://doi.org/10.1111/1365-2435.12589

Gilliham M, Dayod M, Hocking BJ, Xu B, Conn SJ, Kaiser BN, Leigh RA, Tyerman SD (2011) Calcium delivery and storage in plant leaves: exploring the link with water flow. Journal of Experimental Botany 62 (7):2233-2250. doi:10.1093/jxb/err111

Graphpad Prism®. 9.0 edn. GraphPad Software, San Diego, California USA

Grassi G, Millard P, Gioacchini P, Tagliavini M (2003) Recycling of nitrogen in the xylem of *Prunus avium* trees starts when spring remobilization of internal reserves declines. Tree Physiology 23 (15):1061-1068. doi:10.1093/treephys/23.15.1061

Havé M, Marmagne A, Chardon F, Masclaux-Daubresse C (2016) Nitrogen remobilization during leaf senescence: lessons from Arabidopsis to crops. Journal of Experimental Botany 68 (10):2513-2529. doi:10.1093/jxb/erw365

Hayes P, Turner BL, Lambers H, Laliberté E (2014) Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. Journal of Ecology 102 (2):396-410. doi:https://doi.org/10.1111/1365-2745.12196

He Q, Rezai T (2020) Measuring the passive cuticular membrane permeability of potassium with a parallel artificial membrane permeability assay and the implications for foliar nutrient formulations. Chemical and Biological Technologies in Agriculture 7 (1):4. doi:10.1186/s40538-019-0166-z

Hernández Delgado PM, Aranguren M, Reig C, Fernández Galván D, Mesejo C, Martínez Fuentes A, Galán Saúco V, Agustí M (2011) Phenological growth stages of mango (*Mangifera indica* L.) according to the BBCH scale. Scientia Horticulturae 130 (3):536-540. doi:https://doi.org/10.1016/j.scienta.2011.07.027

Hocking B, Tyerman SD, Burton RA, Gilliham M (2016) Fruit calcium: Transport and physiology. Frontiers in Plant Science 7 (APR2016). doi:10.3389/fpls.2016.00569

Holmes R, Hofman P, Barker L (2009) Mango assessment quality manual. The State of Queensland,

Huang X-Y, Salt David E (2016) Plant ionomics: From elemental profiling to environmental adaptation. Molecular Plant 9 (6):787-797. doi:https://doi.org/10.1016/j.molp.2016.05.003

Huett DO, Vimpany I (2006) An evaluation of foliar nitrogen and zinc applications to macadamia. Australian Journal of Experimental Agriculture 46 (10):1373-1378. doi:http://dx.doi.org/10.1071/EA05165

IAEA (2001) Use of isotope and radiation methods in soil and water management and crop nutrition – Training course series No. 14. . International Atomic Energy Agency Austria

IPCC (2006) 2006 IPCC Guidelines for national greenhouse gas inventories. Agriculture, forestry and other land use., vol 4.

IRRI (2019) STAR-Statistical tool for agricultural research., vol 2.0.1.

Isbell RF, NCST (2021) The Australian soil classification. Third edn. National Committee on Soil and Terrain, CSIRO Publishing, Melbourne,

Ishida A, Diloksumpun S, Ladpala P, Staporn D, Panuthai S, Gamo M, Yazaki K, Ishizuka M, Puangchit L (2006) Contrasting seasonal leaf habits of canopy trees between tropical dry-deciduous and evergreen forests in Thailand. Tree Physiology 26 (5):643-656. doi:10.1093/treephys/26.5.643

Johnson G (2000) Introduction of the mango to Australia, vol 109. Royal Society of Queensland. doi:10.3316/ielapa.906536410591585

Karlowsky S, Augusti A, Ingrisch J, Akanda MKU, Bahn M, Gleixner G (2018) Drought-induced accumulation of root exudates supports post-drought recovery of microbes in mountain grassland. Frontiers in Plant Science 9 (1593). doi:10.3389/fpls.2018.01593

Kazakou E, Garnier E, Navas M-L, Roumet C, Collin C, Laurent G (2007) Components of nutrient residence time and the leaf economics spectrum in species from Mediterranean old-fields differing in successional status. Functional Ecology 21 (2):235-245. doi:https://doi.org/10.1111/j.1365-2435.2006.01242.x

Kerstiens G (1996) Cuticular water permeability and its physiological significance. Journal of Experimental Botany 47 (12):1813-1832. doi:10.1093/jxb/47.12.1813

Killingbeck KT (1996) Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency. Ecology 77 (6):1716

Kooyman RM, Laffan SW, Westoby M (2017) The incidence of low phosphorus soils in Australia. Plant and Soil 412 (1-2):143-150. doi:http://dx.doi.org/10.1007/s11104-016-3057-0

Krauss P, Markstädter C, Riederer M (1997) Attenuation of UV radiation by plant cuticles from woody species. Plant, Cell & Environment 20 (8):1079-1085. doi:https://doi.org/10.1111/j.1365-3040.1997.tb00684.x

Laliberté E, Turner BL, Zemunik G, Wyrwoll K-H, Pearse SJ, Lambers H (2013) Nutrient limitation along the Jurien Bay dune chronosequence: response to Uren & Parsons (). Journal of Ecology 101 (5):1088-1092. doi:https://doi.org/10.1111/1365-2745.12123

Laliberté E, Tylianakis JM (2012) Cascading effects of long-term land-use changes on plant traits and ecosystem functioning. Ecology 93 (1):145-155. doi:https://doi.org/10.1890/11-0338.1

Lassaletta L, Billen G, Grizzetti B, Garnier J, Leach AM, Galloway JN (2014) Food and feed trade as a driver in the global nitrogen cycle: 50-year trends. Biogeochemistry 118 (1):225-241. doi:10.1007/s10533-013-9923-4

Lawrence BT, Melgar JC (2018) Variable fall climate influences nutrient resorption and reserve storage in young peach trees. Frontiers in Plant Science 9 (1819). doi:10.3389/fpls.2018.01819

Léchaudel M, Génard M, Lescourret F, Urban L, Jannoyer M (2005a) Modeling effects of weather and source–sink relationships on mango fruit growth. Tree Physiology 25 (5):583-597. doi:10.1093/treephys/25.5.583

Léchaudel M, Joas J, Caro Y, Génard M, Jannoyer M (2005b) Leaf:fruit ratio and irrigation supply affect seasonal changes in minerals, organic acids and sugars of mango fruit. Journal of the Science of Food and Agriculture 85 (2):251-260. doi:https://doi.org/10.1002/jsfa.1968

Ledger S, Barker L, Hofman P, Campbell J, Jones V, Holmes R, Campbell T, Weinert M (2012) Mango ripening manual.

Leger S, Campbell T, Holmes R, Kernot I, Hofman P (2003) Improving knowledge and practices in mango supply chains. Paper presented at the Australasian Postharvest Horticulture Conference, Brisbane,

Levin A (2018) Improving fertiliser and water use efficiency in mango cultivation. Achieving sustainable cultivation of mangoes. Burleigh Dodds Science Publishing. doi:https://doi.org/10.4324/9781351114431

Likens GE, Keene WC, Miller JM, Galloway JN (1987) Chemistry of precipitation from a remote, terrestrial site in Australia. Journal of Geophysical Research: Atmospheres 92 (D11):13299-13314. doi:https://doi.org/10.1029/JD092iD11p13299

Liu J, Ma K, Ciais P, Polasky S (2016) Reducing human nitrogen use for food production. Scientific Reports 6 (1):30104. doi:10.1038/srep30104

Liu T, Ren T, White PJ, Cong R, Lu J (2018) Storage nitrogen co-ordinates leaf expansion and photosynthetic capacity in winter oilseed rape. Journal of Experimental Botany 69 (12):2995-3007. doi:10.1093/jxb/ery134

Lovatt CJ (1999) Timing citrus and avocado foliar nutrient applications to increase fruit set and size. HortTechnology 9 (4):607. doi:10.21273/horttech.9.4.607

Lovatt CJ (2013) Properly timing foliar-applied fertilizers increases efficacy: A review and update on timing foliar nutrient applications to citrus and avocado. HortTechnology 23 (5):536-541. doi:10.21273/horttech.23.5.536

Lu P, Chacko EK, Bithell SL, Schaper H, Wiebel J, Cole S, Müller WJ (2012) Photosynthesis and stomatal conductance of five mango cultivars in the seasonally wet and dry tropics of northern Australia. Scientia Horticulturae 138:108-119. doi:http://dx.doi.org/10.1016/j.scienta.2012.02.019

Luo Z, Kong X, Dai J, Dong H (2015) Soil plus foliar nitrogen application increases cotton growth and salinity tolerance. Journal of Plant Nutrition 38 (3):443-455. doi:10.1080/01904167.2014.912324

Majumdar M, Sanyal SK, Dutta SK, Satyanarayana T, Singh VK (2016) Nutrient mining: addressing the challenges to soil resources and food security. Biofortification of Food Crops. New Delhi, India. doi:https://doi.org/10.1007/978-81-322-2716-8_14

Manzoni S, Trofymow JA, Jackson RB, Porporato A (2010) Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecological Monographs 80 (1):89-106. doi:https://doi.org/10.1890/09-0179.1

Marschner P (2012) Marschner's mineral nutrition of higher plants. Third edn. Academic Press,

McGuire R (1992) Reporting of objective color measurements. Horticultural Science 27:1254-1255

McLaughlin SB, Wimmer R (1999) Tansley Review No. 104. Calcium physiology and terrestrial ecosystem processes. New Phytologist 142 (3):373-417. doi:https://doi.org/10.1046/j.1469-8137.1999.00420.x

