Preliminary investigation of water use efficiency of Avocado varieties, irrigation and intra-canopy variation.

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Summary

- This project has identified a relationship between foliar $\delta^{13}C$ and foliar $\delta^{18}O$ suggesting that Avocado water use efficiency (WUE) (as indicated by foliar $\delta^{13}C$), grown in nursey conditions, varies with transpiration. This result has not been previously been identified in avocado in the literature and may be a useful to explore in avocado and other horticultural crops breeding programs in Australia.
- We have also shown that management influences long-term water use efficiency where the avocado variety Shepard had very different foliar $\delta^{13}C$ with the two different irrigation treatments (Sprinkler and drip irrigation). Although not conclusive, the foliar $\delta^{13}C$ was higher under the sprinkler irrigation which was likely in response to increased growth or water stress at this site.
- The relationship between foliar N, chlorophyll and the chlorophyll SPAD meter indicates that the N could be monitored with the SPAD meter in avocado orchards. This may be used to supplement dry matter analysis and help improve N use efficiency in North Queensland catchments draining into the Great Barrier Reef, increasing sustainable horticultural outcomes.
- The relationship between foliar N and $\delta^{13}C$ (less negative $\delta^{13}C$), indicates that the north facing canopy had a greater variation in N and WUE compared to the south facing and the shoot function site.
- There was variation in nitrogen (N) within the canopies particularly when the shoot function and canopy sampling position samples were pooled, indicating a potential response in the canopy to N cycling at the different sites. This is suggested as a response to Nitrogen management at the different sites. Further work would be required to better understand the mechanisms behind this relationship.
- Finally, the correlation between $\delta^{15}N$ and $\delta^{13}C$ at the pooled sites indicates how increasing foliar $\delta^{15}N$ was related to increasing WUE (less negative $\delta^{13}C$), showing potentially how N availability across sampled orchards influences growth. Again further work would be required to better understand the mechanisms behind these relationships.

Key Messages

The application of natural stable carbon, nitrogen and oxygen isotopes in this project has shown differences between tree and orchard WUE, by genetics and irrigation, and orchard nitrogen cycling. These techniques offer a tool to further progress our understanding in increasing productivity and water use in horticultural tree crop breeding and orchard management. This relationship may be useful to explore for avocado and other horticultural crop breeding and intensification programs in Queensland. It also seems a critical step to increasing the Horticultural industry’s efficient use of water and nitrogen, particularly in catchments flowing into the Great Barrier Reef.

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Background

Horticultural crops are highly reliant on irrigation water and fertilizers for productivity. There are some real threats to the availability of irrigation water as it is considered a scarce resource in some regions. Water scarcity and increasing costs of water, competition for agricultural land, the need for increasing crop production with increasing population and climate change are critical issues when it comes to horticultural production. The current trend for horticulture has potential to exacerbate the demand on water resources. This is particularly critical with Avocado as the Industry contributes an estimated $226 million GVP to the State of Queensland. In addition, there has been a doubling of avocado production area in Far North Queensland (where this study was conducted) since 2015, where orchard expansion has gone from 950 ha in 2015 (Dickinson 2015) to 2250 ha in 2018 (Tyas 2018), with over 50 % of its orchard area being less than 5 years of age.

Water use efficiency can be measured at different scales such as the regional scale, the Irrigation Systems scale, the farm, the field or the crop level and while substantial work has targeted vegetable crops, less research has been invested into tree crops. At the Regional through to farm level the introduction of smart irrigation technologies such as real-time monitoring, automated solutions, water flow controllers, wastewater management and sensor technology aim to better inform plant water requirements and increase the performance of irrigation systems (Katerji and Mastrorilli 2014). At the crop level there are a range of methods we can use to measure water-use efficiency to reduce water use and maintain production. The first is the agronomic approach or crop WUE (kg m^{-3}), which is the ratio between yield (kg m^{-2}) (or biomass) and water consumption (m^3 m^{-2}) at the tree level; the second being the physiological method which compares carbon assimilation and transpiration rates at the leaf scale. This project aim was to use the physiological method to investigate the use of foliar carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotopes to identify if avocado genetics influences leaf level water-use efficiency. If it does, then there maybe potential to use foliar $\delta^{13}C$ to investigate other aspects of avocado trees such as irrigation management, intra-canopy variability and shoot functioning.

