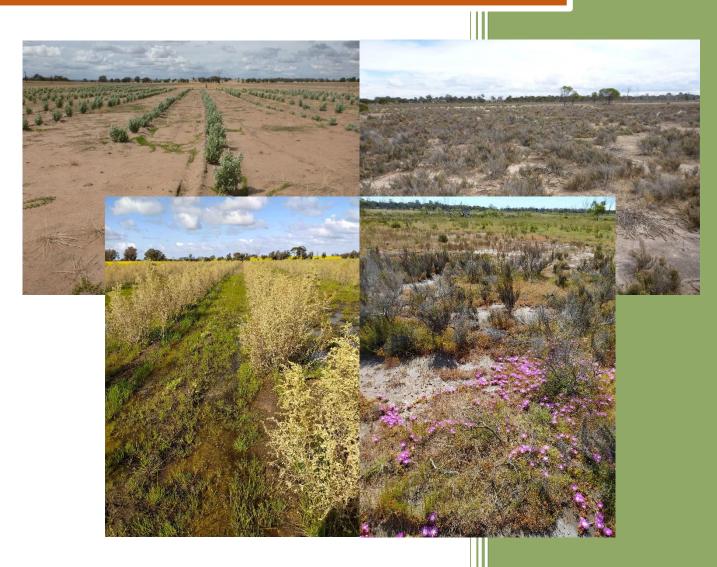
# Saline Bush Foods - Developing a Paddock to Plate Supply Chain to Restore Degraded Land, Badgebup WA

# ENVIRONMENTAL REPORTING TRAINING DAY HANDOUT







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Dr Bede Mickan (Contributed the DNA scope of work)

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## INTRODUCTION

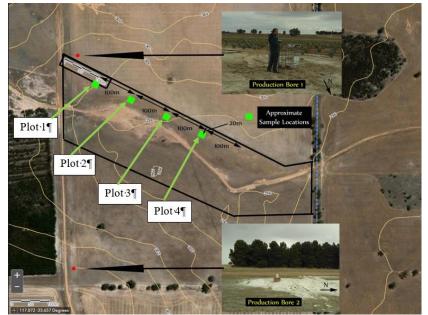
The Environmental Scope of the project was focused on two production strategies:

- 1. Plantation Site "salt scalded" bare area approximately 200m long x 80m wide immediately north of a saline drainage area between two broad acre cropping fields. Planted as a pseudo-monoculture of saltbush in rows in 2019.
- 2. Wild harvest from existent plants in a lower lying saline drainage area with existing native halophytes that have been previously used for wild harvests (ice-plant, samphire, pigface), was scarified using a tractor mounted rake in Autumn 2020 to promote new growth. Non-scarified area left to serve as control.

Samples were taken at end of rainy season in 2020 and 2021.

This assessment focused most particularly on the observable impacts on:

- Soil health i.e. salinity, carbon, nutrient availability, microbial activity.
  - Groundwater i.e. salinity variation, plant salinity extraction, water depth.
- Plant behaviour i.e. soil coverage, plant profile, defining habitat creation.



#### Plantation Project Area Map

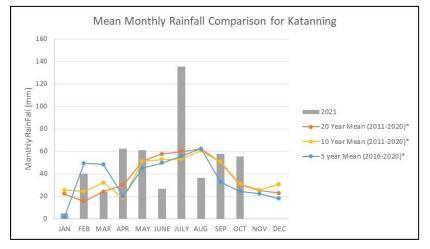
Wild Harvest Project Area Map



# **RESULTS AND CONCLUSIONS**

**Project Context:** data provided was collected with just 24 months of plant development following significant plantation site disturbance and is an indication of potential direction of future change.

**Climate context:** higher and later rainfall than typical for region in winter/spring 2021 highlighted the importance of the comparison of the Plantation Site results across the three years of sampling in absence of a control area and retention of control (non-scarified) area in Wild Harvest Site.



Comparison of Monthly Rainfall for Katanning –5, 10, and 20 year historical averages. \*Note results are averaged from available data points within Bureau of Meteorology data sets (http://www.bom.gov.au/isp/ncc/cdio/weatherData - Station 010916)

# 1. Plantation Site Base Line Results – 2019

The analysis identified high salinity soil and poor nutrient availability compared to a standard agricultural expectation highlighting appropriateness of location for alternate production options. High level summary of trial commencement soil:

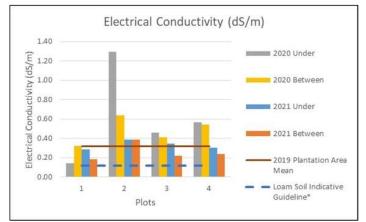
- Very high sulfur ~3 times indicative guidelines.
- Generally high magnesium (Mg), Sodium (Na), Sulfur (S), and the micronutrient iron (Fe).
- Moderately low pH.
- Moderately high Electrical Conductivity (EC).
- Moderately low carbon (C).
- Generally low calcium (Ca), potassium (K), phosphorus (P), aluminium (Al), hydrogen (H), and the micro nutrients zinc (Zn), manganese (Mn), copper (Cu), Boron (B), and Silicon (Si).
- Low nitrogen (N) content relative to carbon content, lower nitrate relative contribution compared to ammonium (and relative to Laboratory Indicative Guidelines See Appendix 2).
- Low Ca and high Mg has led to low Ca/Mg ratio results.