Menge DNL, Ballantyne F, Weitz JS (2011) Dynamics of nutrient uptake strategies: lessons from the tortoise and the hare. Theoretical Ecology 4 (2):163-177. doi:10.1007/s12080-010-0110-0

Millard P, Grelet G-a (2010) Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. Tree Physiology 30 (9):1083-1095. doi:10.1093/treephys/tpq042

Mueller ND, Gerber JS, Johnston M, Ray DK, Ramankutty N, Foley JA (2012) Closing yield gaps through nutrient and water management. Nature 490 (7419):254-257. doi:10.1038/nature11420

Murovhi NR, Materechera SA (2015) Decomposition of subtropical fruit tree leaf litter at Nelspruit, South Africa. Communications in Soil Science and Plant Analysis 46 (7):859-872. doi:10.1080/00103624.2015.1011750

Musvoto C, Campbell BM, Kirchmann H (2000) Decomposition and nutrient release from mango and miombo woodland litter in Zimbabwe. Soil Biology and Biochemistry 32 (8):1111-1119. doi:https://doi.org/10.1016/S0038-0717(00)00023-7

Nacry P, Bouguyon E, Gojon A (2013) Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant and Soil 370 (1):1-29. doi:10.1007/s11104-013-1645-9

Naik SK, Maurya S, Mukherjee D, Singh AK, Bhatt BP (2017) Rates of decomposition and nutrient mineralization of leaf litter from different orchards under hot and dry sub-humid climate. Archives of Agronomy and Soil Science 64 (4):560-573. doi:10.1080/03650340.2017.1362104

Nair R, Weatherall A, Perks M, Mencuccini M, Näsholm T (2014) Stem injection of $^{15}N–NH_4NO_3$ into mature Sitka spruce (*Picea sitchensis*). Tree Physiology 34 (10):1130-1140. doi:10.1093/treephys/tpu084

Nasreen S, Kamal AM, Siddiky MA, Rannu RP, Islam MS (2014) Effect of nitrogen, phosphorus, potassium and sulfur on the yield of mango. Bangladesh Journal of Agricultural Research 39:631-640

Neilsen D, Millard P, Neilsen GH, Hogue EJ (2001) Nitrogen uptake, efficiency of use, and partitioning for growth in young apple trees. Journal of the American Society for Horticultural Science 126 (1):144. doi:10.21273/jashs.126.1.144

Nguyen H, Hofman P, Holmes R, Bally I, Stubbings B, McConchie R (2004) Effect of nitrogen on the skin colour and other quality attributes of ripe Kensington Pride mango (*Mangifera indic*a L.) fruit. The Journal of Horticultural Science and Biotechnology 79 (2):204-210. doi:10.1080/14620316.2004.11511749

Nufarm (2006) LI 700 Surfactant. Give your crop the advantage.

Nutrien AS (2018) Loveland Industries LI 700 Surfactant. Loveland Products. https://www.lovelandproducts.com/sites/default/files/pdf/sellsheets/LI_700.pdf. Accessed 10 March 2021

Olano C, Schnell R, Quintanilla WE, Campbell R (2005) Pedigree analysis of Florida mango cultivars. Proceedings of the Florida State Horticultural Society 118:192-197

Oosthuyse SA (1993a) Effect of spray application of KNO_3, urea and growth regulators on the yield of Tommy Atkins mango. In: South African Mango Grower's Association Yearbook, vol 13. pp 58-62

Oosthuyse SA (1993b) Ripening behaviour and quality on ripening of Sensation mango in relation to cold-storage temperature and tree fertilisation. In: South Africa Mango Grower's Association Yearbook, vol 13. pp 67-75

Pandeya HR (2021) Carbon and nitrogen flux dynamics in highly weathered tropical soils: Interaction effect of leaf litter and fertiliser. PhD thesis

Pandeya HR, Friedl J, De Rosa D, Asis C, Tilbrook J, Scheer C, Bristow M, Grace PR, Rowlings DW (2020) Combined effect of nitrogen fertiliser and leaf litter carbon drive nitrous oxide emissions in tropical soils. Nutrient Cycling in Agroecosystems 118 (2):207-222. doi:10.1007/s10705-020-10094-6

Parsons SA, Congdon RA, Lawler IR (2014) Determinants of the pathways of litter chemical decomposition in a tropical region. New Phytologist 203 (3):873-882. doi:https://doi.org/10.1111/nph.12852

Patrick JW, Zhang W, Tyerman SD, Offler CE, Walker NA (2001) Role of membrane transport in phloem translocation of assimilates and water. Functional Plant Biology 28 (7):697-709. doi:https://doi.org/10.1071/PP01023

Peguero G, Sardans J, Asensio D, Fernández-Martínez M, Gargallo-Garriga A, Grau O, Llusià J, Margalef O, Márquez L, Ogaya R, Urbina I, Courtois EA, Stahl C, Van Langenhove L, Verryckt LT, Richter A, Janssens IA, Josep P (2019) Nutrient scarcity strengthens soil fauna control over leaf litter decomposition in tropical rainforests. Proceedings of the Royal Society of Britain 130020191300 286:20191300. doi:http://doi.org/10.1098/rspb.2019.1300

Pérez V, Herrero M, Hormaza JI (2019) Different factors involved in the low fruit set of mango (*Mangifera indica*). Acta Horticulturae 1231:43-48. doi:10.17660/ActaHortic.2019.1231.8

Protacio CM A model for potassium nitrate-induced flowering in mango. In, 2000. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp 545-552. doi:10.17660/ActaHortic.2000.509.62

Raymond L, Schaffer B, Brecht JK, Crane JH (1998) Internal breakdown in mango fruit: symptomology and histology of jelly seed, soft nose and stem-end cavity. Postharvest Biology and Technology 13 (1):59-70. doi:https://doi.org/10.1016/S0925-5214(97)00074-4

Russo SE, Kitajima K (2016) The ecophysiology of leaf lifespan in tropical forests: Adaptive and plastic responses to environmental heterogeneity. Tropical Tree Physiology, vol 6. Springer, Cham. doi:doi.org/10.1007/978-3-319-27422-5_17

Serrano M, Coluccia F, Torres M, L'Haridon F, Métraux J-P (2014) The cuticle and plant defense to pathogens. Frontiers in Plant Science 5 (274):5293-5306. doi:10.3389/fpls.2014.00274

Silcox S, Holloway PJ (1986) A simple method for the removal of folar deposits of agrochemicals using cellulose acetate film stripping. Aspects of Applied Biology 11:13-17

Slattery WJ, Conyers MK, Aitken RL (1999) Soil pH, aluminium, manganese and lime requirement. Soil analysis, an interpretation manual. CSIRO Publishing, Australia

Smith S, Hill J (2011) Supporting sustainable development-risks and impacts of plant industries on soil condition.

Stassen PJC, Janse van Vuuren BPH Seasonal uptake and utilization of nitrogen by "Sensation" mango trees. In, 1997. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp 351-358. doi:10.17660/ActaHortic.1997.455.45

Stassen PJC, Mostert PG, Smith B (1999) Mango tree nutrition. A crop perspective. Neltropika Bulletin (January):41-51

Subedi PP, Walsh KB, Owens G (2007) Prediction of mango eating quality at harvest using short-wave near infrared spectrometry. Postharvest Biology and Technology 43 (3):326-334. doi:https://doi.org/10.1016/j.postharvbio.2006.09.012

Sudha R, Balamohan, T. N., & Soorianathasundaram, K. (2012) Effect of foliar spray of nitrogenous chemicals on flowering, fruit set and yield in mango (*Mangifera indica* l.) cv. Alphonso. Journal of Horticultural Sciences 7:190-193

Taiz L, Zeiger E (1991) Plant physiology. Benjamin Cummings, California

Tanou G, Ziogas V, Molassiotis A (2017) Foliar nutrition, biostimulants and prime-like dynamics in fruit tree physiology: New insights on an old topic. Frontiers in Plant Science 8 (75). doi:10.3389/fpls.2017.00075

Tateno R, Takeda H (2010) Nitrogen uptake and nitrogen use efficiency above and below ground along a topographic gradient of soil nitrogen availability. Oecologia 163 (3):793-804. doi:10.1007/s00442-009-1561-0

Tegeder M, Masclaux-Daubresse C (2018) Source and sink mechanisms of nitrogen transport and use. New Phytologist 217 (1):35-53. doi:https://doi.org/10.1111/nph.14876

Thorburn PJ, Biggs JS, Webster AJ, Biggs IM (2011) An improved way to determine nitrogen fertiliser requirements of sugarcane crops to meet global environmental challenges. Plant and Soil 339 (1):51-67. doi:10.1007/s11104-010-0406-2

Urban L, Alphonsout L (2007) Girdling decreases photosynthetic electron fluxes and induces sustained photoprotection in mango leaves. Tree Physiology 27 (3):345-352. doi:10.1093/treephys/27.3.345

Urban L, Jegouzo L, Damour G, Vandame M, Francois C (2008) Interpreting the decrease in leaf photosynthesis during flowering in mango. Tree Physiol 28 (7):1025-1036. doi:10.1093/treephys/28.7.1025

Van Bel AJE (2003) The phloem, a miracle of ingenuity. Plant, Cell & Environment 26 (1):125-149. doi:https://doi.org/10.1046/j.1365-3040.2003.00963.x

Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecological Monographs 82 (2):205-220. doi:10.1890/11-0416.1