Foliar $\delta^{13}C$ is accepted as a long-term index of carbon assimilation due to the ability of the C fixing enzyme ribulose bisphosphate carboxylase/ oxygenase (Rubisco) to discriminate against the heavier $^{13}CO_2$ compound, preferentially using the lighter $^{12}CO_2$ compound (Farquhar et al. 1982, Farquhar and Richards 1984 and Warren et al. 2001). If a plant is under water stress and reduces its stomatal conductance, this discrimination decreases as it uses up the remaining internal CO_2 thereby incorporating the heavier $^{13}CO_2$ into the leaf during photosynthesis. While this relationship can result from reducing soil moisture, it cannot be attributed to stomatal conductance alone (through transpiration). This is because foliar $\delta^{13}C$ is also influenced by the plant's capacity for carbon assimilation (through photosynthesis).

Foliar $\delta^{18}O$ has been shown to negatively relate to stomatal conductance and the relationship is independent of Rubisco activity. Hence a positive relationship between foliar $\delta^{18}O$ and foliar $\delta^{13}C$ indicates that foliar $\delta^{13}C$ is driven by stomatal conductance (Saurer et al. 1997, Barbour et al. 2000, Barbour 2007, Keitel et al. 2003). Hence foliar $\delta^{18}O$ can serve as a proxy for how stomatal conductance influences WUE. In addition, and due to close relationship between water-use, growth and nitrogen, foliar nitrogen (N) and foliar nitrogen ($\delta^{15}N$) isotopes will also be considered.

Project Objectives

This project aims to investigate:

- The use of carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$) and nitrogen ($\delta^{15}N$) isotopes to identify how plant water and nitrogen use varied in four highly productive commercial avocado varieties.
- How carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes vary with irrigation systems in two commercial orchards grown in Far North Queensland.
- If there is intra-canopy variability between the different canopy positions or shoot functions in mature Avocado canopies.
- If there is a relationship between leaf N dry matter analysis and chlorophyll in Avocado leaves to identify if rapid nutrient diagnostic instrumentation such as the SPAD chlorophyll meter maybe useful for rapid N diagnostics.

Methodology

Avocado genetics

For the genetic experiment, 6 samples of leaves (with 6-8 leaves in each sample) were collected from nursery trees of Velvick, Hass, Shepard and Maluma trees growing under the same environmental conditions, on two occasions. The first collection was on the 1st of December 2017 and a second sampling was taken on the 15th of February 2018. Each sampling was taken between the time of 11 am and 12 pm, and fell between the mid-morning and midday irrigation. The leaves sampled were taken from the most recently matured leaf at the top of the plant. All trees except the Velvick were approximately 6 months old and were grafted scions on a Velvick seedling rootstock. The Velvick was also the same age but was an ungrafted seedling.

Irrigation management

The investigation into irrigation management looked at two commercial orchard sites. At the first site the two varieties were Turner Hass (11 years old) and Shepard (15 years old) growing and irrigated with sprinklers (17°9'3.06"S, 145º5'3.35"E) (sprinkler (96 L / tree hour) (soil type Algoma), while the second irrigation site the varieties included Shepard and Maluma (both 3 years old) were growing with drip irrigation (drippers 2 L / emitter hour) (17°9'52.99"S, 145º5'0.69"E)(soil type Dimbulah).
Both sites were monitored with soil water monitoring devices and irrigation which were scheduled to drain and rewet to the frequency monitored by the grower. Generally, the sprinkler irrigation was irrigated twice a day (3 times in hot weather) for 1-3 hours depending on conditions a day, with one irrigation scheduled for 2.30 in the afternoon (15-20 mins). Soil water was monitored to 40cm depth. At the drip irrigation site, the Maluma and Shepard were watered with 3 laterals (1.5 m apart) per row, with emitters at 0.4 m apart, irrigated 5 times for 30 minutes, each day.

In each variety, eight trees were monitored for a mid-summer flush. When the flush was a couple of days old, 15 shoots of the same developmental stage in the mid-section of the north facing canopy facing were marked with flagging tape. Samples were allowed to mature and then collected. Due to uneven canopy flushing at the two sites, samples from the two sites were collected at different times. The sprinkler site samples were collected on the 11th of January 2018 while the drip irrigation site samples were collected on the 15th of February 2018. Once collected, samples were placed into labelled brown paper bags and transported in an cooled in an insulated box back to the Department of Agriculture and Fisheries (DAF), Tropical Agriculture Research Facility, at Mareeba where they were oven dried at 70ºC for 48 hours and later sent for analysis.

Foliar N, Chlorophyll and SPAD

A method to rapidly diagnose canopy N can saving growers time and money in assessing critical N nutrient concentrations. The aim of this experiment was to identify if there was a relationship amongst the leaf total N concentrations, chlorophyll concentrations and the rapid assessment chlorophyll SPAD meter. Eight samples (several leaves per sample) of leaves with different chlorophyll levels were collected (yellow leaves, lime green leaves, light green and dark green leaves) from the Turner Hass trees at the sprinkler irrigation site. Once collected, samples were placed into labelled brown paper bags and transported in an insulated box back to the DAF, Centre for Tropical Agriculture Research Facility and were frozen overnight and then sent express on ice to the Chemistry Centre Laboratory (Brisbane) for determination. SPAD readings were conducted on each leaf before freezing.

Intra-canopy variation

Intra-canopy variability may influence synchronization of phenological stages within the canopy, and influence production adversely. This experiment was carried out at a commercial orchard in Walkamin, Far North Queensland (17°6'2.56"S, 145º26'44.67"E). Eight trees were monitored for a summer flush and when it occurred 15 shoots with similarly aged flush were marked with flagging tape on the north and south facing side of the trees. Shoots were sampled from the mid-section of the tree. Once collected, samples were placed into labelled brown paper bags and transported in an insulated box back to the DAF, Centre for Tropical Agriculture Research Facility at Mareeba where they were oven dried at 70ºC for 48 hours.

Shoot function

The crop load experiment was carried out at the Walkamin Research Station (17°8'17"S 145°25'41"E) on Shepard trees. Eight trees were monitored for a mid-summer flush. When the flush was a couple of days old, 8 shoots of the same developmental stage in the mid-section canopy, facing a northern aspect were marked with flagging tape. Shoot types represented vegetative (without fruit) and fruiting shoots and were marked with different colour flagging tape. Shoots with fruit were selected where the base of the shoot was directly in contact with a fruit penduncle. Mature leaf samples from each shoot was collected on the 15th of January 2018. Due to the small amount of shoot samples available from the same age, three leaves from each shoot type per tree were composited together to provide one sample per shoot type per tree.

Stable carbon and nitrogen isotope analysis

Samples for the irrigation sites, canopy sampling position and shoot function were sent to the Stable Isotope Laboratory, Australian Rivers Institute (ARI), Griffith University, Nathan, Brisbane, for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes analysis. Samples for the Genetics experiment and chlorophyll data for the rapid N assessment tool were sent to the Queensland Department of Environment and Science (DES), Science Delivery, Chemistry Centre Laboratory, Dutton Park, Brisbane, for analysis of stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope analysis and N and C (%) and chlorophyll determination.