Vickery BS (2019) The limit to soil organic carbon sequestration in tropical soils. Honours, Queensland University of Technology,

Wang Z, Fan Z, Zhao Q, Wang M, Ran J, Huang H, Niklas KJ (2018) Global data analysis shows that soil nutrient levels dominate foliar nutrient resorption efficiency in herbaceous species. Frontiers in Plant Science 9:1431. doi:10.3389/fpls.2018.01431

Weinbaum SA, Johnson RS, DeJong TM (1992) Causes and Consequences of Overfertilization in Orchards. HortTechnology 2 (1):112-132. doi:10.21273/HORTTECH.2.1.112b

White PJ, Broadley M (2003) Calcium in plants. Annals of Botany 92 (4):487-511. doi:10.1093/aob/mcg164

White PJ, Brown PH (2010) Plant nutrition for sustainable development and global health. Annals of Botany 105 (7):1073-1080. doi:10.1093/aob/mcq085

Williams A, de Vries FT (2020) Plant root exudation under drought: implications for ecosystem functioning. New Phytologist 225 (5):1899-1905. doi:https://doi.org/10.1111/nph.16223

Winston T (undated) Understanding crop nutrition. https://www.horticulture.com.au/globalassets/hort-innovation/resource-assets/mg15006-understanding-crop-nutrition-mango.pdf Accessed 24 June 2021

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428 (6985):821-827. doi:10.1038/nature02403

Yuan Z, Chen HYH (2009) Global trends in senesced-leaf nitrogen and phosphorus. Global Ecology and Biogeography 18 (5):532-542. doi:10.1111/j.1466-8238.2009.00474.x

Yuan ZY, Chen HYH (2015) Negative effects of fertilization on plant nutrient resorption. Ecology 96 (2):373-380. doi:https://doi.org/10.1890/14-0140.1

Zhou G, Xu S, Ciais P, Manzoni S, Fang J, Yu G, Tang X, Zhou P, Wang W, Yan J, Wang G, Ma K, Li S, Du S, Han S, Ma Y, Zhang D, Liu J, Liu S, Chu G, Zhang Q, Li Y, Huang W, Ren H, Lu X, Chen X (2019) Climate and litter C/N ratio constrain soil organic carbon accumulation. National Science Review 6 (4):746-757. doi:10.1093/nsr/nwz045

Appendix D: Amendments to improve nitrogen retention in horticultural soils of the Northern Territory

D.1 Background and rationale

Despite being heavily cultivated, Australia's ancient and weathered soils have quite poor nutrient-retention capacities (Thorburn et al. 2011). Large areas of Australia's Northern Territory (NT) are extensively cultivated for mango horticulture. Mango production in the NT alone contributes over 50 % of the total domestic industry, worth $300 million (AMIA, 2020).

Nitrogen (N) is essential to mango production, governing tree growth, and fruit production and quality. Plant-available N is mainly supplemented through the use of synthetic N fertilisers. However, due to often unfavourable soil, climatic and management conditions, a large portion of N that is applied to soils is not utilised by crops. In most agricultural systems, nitrogen uptake efficiency (NUE) is often below 50 % (Ladha et al. 2005). N that is not utilised is lost to the environment, primarily through processes including nitrate leaching, denitrification (resulting in nitrous oxide emissions) and ammonia volatilisation (Bell et al. 2016). Because of the growing costs of poor NUE, both environmental and financial, improving N retention has become one of the most important mandates in agriculture.

For soils with poor physiochemical properties, amendments are often used in an attempt to increase the nutrient-retention capacities of soils or just increase the amount of plant-available N retained. Biochar and hydrochar are amendments both of which are produced through the pyrolysis of organic material (although hydrochar is formed through hydrothermal carbonisation). Both are carbon-rich and past studies show increased biomass production and nutrient retention under these ameliorants (McHenry, 2009). Zeolites, both natural and synthetic are aluminosilicate minerals with high adsorption capacities that can increase N retention (Sepaskhah & Yousefi, 2007; Jakkula & Wani, 2018). Plant residue or mulch is also commonly used as a means to increase soil organic matter and soil fertility. While all these ameliorants and their potential to improve agricultural productivity have been studied individually, their application in Australia remains limited, and only a small number of studies have directly compared the effects of a variety of amendments on N-fertilised soils.

Research questions

The specific research questions being answered through this laboratory incubation were:

- Is leaching of inorganic N reduced in amended soils?
- Are nitrous oxide (N_2O) emissions increased under amendment application?
- Is N that is not leached through the soil lost through a different pathway (i.e. denitrification)?
- Is there a change in soil physiochemical properties as a result of amendments?
- What is the effect of these amendments on N retention? What is the net effect of leaching and nitrous oxide loss pathways

Methodology

Soils used in the laboratory incubation were from the Coastal Plains Research Station in Middle Point, NT. The soil is classified as a red Kandosol under the Australian Soil Classification system, with a sandy loam texture. 20 kg of loose soil from this site was used to repack soil cores to a target bulk density of 1.3 g cm^{-3}. Eighteen intact cores remaining from a previous study were incorporated as well and used to test surface applications of biochar and zeolite. Zeolite, hydrochar and biochar were also tested as an amendment incorporated into the top 10 cm of soil cores (Table D.1). This was achieved by mixing the amendment into the soil while the cores were being packed.

Table D.1: Sampling repetition plan.

Amendment	Soil core	Application	Replicates	Destructive samples	Day 100 cores for gas sampling
Control (urea only)	Repacked	surface	10	day 7 = 3, day 35 = 3, day 100 = 4	4
Biochar + urea	Intact core	surface	9	day 7 = 3, day 35 = 3, day 100 = 3	3
	Repacked	topsoil mixing	10	day 7 = 3, day 35 = 3, day 100 = 4	4
Zeolite + urea	Intact core	surface	9	day 7 = 3, day 35 = 3, day 100 = 3	3
	Repacked	topsoil mixing	10	day 7 = 3, day 35 = 3, day 100 = 4	4
Mulch + urea	Repacked	surface	10	day 7 = 3, day 35 = 3, day 100 = 4	4
Hydrochar + urea	Repacked	topsoil mixing	10	day 7 = 3, day 35 = 3, day 100 = 4	4

The rate of applications was based on kg ha^{-1} rates commonly used in commercial settings (Table D.2).

Table D.2: Soil amendment application rates and corresponding sources.

Amendment	Application	Commercial application rate	Amount per core	Total quantity	Reference
Mulch/litter	Surface	~1 kg ha^{-1}	0.17 g	~2 g	Kumar et al. (2015)
Biochar	Surface	~10 kg ha^{-1}	1.74 g	~20 g	Scheer et al. (2011)
	Topsoil mixing				
Hydrochar (BLP+B180 1:1)	Topsoil mixing	~10 kg ha^{-1}	1.74 g	~20 g	n/a
Natural zeolites	Surface	~100 kg ha^{-1} (upper limit)	17.35 g	~195 g	Akbar Nakhli et al. (2017)
	Topsoil mixing				

Urea fertiliser	Dilute with water and apply	50 kg ha^{-1}	0.1 g/100 mL	~7 g	Ngo & Owens (2002)

Treatment application

Urea: the fertiliser application rate used here matches the current industry standard for northern Australian mango growers: 300 g per mango tree. A technical report from the NT Department of Business, Industry and Resource Development (Ngo & Owens, 2002) advises mango producers that 166 mango trees per hectare allows for adequate spacing between trees; therefore, the amount of fertiliser is roughly 50 kg N ha^{-1}.

Biochar: Scheer et al. (2011) used a biochar application rate of 10 t ha^{-1} when studying the effect of biochar on greenhouse gas exchanges between soil and atmosphere. The same application rate is used here. It is recommended that in order to improve soil fertility – specifically cation exchange capacity (CEC) and water-holding capacity – biochar be mechanically mixed into the topsoil, rather than just applied to the surface (Lehmann & Joseph, 2009).

Zeolite: Akbar Nakhli et al. (2017) summarises various studies using natural and amended zeolites, where application rates range from 0.1 tonne ha^{-1} to 50 tonnes ha^{-1}. By starting at the upper limit of zeolite application rates (~100 tonnes ha^{-1}), the limits of the effectiveness of zeolites as a nutrient-retention amendment can be more easily observed, rather than beginning at the lower limit rate and seeing little or no effect.

Mulch: the amount of mulch used to modify agricultural soil microclimates on commercial scales varies significantly. While fallen leaf litter on mango orchards can reach 15 tonnes ha^{-1}, intentional application of mulch, as used in similar studies, sits at around 1 tonne ha^{-1} (Kumar et al. 2015).

Hydrochar: similar to biochar but is produced as a slurry rather than through hydrothermal carbonisation, where water is used as the reacting medium. The hydrochar used here was generated at a temperature of 180 °C from organic and plant waste.

Experimental setup

The experimental setup was similar to that used by other soil conditioner studies, including Zhao et al. (2014), Sun et al. (2017), and Saporito et al. (2016). The soil cores are 200 mm tall and ~50 mm in diameter made of PVC pipes. The bottom of the columns was sealed using 2 mm fine woven mesh wire to allow for adequate drainage into the collection jar below. Once the column had been sealed, gravel (4–6 mm) covered roughly the bottom 2 cm of the column to ensure that the soil cores did not become waterlogged, and leachate could easily drain from the column. Soil was packed into the columns until roughly a 5 cm headspace remained at the top of the column (Figure D.1).