Statistical analysis

Due to cost and sensitivity of the experiments, no replication (blocking) was undertaken in the genetics and irrigation experiments. For this reason, no formal significance testing was conducted but exploratory data analysis was conducted using 95% confidence intervals. The canopy sampling position and shoot function experiments were analyzed using analysis of variance (ANOVA). Where a significant effect was found, the 95% least significant difference (LSD) was used to make pairwise comparisons. All significance testing was performed at the 0.05 level. Correlations were used to describe relationships between independent variables, while regression models were used to describe casual relationships. All data was analyzed using Genstat for Windows, 18th edition (VSN international, 2018).

Results

Avocado genetics

When both sampling data were combined variety means for foliar $\delta^{13}C$ across both sampling occasions showed Velvick had a higher mean $\delta^{13}C$ (less negative) compared to the other varieties (Figure 1a). There was some variability in the means for foliar $\delta^{18}O$ and foliar N however the trend for means and confidence intervals were different with each variety. Foliar $\delta^{15}N$ and foliar C on the other hand had similar means and confidence intervals across each variety. Table 1 outlines the means, 95% confidence for each variable averaged for collection time.

Further investigation of these variables identified a significant positive relationship (adj R^2 = 66.3, $p<0.001$, df = 52) between foliar $\delta^{13}C$ and $\delta^{18}O$. This relationship suggests that WUE (as indicated by foliar $\delta^{13}C$) increased with increasing foliar $\delta^{18}O$ (an index of transpiration, which is higher when stomatal conductance is low). The relationship showed higher $\delta^{13}C$ levels for Velvick across the range of $\delta^{18}O$ values compared to the other varieties (Figure 2a). Because Velvick in this experiment was a seedling and the other varieties were grafted onto Velvick rootstocks, further work would need to confirm that it was genetics and not a factor of the grafting process that has influenced this result.

The relationship between WUE in different avocado varieties has been identified in the US where 24 avocado varieties were compared using foliar $\delta^{13}C$ where substantial variations of foliar $\delta^{13}C$ (varying between -32.62 to -27.17 ‰) were found (Acosta-Rangel et al. 2018). Furthermore their study was based on testing the relationships between foliar $\delta^{13}C$ and a gas analyzer to determine stomatal conductance and intrinsic Water use efficiency (WUEi (µmol mol^{-1})) which is a ratio of the measure of photosynthesis / stomatal conductance (mol m^{-2} s^{-1}). These authors also found a positive relationship between foliar $\delta^{13}C$ and WUEi and a negative relationship between foliar $\delta^{13}C$ and stomatal conductance.

Irrigation management

Comparison of the two varieties grown under two different irrigation types (sprinkler (96 L / tree hour and drippers 2 L / emitter hour) have shown that the sprinkler irrigation site had a higher mean foliar $\delta^{13}C$ (less negative) compared to the drip irrigation site (figure 3a) suggesting increased WUE. When Shepard was compared under the same irrigation type with the other variety there was little variability in the means for foliar $\delta^{13}C$, foliar N or foliar C. However, comparing Shepard across the two irrigation types showed that the site with sprinkler irrigation had greater WUE. These results indicate that the trees on the sprinkler irrigation site had greater WUE, however other factors may be contributing to this result such irrigation system, irrigation management, tree size and planting configuration, (potentially) soil, and site slope/aspect/elevation/exposure. Despite this the leaves at each site were of a similar physiological age and from the same northern aspect.

There was also considerable variation at the sprinkler irrigation site for foliar $\delta^{15}N$ (figure 3b) between varieties. Variation in foliar $\delta^{15}N$ represents variation in N cycling/ sources. While $\delta^{15}N$ represents N cycling in soils and can be reflected in the tree canopy, the specific mechanisms at work here, thus causing the variation in soil N would require further research, for us to be confident to understand the mechanisms at work in these results (fertilizer, N leaching, volatilization or mineralization).