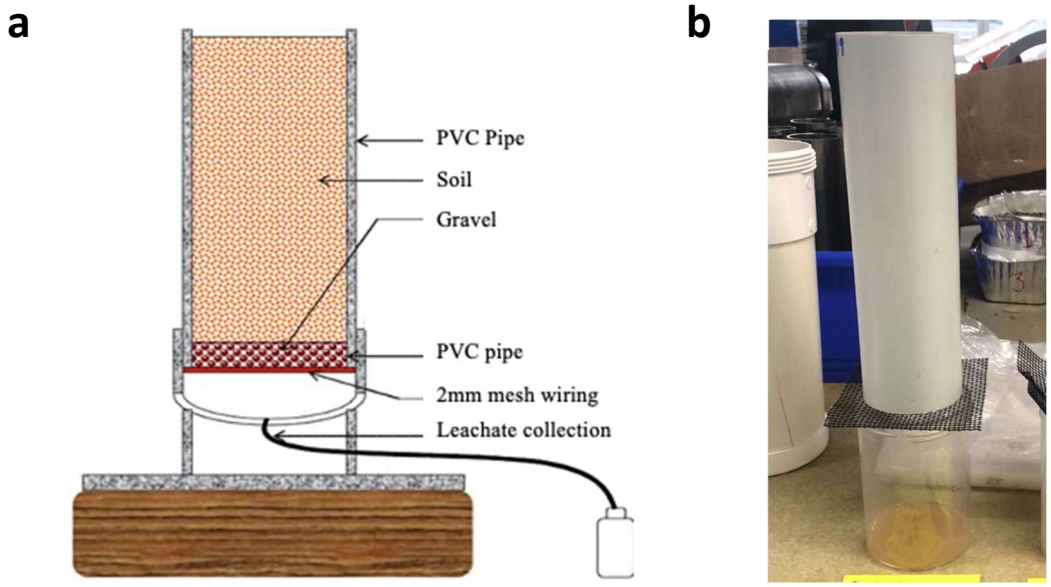


Figure D.1: Soil column setup diagram (a) and actual column setup (b).

The soil cores were repacked following the method of Oliviera et al. (1996), which requires repacking soil in very small increments and using a flat heavy pestle to gently compact each incremental layer added, to ensure uniform porosity and homogeneity across the column is maintained. This ensures the correct bulk density (1.30 g cm^{-3}) is achieved and no preferential flow pathways are created.

Prior to the beginning of the incubation, soil columns were wet to field capacity with treatments added and left to incubate for seven days at 30 °C. Field capacity is the maximum amount of water a soil can hold after excess (gravitational) water has drained. Field capacity was measured using one soil core where the column was placed in a dish with water and left to 'soak up' water until the entire column is wet. After this seven-day period, urea fertiliser diluted in 100 mL of water was applied to the soil cores, which marked the beginning of the 100-day incubation.

Simulated rainfall events

Rainfall directly affects soil water content and, in turn, drainage which is a key driver of N loss (Kodur et al. 2019). It is therefore critical to simulate rainfall events as part of the leaching experiment. Rainfall is highly variable across Australia, both geographically and temporally. To provide the most accurate representation of leaching from NT mango soils, rainfall events were designed so that they mimic actual rainfall trends in Middle Point, NT. N fertiliser application for mangoes is most critical during the 'flush' growth period when mango roots experience sudden bursts of activity and require N for growth. During this flush period, 60–70 % of total annual N fertiliser is applied to soils (Queensland Government, 1999). The 'flush' generally occurs between January and April (post-harvest flush) (Bithell et al. 2010), when average monthly rainfall reaches its highest levels. Therefore, it is in the best interests of this study to investigate how to improve soil nutrient retention under high rainfall, during this post-harvest vegetative flush. These simulated rainfall events consist of 50 mL of deionised water applied to each soil core.

Soil and greenhouse gas sampling protocol

Greenhouse gas sampling frequency increased around simulated rainfall events since the microbial activity is stimulated by increased water contents. Capturing greenhouse gas fluxes during periods of increased soil moisture is crucial. Table D.3 shows the sampling regime for the 100-day period. For greenhouse gas sampling, soil columns were moved into larger cylindrical chambers that were sealed with a tap through which gas samples could be extracted and transferred to 25 mL evacuated vials. Per sampling event, samples were taken at time 0 (start), after 60 minutes and after 120 minutes. This allows for the calculation of a flux value (GHG g ha^{-1} day^{-1}). All samples are analysed using a gas chromatograph, which determines concentrations of N_2O, methane (CH_4) and carbon dioxide (CO_2). Analysis was conducted by the Central Analytical Research Facility at QUT Gardens Point.

Table D.3: Simulated rainfall, gas and soil sampling schedule for 100-day incubation.

Destructive cores	Gas sampling			Rainfall events				
	M	T (am)	(pm)	W	Th (am)	Th (pm)	F	Sa	Su
Week 1	incubation @ 30 °C with urea and treatment								
Week 2									
Week 3									
Week 4									
Week 5									
Week 6									
Week 7									
Week 8									
Week 9									
Week 10									
Week 11									
Week 12									
Week 13									
Week 14									
Week 15									
Week 16				100					

Simulated rainfall events occurred twice weekly, every four weeks. Gas sampling was conducted immediately following these rainfall events and also once weekly when there were no rainfall events (Table D.3). Destructive sampling refers to the extraction of soil cores which was done on days 7, 35 and 100 as described. Destructive sampling was done to quantify soil mineral N (nitrate and ammonium) levels, pH and CEC over time. See Perrone et al. (2020) for a detailed method of these sampling protocols. Statistical analyses (ANOVA, mean, standard error) were conducted using SPSS.

D.2 Results and discussion

Soil physiochemical properties

Soil pH and CEC were measured as indicators of soil physiochemical fertility. CEC is an important indicator of a soil's capacity to retain nutrients, which is correlated with soil pH (as well as other physical and chemical soil properties). As soil pH increases, the number of negative ions on the soil particles increases, leading to higher CEC. pH levels of the amendments alone were tested prior to the incubation and found that biochar had a pH of 7.50, zeolite, 8.45 and hydrochar, 4.71.

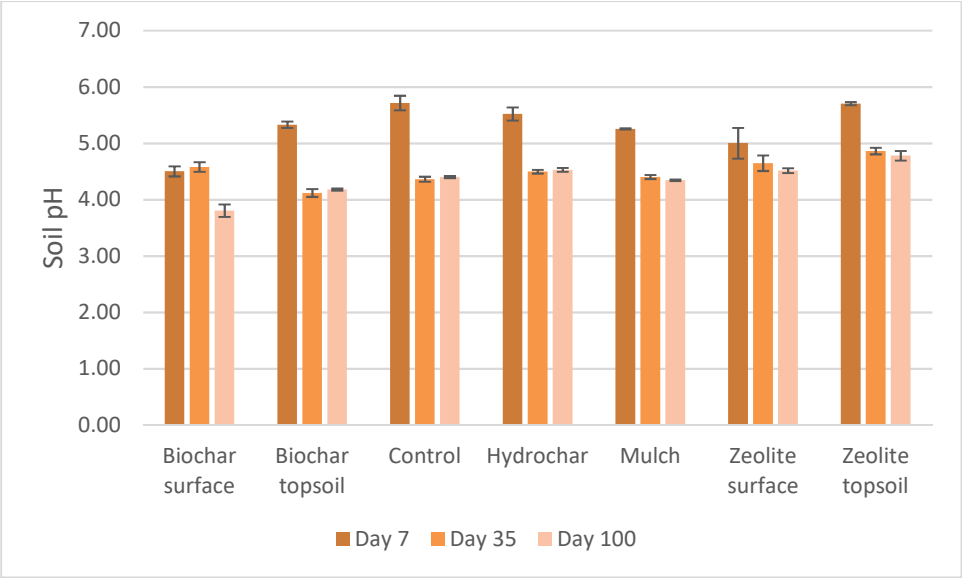


Figure D.2: Soil pH of all treatments after 7, 35 and 100 days of incubation.

The pH of the soil prior to treatment was 5.09. Soil pH declined across all treatments except surface biochar (Figure D.2). This noticeable decline across almost all treatments can be attributed to the acidifying effect of urea application and nitrification. Urea hydrolysis and ammonification alone do not cause soil acidification, but nitrification (conversion of $NH_4^+ \rightarrow NO_3^-$) releases large amounts of H^+ ions which causes acidification (Barak et al. 1997; Cai et al. 2014). A decrease in pH may also be due to the leaching of base cations from the soil during the simulated rainfall events. The evolution of soil pH over the 100-day period shows that urea was transformed and nitrified within the first few weeks of the incubation and soil pH stabilised over time.

Topsoil biochar and mulch-treated soils saw a slight increase in soil pH between day 35 and day 100. This is most likely due to the inherent neutral pH level of biochar and anion concentrations in the decomposing mulch plant material (Ritchie & Dolling, 1985). The effect observed here is not necessarily a problematic result; other soil ameliorant studies, specifically zeolite studies, often report significantly greater soil alkalinity as a result of zeolite application that often leads to crop failure (Omar et al. 2015).

The natural CEC of the soil at the Coastal Plains Research Farm (CPRF) is below 1.00 cmol kg^{-1}. CEC was quantified after 35 and 100 days of incubation (Figure D.3). Based on this initial level, all treatments and the control soils experienced significant increases in CEC, most notably those soils treated with zeolite. This significant increase in zeolite-treated soil CEC was expected as the CEC of natural zeolites can range between 100 and 300 cmol kg^{-1} (Mehrab et al. 2016).