The relationship between foliar N, chlorophyll and SPAD

When leaves of the variety Turner Hass were assessed for different levels of chlorophyll, there were strong relationships for foliar N to chlorophyll (adj R^2= 85.1, p<0.001, n=24), for foliar N to SPAD (adj R^2= 87.3, p<0.001, n=24) and for chlorophyll to the SPAD meter (adj R^2= 85.1, p<0.001, n=24).

Intra-canopy variation

When shoot canopy position was assessed at one site in leaves with either a northerly or southerly aspect and of the same physiological age, there was no significant differences for foliar N (p=0.920), C (p=0.972) or $\delta^{13}C$ (p=0.257). There was significant difference for $\delta^{15}N$ (p = 0.014, 95% lsd = 0.43, df =15) where the mean $\delta^{15}N$ was lower in the northerly aspect (0.1‰) compared to the southerly aspect (0.7 ‰). When relationships were investigated there was a significant relationship between foliar C and foliar N (adj R^2= 33.4, p=0.011, n=15) which showed that when foliar N increased so did foliar C, but where this relationship did not vary with canopy sampling position at this site.

Shoot function

When shoot function was assessed in leaves from vegetative compared to fruiting shoots, with each selected from a northerly aspect and of the same physiological age, there was no significant differences for foliar N (p=0.641), C (p=0.179), $\delta^{13}C$ (p=0.490) or $\delta^{15}N$ (p=0.160) at this site. When relationships were further investigated there was a significant relationship between foliar $\delta^{15}N$ and $\delta^{13}C$ (R^2= 25.8, p=0.026, n=15) which suggests as foliar $\delta^{15}N$ increased so too did foliar $\delta^{13}C$, but this relationship did not vary with shoot function (vegetative compared to fruiting).

Combining intra-canopy and shoot function sites

When the intra-canopy and shoot function samples from both sites were combined, there was a highly significant positive relationship between foliar N and foliar $\delta^{15}N$ (R^2= 76.1, p<0.001, n=32) (figure 4a) where shoot location/ function was significant. In this relationship shoot location/ function at canopy location 1 (north) was significantly different and canopy location 2 (south) but not 3 (vegetative) or 4 (fruiting). There was also a significant relationship between foliar N and foliar $\delta^{13}C$ (R^2= 35.7, p<0.011, n=32) (figure 4b) where shoot location/ function was significant and where each shoot location/ function relationship was significantly different from canopy location 1 (north). A significant positive correlation between foliar $\delta^{13}C$ and foliar $\delta^{15}N$ was also significant (R= 45.20, p=0.0094, n=32) (figure 4c).

Conclusions/Significance/Recommendations

- This project has identified a relationship between foliar $\delta^{13}C$ and foliar $\delta^{18}O$ suggesting that Avocado water use efficiency (WUE) (as indicated by foliar $\delta^{13}C$), grown in nursey conditions, varies with transpiration. Further work would be required to better understand the mechanisms behind these variations, particularly how grafted and seedling trees differ.
- We have also shown that irrigation management can influence long-term water use efficiency where the avocado variety Shepard had very different foliar $\delta^{13}C$ with the two different irrigation treatments (Sprinkler and drip irrigation). Although not conclusive (due to other potentially compounding factors), the foliar $\delta^{13}C$ was higher under the sprinkler irrigation which was likely in response to increased growth or water stress at this site.
- The relationship between foliar N, chlorophyll and the chlorophyll SPAD meter indicates that the N could be monitored with the SPAD meter in avocado orchards. This may be used to supplement dry matter analysis and help improve N use efficiency in orchards.
- The relationship between foliar N and $\delta^{13}C$ (less negative $\delta^{13}C$), indicates that the north facing canopy had a greater variation in N with WUE, compared to the south facing canopy position and the different shoot functions (vegetative vs fruiting shoots).
- The variation in nitrogen (N) within the canopies, particularly when the shoot function and canopy sampling position samples were pooled, indicates a potential response in the canopy to N cycling at the different sites. This is suggested as a response to N cycling at the different sites.
- Finally, the correlation between $\delta^{15}N$ and $\delta^{13}C$ at the pooled sites indicates how increasing foliar $\delta^{15}N$ was related to increasing WUE (less negative $\delta^{13}C$), showing potentially how N availability across sampled orchards influences growth. Further work would be required to better understand the mechanisms behind soil and foliar N and $\delta^{15}N$ relationships.