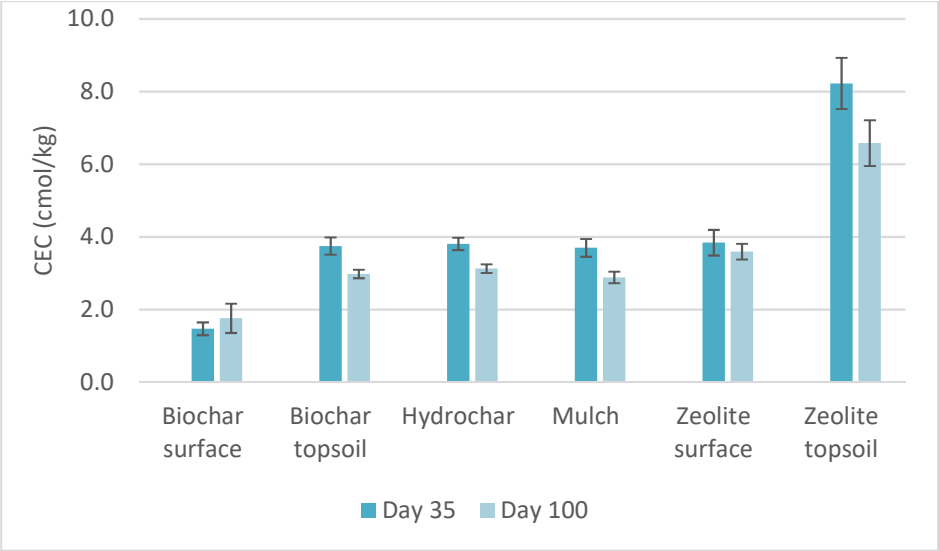


Figure D.3: CEC (cmol kg^{-1}) of all treatments after 35 and 100 days of incubation. Bars indicate standard error.

Topsoil zeolite was the only amendment that caused a significant increase in CEC relative to the CEC of control samples (p=0.04). Surface application of biochar caused the smallest increase in CEC. This was expected as the amendment was not mixed into the soil and therefore failed to bind with soil aggregates to increase CEC. All treatments except surface biochar experienced statistically insignificant declines (p=0.28) between day 35 and 100. Decreases may again be attributed to base cation leaching due to rainfall events and the acidification of soil pH. Despite this decrease in CEC, topsoil zeolite remained the only amendment to significantly increase soil CEC, and thereby increase soil nutrient-retention capacity.

Soil mineral N

Overall, soil NO_3^- levels increased across the incubation period, and NH_4^+ levels decreased as urea fertiliser was hydrolysed to ammonium and subsequently nitrified. Mineral N concentrations were scaled to represent the total concentrations (mg) of mineral N per soil core.

After 100 days, topsoil-mixed zeolite and biochar amendments showed the greatest retention of soil NO_3^- (Figure D.45a, Table D.4), closely followed by topsoil biochar and hydrochar.

Topsoil zeolite retained, on average, 31.7 % more soil NO_3^- than control, while zeolite that was only applied to the surface retained 79.2 % **less** NO_3^- than the control soils. The same trend was observed for biochar treatments; biochar mixed into the topsoil was far more effective than surface application. Mulch was also a poor performer of nitrate retention, averaging 40.5 mg per core (21.8 % less than control).

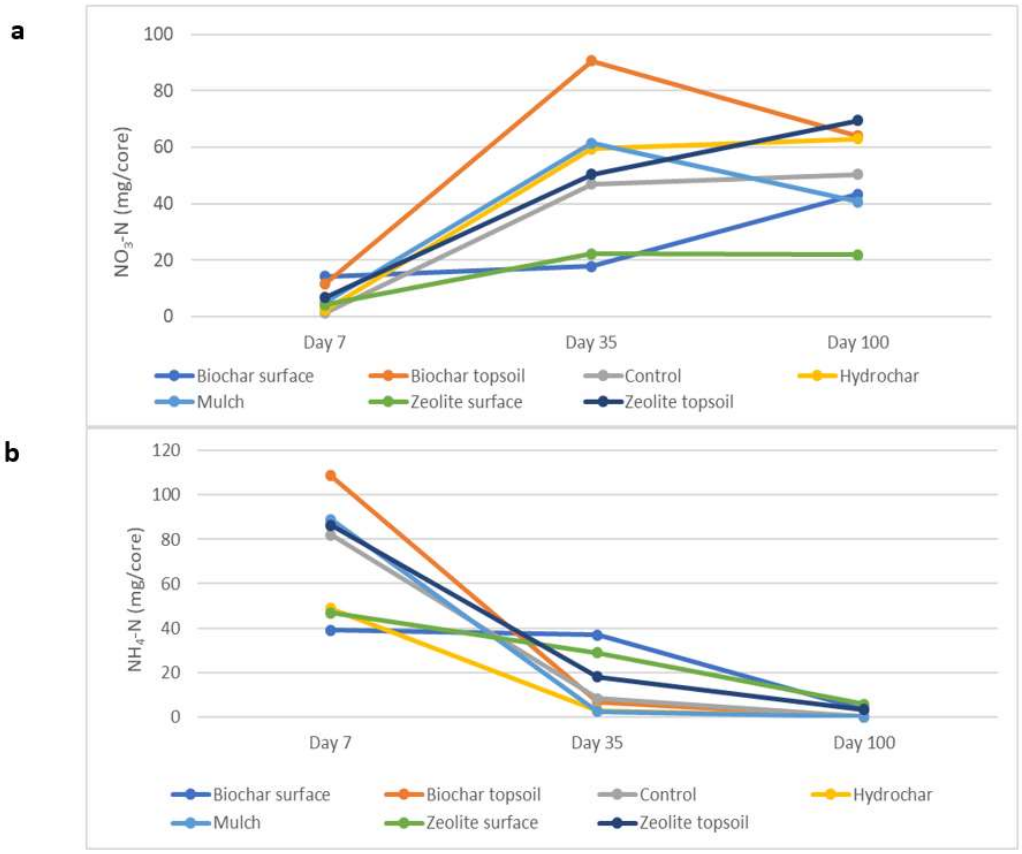


Figure D.4: Soil nitrate (a) and ammonium (b) concentrations remaining in soil cores for all treatments after 7, 35 and 100 days. Standard error is shown in Table 4 below.

Table D.4: Standard error (SE) for each treatment at day 7, 35 and 100 sample events. Associated with Figure D.4.

	Standard Error		
	Day 7	Day 35	Day 100
Biochar surface	1.63	1.47	3.75
Biochar topsoil	1.81	10.74	6.36
Control	1.67	4.23	3.98
Hydrochar	0.45	7.15	3.93
Mulch	0.60	6.45	3.03
Zeolite surface	1.22	1.99	0.47
Zeolite topsoil	0.18	1.18	4.14

The NO_3^- response to treatments is variable between day 35 and day 100, while continuously decreasing NH_4^+ concentrations suggest that nitrification of available NH_4^+ took place within the first few weeks of the incubation (Figure D.4b, Table D.4). Day 100 concentrations of NO_3^- varied significantly (p=0.0002), while final NH_4^+ concentrations were relatively homogenous across all treatments (p=0.075). Topsoil zeolite soils showed a steady increase in NO_3^- concentration over time,

corroborating findings of other studies which show zeolites to behave as a slow-release fertiliser (Mondal et al. 2021).

Mineral N leaching

Across the duration of the incubation experiment, eight rainfall events were simulated. As described in the Experimental Design section, the soil columns were placed on top of collection jars which captured all leachate. This leachate was then analysed for mineral N concentrations. Cumulative concentrations of NO_3^- and NH_4^+ are presented below. NO_3^- is a highly mobile form of N, and NO_3^- leaching is one of the largest problems in intensive agriculture around the world. It is immediately obvious from Figure D.5a (and Table D.5) that topsoil-mixed biochar lost large amounts of NO_3^- through leaching. During the eighth and last rainfall event alone, the average NO_3^- leachate concentration was on average 2083.9 mg L^{-1} (cumulatively, 4389.7 mg L^{-1}). All other treatments outperformed the control in terms of nitrate leaching, with topsoil zeolite producing the lowest amount of nitrate leachate. It should be noted that for all rainfall events after day 31, topsoil zeolite soils produced no leachate at all (Figure D.5a, b), most likely due to the high water-holding capacity of zeolites (Akbar Nakhli et al. 2017).

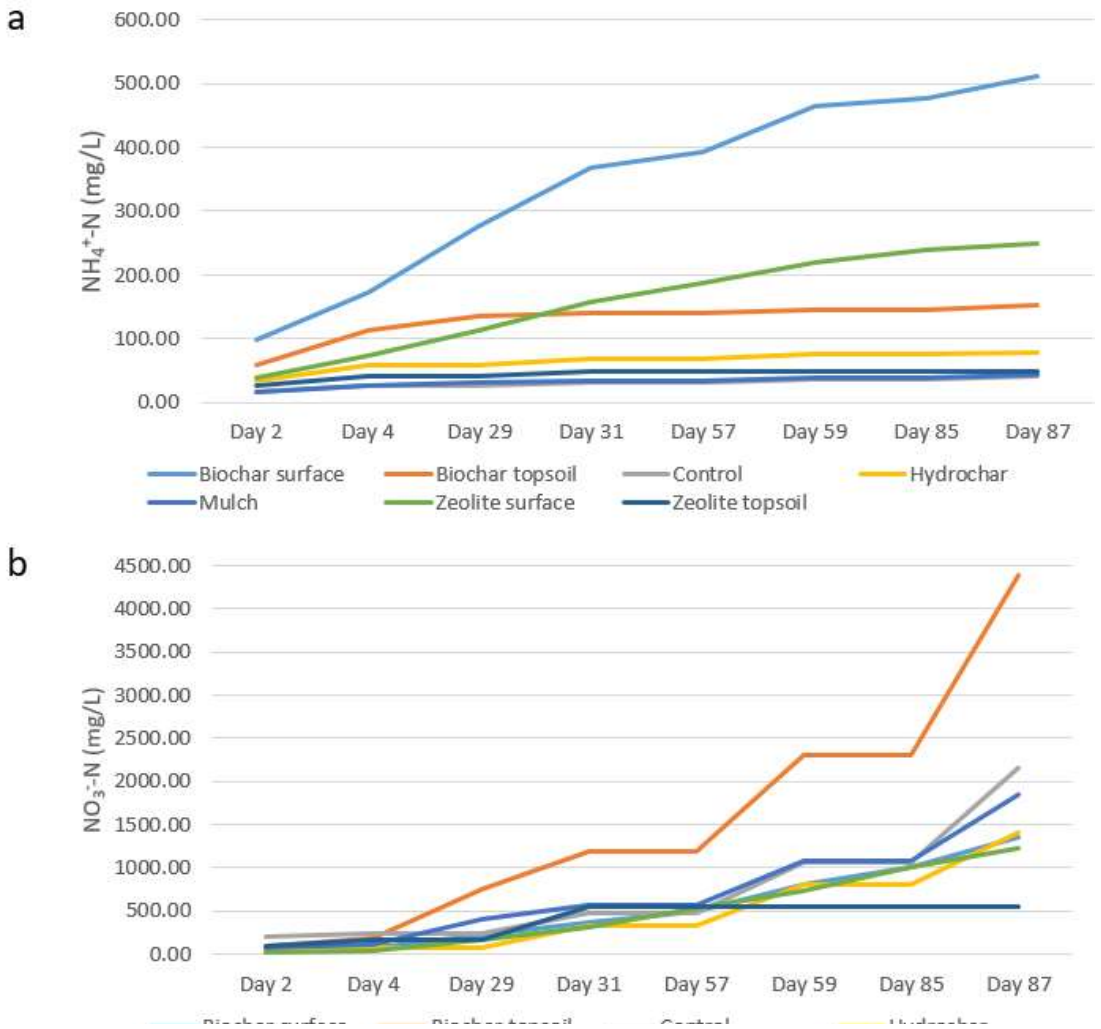


Figure D.5: Cumulative ammonium (a) and nitrate (b) leachate loss for all treatments over all eight simulated rainfall events. Concentrations in mg L^{-1}. Standard error shown below in Table D.5.

Table D.5: Standard error (SE) for all treatments for leachate concentrations following all simulated rainfall events.

	Day 2	Day 4	Day 29	Day 31	Day 57	Day 59	Day 85	Day 87
NO_3^- Standard Error								
Biochar surface	1.91	10.21	35.34	22.76	0.00	71.21	0.00	70.14
Biochar topsoil	13.29	10.79	0.00	13.60	0.00	172.92	0.00	62.41
Control	10.88	8.01	0.00	15.92	0.00	37.67	0.00	223.37
Hydrochar	6.74	4.02	0.00	26.63	0.00	0.00	0.00	0.00
Mulch	11.67	8.71	19.60	12.33	0.00	47.36	0.00	282.64
Zeolite surface	5.08	5.38	25.13	15.94	32.27	14.86	81.66	12.79
Zeolite topsoil	6.42	6.09	0.00	57.43	0.00	0.00	0.00	0.00
NH_4^+ Standard Error								
Biochar surface	13.89	8.47	25.27	7.15	0.0	20.55	0.0	11.42
Biochar topsoil	4.45	2.15	0.0	0.93	0.0	0.56	0.0	0.07
Control	2.06	0.75	0.0	3.23	0.0	0.32	0.0	1.15
Hydrochar	1.83	1.26	0.0	1.66	0.0	0.0	0.0	0.0
Mulch	1.22	0.67	1.66	1.24	0.0	0.147	0.0	0.44
Zeolite surface	3.27	3.18	4.56	4.54	4.79	10.19	8.89	2.81
Zeolite topsoil	2.02	1.05	0.0	1.26	0.0	0.0	0.0	0.0

In terms of NH_4^+ leachate loss, the control outperformed all treatments. Surface-applied biochar lost the largest amount of NH_4^+ via leaching, accumulating 510.6 mg L^{-1} over the eight rainfall events, followed by surface zeolites accumulating 250.3 mg L^{-1} Referring back to Figure D.4a, it can be seen that surface biochar soils had a relatively slow rate of nitrification. So, despite NH_4^+ being less mobile and susceptible to leaching, greater amounts of NH_4^+ were able to be leached through the soil. Figure D.3 also showed how low surface biochar CEC remained, suggesting that leaching and general loss of mineral N compounds could occur more rapidly and easily. Biochar materials are highly porous and have an inherent bulk density of 0.6 g cm^{-3} (Kavitha et al. 2018; Yu et al. 2019). This, in conjunction with the failure to significantly increase soil CEC, may have allowed for mineral N to easily move through the soil cores, causing higher leachate concentrations.

Topsoil-mixed zeolite on the other hand consistently reduced mineral N leachate compared to other treatments and the control. This can be attributed to the higher soil CEC observed under this treatment and the high adsorption capacity of zeolites. This high adsorption property is a product of the small pores within zeolites' crystalline structure where nitrogenous compounds like NH_4^+ can easily be absorbed, slowing the nitrification rate and susceptibility of mineral N to loss pathways (Mondal et al. 2021). Additionally, Bigelow et al. (2001) found that under zeolite amendments, the reduction in NH_4^+ and NO_3^- leaching was directly proportional to the increase in soil CEC.

Soil greenhouse gas emissions

Quantifying greenhouse gases, specifically N_2O and CO_2, provides valuable information on N transformation pathways and microbial activity. Cumulative N_2O and CH_4 emissions are graphed below according to mg $core^{-1}$ day^{-1}, and CO_2 emissions are expressed in g $core^{-1}$ day^{-1} (Figure D.6).

N_2O is produced predominantly as a by-product of nitrification and as an intermediate step of denitrification. N_2O emissions mostly plateau after the first fortnight. Small spikes in fluxes after rainfall events are continuously observed across the 100-day period, but these spikes become less pronounced over time as the incubation progresses and soil N is consumed, transformed and lost via leaching, as already observed. The spikes in N_2O activity after the simulated rainfall events indicates that most N_2O was produced via denitrification since this process requires anaerobic, low oxygen environments which occur when soil pores are saturated with water due to irrigation or rainfall events.

Topsoil-mixed zeolite was the only treatment to consistently produce lower N_2O emissions than those produced by the unamended control cores.

The largest emitter of N_2O, surface-applied zeolite soils, was among the treatments to show low mineral N concentrations in leachate but still quite low soil N levels at the end of the incubation. By quantifying N_2O emissions, which varied significantly between treatments ($p=0.002$), it is evident that the most significant loss pathway for this treatment type was denitrification. Similarly, mulch-treated soils showed low leachate concentrations but quite high N_2O levels in comparison, making denitrification the dominant loss pathway, as well. Hydrochar N_2O emissions very closely resemble the control trends, while most treatments generally appear to stimulate N_2O-producing processes, most likely through rapid nitrification and poor retention.