Key Messages

The application of natural stable carbon, nitrogen and oxygen isotopes in this project has indicated potential differences between tree and orchard WUE, with genetics and irrigation, and orchard N cycling. These techniques offer a tool to further progress our understanding in the relationships between productivity and water and N use in horticultural tree crop breeding and orchard management. These tools may be useful to explore in horticultural tree crop breeding and intensification programs in Queensland. This seems a critical step to increasing the Horticultural industry's efficient use of water and N, particularly in catchments flowing into the Great Barrier Reef.

Where to next

There is potential to further explore the use of stable carbon isotopes in breeding lines for horticultural crops in Queensland. This is important with more and more orchards destined to be established in more marginal areas and as water resources become more scarce and expensive. Further testing of tree specific water use and agronomic WUE would also be important with links to sensor measurement of water use in orchards and help growers better understand how much water trees are using and how much they need. A commercial partner has already expressed interest in working in with the author to test a range of tree water use monitoring instruments in horticultural tree crops.

There is also some interest with our collaborator Griffith University, Environmental Futures Centre to continue this collaboration and to better understand the mechanism behind these results with the request to provide site access to a student in their Masters' program to build on this research in a project on "The use of C and N natural stable isotopes, NRMS and functional gene discovery in horticultural tree crop orchards looking at WUE, N cycling and fruit quality". This type of work has potential to lead to a potential future ARC grant. Some seed funding by DAF would be requested to further these opportunities.

Budget Summary

Contractors Operational (excludes Payroll tax)	847.11
***Travel Accommodation & Meals**	17.30
***Travel Expense Other**	205.30
***Travel Expense Other Total**	76.37
***Travel Airfares Total**	415.71
Freight and cartage	288.35
#(inc. Animal feed)	18.55
Analytical and data collection services	4,764.00
#(inc. under Lab consumables)	2,520.00
Lab consumables	9.09
Star pickets	12.36
Total cost	9,174.14

N.B.*Travel was to present the information at a Horticultural forum ' Future of Horticulture' in Brisbane plus 1 night accommodation.

#The preceding heading includes these costs as they have incorrect Cost collectors attributed to them.

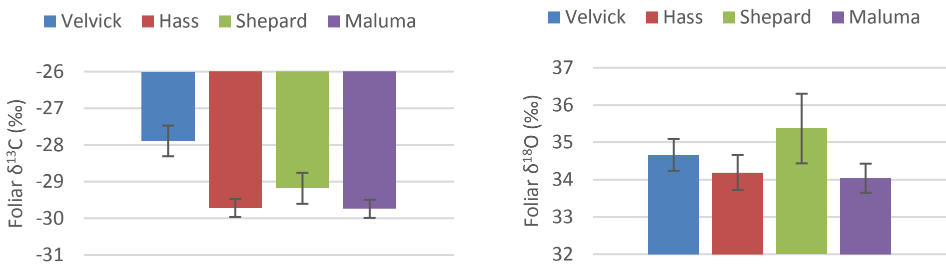
Table 1 – Means for Foliar $\delta^{13}C$ (‰), $\delta^{15}N$ (‰), $\delta^{18}O$ (‰), N (%) and C (%) for six month old seedlings of four avocado varieties Velvick, Hass, Shepard and Maluma grown under nursery condition in Walkamin in Far North Queensland. (95% confidence limits in parenthesis).