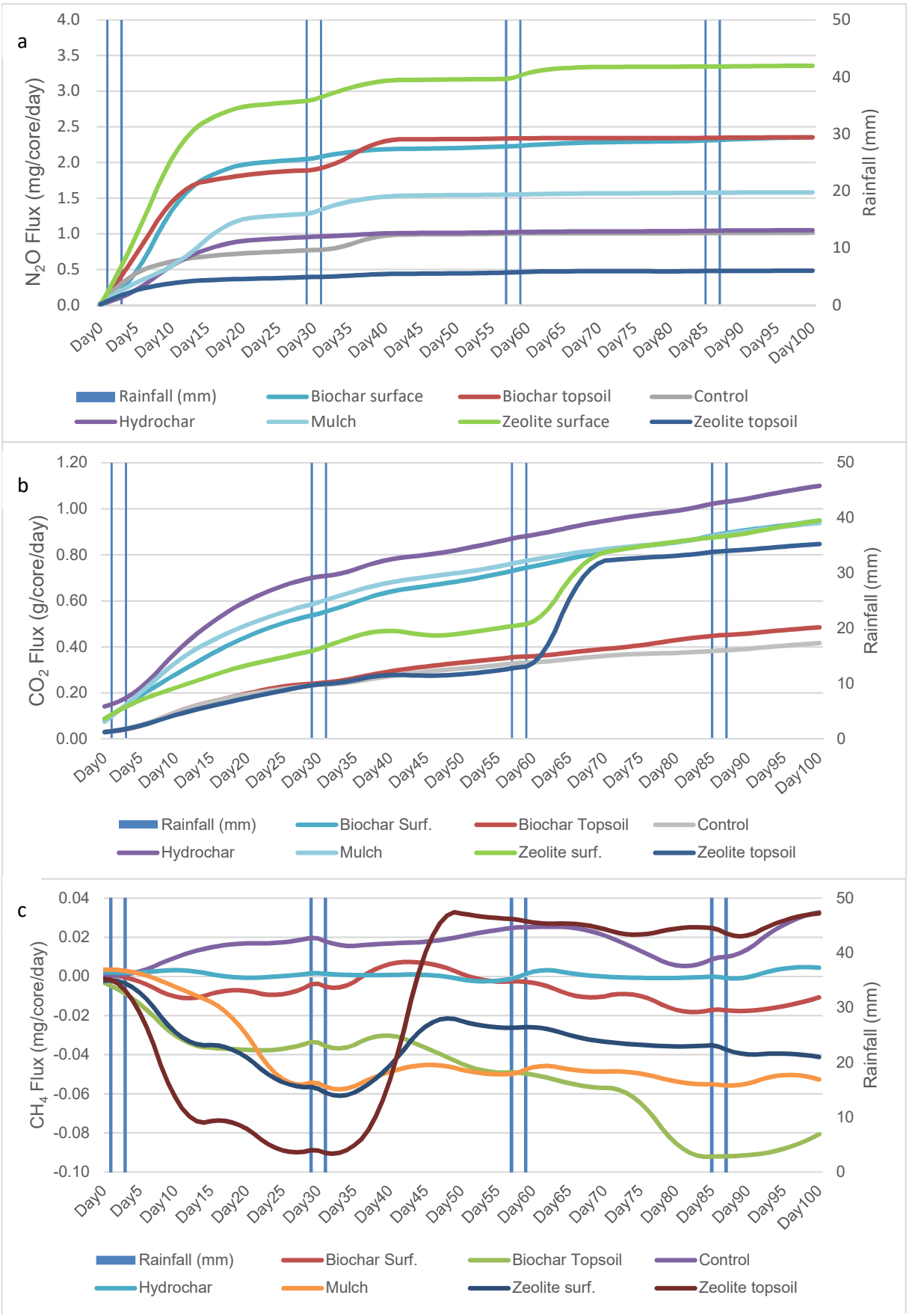


Figure D.6: Cumulative N_2O (a), CO_2 (b) and CH_4 (c) emissions per soil core per day graphed against rainfall (mm) for all treatments over 100-day incubation. Standard error is tabulated below.

Table D.6: Cumulative day 100 N_2O, CO_2 and CH_4 emissions and cumulative standard error (SE) for all treatments.

	N_2O		CO_2		CH_4	
	Day 100	**SE**	**Day 100**	**SE**	**Day 100**	**SE**
Biochar surface	2.35	0.62	0.95	0.09	-0.01	0.02
Biochar topsoil	2.35	0.33	0.49	0.04	-0.08	0.03
Control	1.02	0.23	0.42	0.01	0.03	0.02
Hydrochar	1.05	0.13	1.10	0.05	0.00	0.01
Mulch	1.58	0.30	0.94	0.02	-0.05	0.01
Zeolite surface	3.36	0.74	0.95	0.18	-0.04	0.03
Zeolite topsoil	0.48	0.15	0.85	0.18	0.03	0.03

While N_2O emissions plateau, CO_2 production consistently increased over the incubation period. All treatments produced more CO_2 emissions compared to the control soils. The difference in final cumulative CO_2 flux values between treatments was statistically significant ($p=0.0002$). Given that all treatments comprised organic material, the input of organic matter into the soil matrix most likely stimulated microbial respiration and CO_2 production. While hydrochar-treated soils produced very little N_2O, they produced the greatest amount of CO_2. Many recent studies report that hydrochar rapidly and effectively increases soil organic matter, stimulating microbial reproduction (Zhang et al. 2018). A rapidly growing microbial population in an environment with high-quality organic matter available would explain the large CO_2 flux. Mulch application would cause a similar trend, as decomposing plant material is likely carbon-rich, stimulating microbial respiration. Zeolite-treated soils were the only soils to behave as a CO_2 sink for a limited amount of time (between days 42 and 47). The zeolite treatments show a large response to the rainfall events at days 57 and 59, as increasing soil moisture rapidly stimulates microbial respiration. Low CO_2 emissions from topsoil biochar-treated soils is most likely a result of biochar recalcitrance. Most biochar amendments are initially very resistant to microbial decomposition, limiting substrate available for microbial consumption and respiration (Kavitha et al. 2018). This same trend is not observed in surface-applied biochar as this application method does not incorporate the biochar into the soil.

Control soils remain a source of CH_4 throughout the entire incubation period, while all amended soils behave as a sink for, at least, some period of time. Hydrochar and topsoil zeolite soils are the only treatments that are net emitters of CH_4. The first 30 days see topsoil zeolite-treated soils behaving as the largest CH_4 sink before the third and fourth rainfall events trigger large increases in CH_4 production. The difference in day 100 cumulative CH_4 emissions between treatments was statistically insignificant ($p=0.35$). The net effect of greenhouse gas emissions between treatments is difficult to discern; while topsoil zeolite and hydrochar treatments showed significantly lower N_2O values, they produced greater amounts of CO_2 and CH_4 relative to other treatments.

N budget

Simplified N budgets were calculated using total N retention and loss across the different treatments. The amount of N applied to soils was 100 mg and preliminary tests of soils found that the existing pool of mineral N was negligible (0.04 mg g^{-1}). As the results of the experiment accounted for the major N loss pathways, the relative contributions of these loss pathways can be broken down. N retention was outweighed by N loss in soils amended with surface-applied biochar, zeolite and

mulch. N_2O was a minor loss pathway across all treatments. Unaccounted for loss pathways (most likely N immobilisation) dominated mulch and surface zeolite soils but played a significant role in the overall N dynamics of all treatments.

This finding indicates that even in poorly structured, sandy soils with limited water and nutrient retention properties, where leaching is expected to dominate N loss pathways, denitrification can still be a major cause of N loss.

N loss still occurred to some extent across all treatments, the best result being 26 % loss. Soil N retention exceeded loss in all treatments except mulch and surface applications of zeolite and biochar. The most effective treatment, based on the comparisons (Figure D.7, D8) was topsoil-mixed zeolite, followed by mixed biochar and hydrochar.

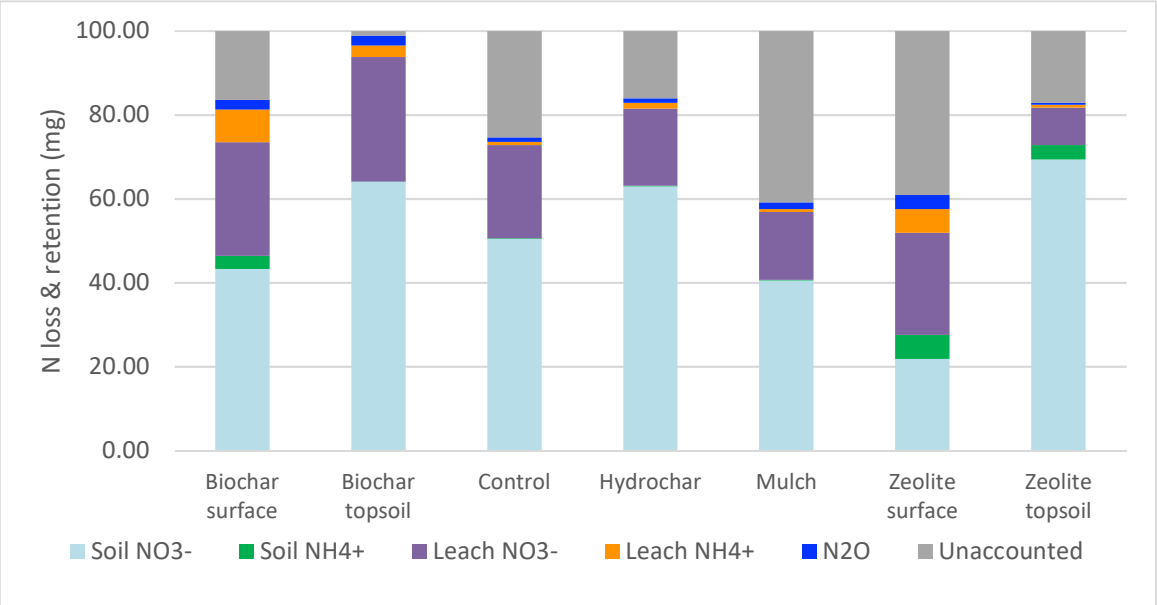


Figure D.7: Breakdown of N loss pathways and N retention for each treatment based on final (day 100) values.

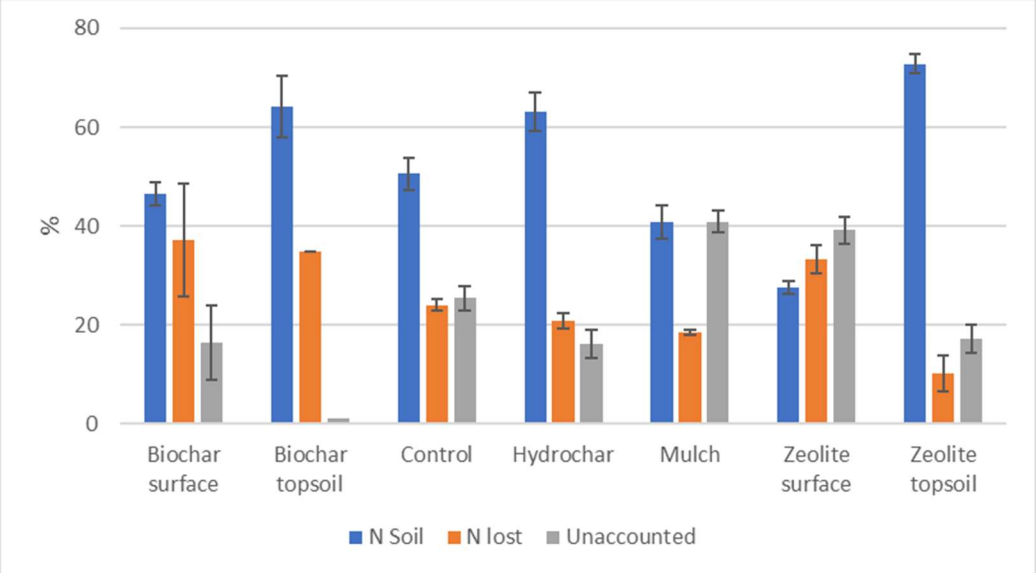


Figure D.8: Percentage and comparison of soil N retained, N lost and unaccounted for loss pathways at the end of incubation for all treatments. Bars indicate standard error. N loss includes leachate and N_2O emissions.