Variety	Foliar $\delta^{13}C$ (‰)	Foliar $\delta^{15}N$ (‰)	Foliar $\delta^{18}O$ (‰)	Foliar N (%)	Foliar C (%)
Velvick	-27.89 (-28.31, -27.47)	-2.437 (-3.283, -1.591)	34.66 (34.23, 35.08)	2.807 (2.681, 2.932)	47.01 (46.11, 47.91)
Hass	-29.72 (-29.96,- 29.47)	-2.34 (-3.348, -1.332)	34.19 (33.72, 34.66)	2.218 (2.105, 2.331)	47.24 (46.13, 48.36)
Shepard	-29.18 (-29.6, -28.75)	-2.797 (-3.537, -2.058)	35.37 (34.43, 36.3)	2.198 (1.979, 2.418)	46.43 (45.04, 47.81)
Maluma	-29.74 (-29.99, -29.49)	-2.127 (-3.591, -0.6636)	34.04 (33.63, 34.43)	2.292 (2.09, 2.493)	46.13 (44.71, 47.56)

Image 1– Location of (a) the study, (b) the irrigation sites and (c) the genetics, shoot function and canopy position experiment in Far North Queensland.

(a) (b) (c)

Figure 1 – The foliar (a) δ^{13}C and (b) δ^{18}O for four commercial Avocado varieties grown in nursery condition in Far north Queensland.



(a) (b)

Figure 2 – The relationship between foliar δ^{13}C ‰ and δ^{18}O for four commercial Avocado varieties grown in greenhouse condition in Far north Queensland.

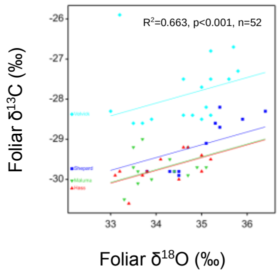


Figure 3 – Means and 95 % confidence intervals of (a) foliar δ^{13}C (‰) and (b) foliar δ^{15}N (‰) under two irrigation types for Turner Hass, Shepard and Maluma avocado varieties, at two commercial orchards, in Far north Queensland.

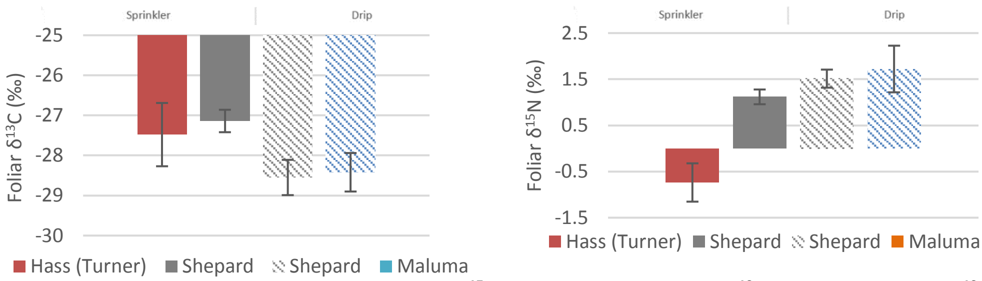
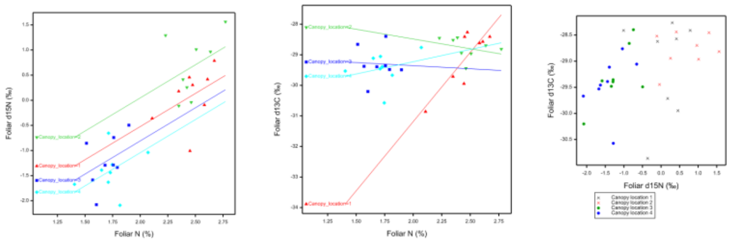


Figure 4 – The relationships between (a) foliar N and δ^{15}N, (b) foliar N and foliar δ^{13}C (‰) and (c) foliar δ^{13}C and δ^{15}N, combining different shoot positions (northerly [1] and southerly [2] aspects), and function (vegetative [3] and fruiting [4]), for the avocado variety Shepard in two avocado orchards, in Far North Queensland.



(a) **(b)** **(c)**