Of course, this breakdown of N retention and loss does not account for plant uptake, which was not part of this laboratory study. Ideally, in an in situ study, plant N uptake would constitute a large proportion of the total N budget. However, for this laboratory incubation, to achieve mineral N retention greater than 50 % over an extended period of time is a very promising result for these amendments.

D.3 Conclusions and recommendations

This experiment compared and quantified soil N loss and retention across a variety of soil ameliorants in a laboratory incubation setting spanning 100 days. The results of this experiment indicate that the application of natural zeolites that are mixed into the top layer of soil has substantial potential to reduce leaching of mineral N from soil and reduce nitrous oxide emissions relative to unamended, fertilised soils. Other amendments like biochar and hydrochar that are also mixed into topsoil show some promise but further development and understanding of their inherent properties and effect on soil biochemistry may be required first. Over the 100-day period, most of the N that was lost from zeolite-treated soils occurred in the first 20–30 days, showing great potential for longer-term nutrient retention following an initial period of loss before zeolites are properly incorporated into the soil and able to increase the nutrient and water retention capabilities of soils.

It is important to reiterate that this study was conducted in a controlled laboratory setting on a very small scale. To confirm that these results and trends hold, a larger-scale field study should be conducted, which may also help to answer questions about the effects of these amendments on plant N uptake. Future research may also study different application methods and rates; for example, other zeolite studies frequently study the effect of zeolites on soil and plant dynamics at different rootzone depths (Bigelow et al. 2001). Additionally, longer-term studies may prove useful given that zeolites do not break down over time, but the permanence of their effect on soil physiochemical properties remains disputed (Omar et al. 2015).

No two agricultural soils are identical; N loss and retention are influenced by soil physiochemical qualities, microbial communities, local climate, and management more than they are influenced by a single soil ameliorant. For the particular soil type studied here, zeolite was an effective ameliorant, but under different conditions, other amendments may prove more or less useful. The development of zeolite as a commercial soil amendment will be depend heavily on economic feasibility. If an application method similar to the one replicated here were used (100 kg ha^{-1}), the cost is estimated at around $50,000 per hectare. If the avoided cost of N loss per hectare as a result of zeolite, or other amendment applications, can be quantified in monetary terms, this may help to advance soil ameliorants on a commercial scale.

Mango horticulture is a critical part of the NT's economy and agricultural sector. Improving the productivity of soils and the sustainability of horticultural activities is a significant factor in securing this industry's future. The development and adoption of soil ameliorants, as shown by this study, has the potential to significantly advance the mango industry in these respects.

D.4 References

Akbar Nakhli S, Delkash M, Bakhshayesh B, Kazemian H (2017) Application of zeolites for sustainable agriculture : a review on water and nutrient retention. Water, Air, & Soil Pollution.

Australian Mango Industry Association (2020) Hort stats handbook: 2019-20 mangoes. Brisbane.

Barak P, Jobe B, Krueger A, Petersen L, Laird D (1997) Effects of long-term soil acidification due to nitrogen fertiliser inputs in Wisconsin. Plant and Soil, 197, 61–69.

Bell M, Schwenke G, Lester D (2016) Understanding and managing N loss pathways.

Bigelow CA, Bowman DC, Cassel DK (2001) Nitrogen leaching in sand-based rootzones amended with inorganic soil amendments and sphagnum peat. 126(1), 151–156.

Bithell SL, Diczbalis Y, Moore C (2010) Review of mango irrigation research in the Northern Territory.

Cai Z, Wang B, Xu M, Zhang H, Zhang L, Gao S (2014) Nitrification and acidification from urea application in red soil (Ferralic Cambisol) after different long-term fertilization treatments. Journal of Soils and Sediments, 14(9), 1526–1536. https://doi.org/10.1007/s11368-014-0906-4

Kavitha B, Reddy PVL, Kim B, Lee SS, Pandey SK, Kim KH (2018) Benefits and limitations of biochar amendment in agricultural soils: A review. Journal of Environmental Management, 227(September), 146–154. https://doi.org/10.1016/j.jenvman.2018.08.082

Kodur S, Babu U, Narayan T, Deo RC (2019) Environmental and economic impacts and trade-offs from simultaneous management of soil constraints, nitrogen and water. Journal of Cleaner Production, 222, 960–970. https://doi.org/10.1016/j.jclepro.2019.03.079

Kumar D, Pandey V, Nath V (2008) Effect of organic mulches on moisture conservation for rain fed turmeric production in mango orchard. Indian Journal of Soil Conservation, 36, 188-191.

Ladha JK, Pathak H, Krupnik TJ, Six J, van Kessel C (2005) Efficiency of fertiliser nitrogen in cereal production: Retrospects and prospects. Advances in Agronomy, 87, 85–156. Retrieved from https://www.sciencedirect.com/science/article/pii/S0065211305870038?via%3Dihub

Lehmann J, Joseph S (2009) Biochar for Environmental Management. London: Earthscan.

Mchenry MP (2009) Agriculture , ecosystems and environment agricultural bio-char production, renewable energy generation and farm carbon sequestration in Western Australia: Certainty, uncertainty and risk. 129, 1–7. https://doi.org/10.1016/j.agee.2008.08.006

Mehrab N, Chorom M, Hojati S (2016) Effect of raw and $NH4^{+}$-enriched zeolite on nitrogen uptake by wheat and nitrogen leaching in soils with different yextures. Communications in Soil Science and Plant Analysis, Vol. 47, pp. 1306–1316. https://doi.org/10.1080/00103624.2016.1166377

Mondal M, Biswas B, Garai S, Sarkar S, Banerjee H, Brahmachari K, Hossain A (2021) Zeolites enhance soil health, crop productivity and environmental safety. Agronomy, 11(3), 448. https://doi.org/10.3390/agronomy11030448

Ngo H, Owens G (2002) The profitability of mangoes in the Top End.

Oliviera IB, Demond AH, Salehzadeh A (1996) Packing of sands for the production of homogeneous porous media. Soil Science Society of America, 60, 49–53. https://doi.org/10.2136/sssaj1996.03615995006000010010x

Omar L, Haruna O, Majod N (2015) Improving ammonium and nitrate release from urea using clinoptilolite zeolite and compost produced from agricultural wastes. The Scientific World Journal, 1–12.

Perrone S, Grossman J, Liebman A, Sooksa-nguan T, Gutknecht J (2020) Nitrogen fixation and productivity of winter annual legume cover crops in Upper Midwest organic cropping systems. Nutrient Cycling in Agroecosystems, 117(1), 61–76. https://doi.org/10.1007/s10705-020-10055-z

Queensland Government (1999) Mango Information Kit.

Ritchie G, Dolling P (1985) The role of organic matter in soil acidification. Australian Journal of Soil Research, 23(4), 569–576.

Saporito LS, Bryant RB, Kleinman PJA (2016) A protocol for collecting and constructing soil core lysimeters. Journal of Visualised Experiments, 112. https://doi.org/10.3791/53952

Scheer C, Grace PR, Rowlings DW, Kimber S (2011) Effect of biochar amendment on the soil-atmosphere exchange of greenhouse gases from an intensive subtropical pasture in northern New South Wales, Australia. 47–58. https://doi.org/10.1007/s11104-011-0759-1

Sepaskhah AR, Yousefi F (2007) Effects of zeolite application on nitrate and ammonium retention. (1988), 368–373.

Sun H, Lu H, Chu L, Shao H, Shi W (2017) Biochar applied with appropriate rates can reduce N leaching , keep N retention and not increase NH_3 volatilization in a coastal saline soil. Science of the Total Environment, 575, 820–825. https://doi.org/10.1016/j.scitotenv.2016.09.137

Thorburn,PJ, Biggs JS, Attard SJ, Kemei J (2011) Environmental impacts of irrigated sugarcane production: Nitrogen lost through runoff and leaching. Agriculture, Ecosystems and Environment, 144(1), 1–12. https://doi.org/10.1016/j.agee.2011.08.003

Yu H, Zou W, Chen J, Chen H, Yu Z, Huang J, Gao B (2019) Biochar amendment improves crop production in problem soils: A review. Journal of Environmental Management, 232(November 2018), 8–21. https://doi.org/10.1016/j.jenvman.2018.10.117

Zhang S, Zhu X, Zhou S, Shang H, Luo J, Tsang DCW (2018) Hydrothermal carbonization for hydrochar production and its application. In Biochar from Biomass and Waste: Fundamentals and Applications. https://doi.org/10.1016/B978-0-12-811729-3.00015-7

Zhao X, Wang S, Xing G (2014) Nitrification, acidification, and nitrogen leaching from subtropical cropland soils as affected by rice straw-based biochar: laboratory incubation and column leaching studies. 471–482. https://doi.org/10.1007/s11368-013-0803-2