Plot Specific Notes:

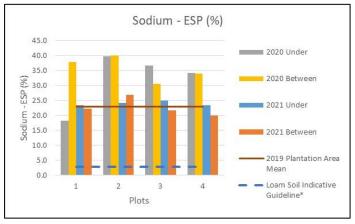
- Plot 1 Lowest Mg across the board.
- Plot 2 Was often different other plots: high nitrogen outliers; higher/recommended Exchangeable Calcium compared to all other plots (multiple samples within plot had this higher value); and lower Phosphorus.
- Plot 3 higher Phosphorus.
- Plot 4 Lower Base Saturation calcium; and extremely high Nitrate Nitrogen outlier.

# 2. Plantation Site Results - 2020/2021

**SALINITY** – in 2020 indicators of salinity (electrical conductivity, exchangeable sodium, and exchangeable sodium as a percentage (ESP) of effective cation exchange capacity (ECEC)) are all higher under plants rather than between. Most likely the tilling of soil bringing up more saline subsoils combined with plant coverage partially protecting area from rainfall - reducing soil washing effect. Whilst under plant samples are higher than between plant samples, 2020 values were in excess of 2019 levels with the exception of Plot 1. By 2021 these salinity indicators had dropped back to towards 2019 level, suggesting a recovering system from the initial impact of soil inversion / establishing a new trajectory. Except Plot 1 under, all results saw a reduction in EC from 2020 to 2021. ESP displayed a similar trend, but with values in excess of the 5% indicates a potential salt issue (See below graphs).



Electrical Conductivity Comparison across Plots.



Exchangeable Sodium Potential Comparison across Plots.

**GENERAL** – If it is assumed that the 2020 to 2021 trajectory best represents the recovery of the Plantation Site system, then this snapshot indicates (See Graphs over page):

- Increasing 2020 to 2021, total data set total nitrogen %, exchangeable calcium, exchangeable aluminium, calcium %, aluminium %, hydrogen %, Manganese and Copper
- Decreasing 2020 to 2021, total data set Electrical Conductivity, sulfur, exchangeable sodium, and sodium %.
- The total nitrogen, aluminium (exchangeable and total %), and calcium % increasing results were replicated both under and between plants.

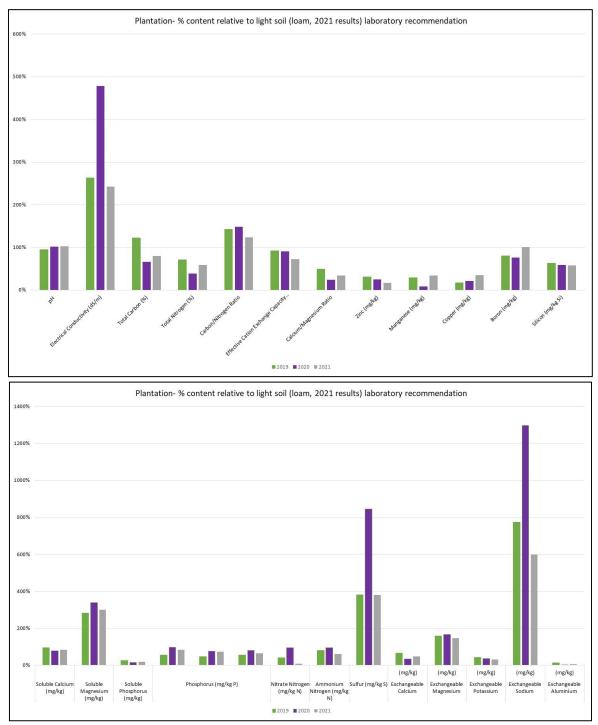
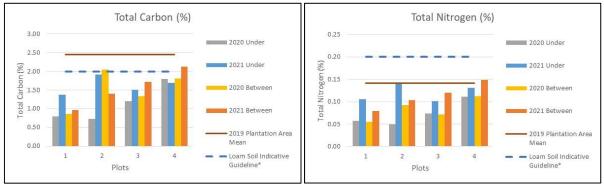


Figure 4.2.1 (a) and (b): Plantation Site - Average 0-10cm sample data as a Percentage of the Laboratory Indicative Guidelines- Part 1

**CARBON** - By 2021, the total carbon was re-established following the initial drop due to the planting preparation process. Future carbon behaviour is anticipated to continue increasing. Salt bush harvesting, excluding the incident of compaction (risk of increased traffic), should promote soil carbon through regular root shedding as plant foliage is pruned and promotion of new root systems during regrowth. The 2021 total nitrogen demonstrated a similar trend to the carbon, with 2021 data sets all higher than 2020 and approaching 2019 original levels. This highlights the importance of minimum tillage and soil coverage to minimize the impact of the topsoil's (and its ecosystems) exposure to diurnal / seasonal weather extremes and the potential for erosion - a short-term risk of commercial vs rehabilitative planting. The soil coverage results bode well for preservation and growth of the soil flora and fauna community, further increasing both carbon and the system's resilience to extreme events impacting plant and soil health.

**UNDER VS BETWEEN** – the impact of planting may have only been partially reversed by 2021 with soil results indicating differences (based on a 3 of 4 plot criteria) to be:

- Under plants higher than between pH, EC, nitrate nitrogen\*, ammonium nitrogen\*\*, calcium (sol., exch.) \*, magnesium (exch., %), potassium (sol., exch.), phosphorus (soluble, Colwell, Bray 2)\*\*, sulfur\*, ECEC, sodium (exchangeable and ESP), and silicon.
  - \*additional to 2020 results.
  - \*\*different relationship compared to 2020 results.
- Between plants higher than under exch. aluminium, total magnesium, aluminium %, calcium / magnesium ratio, zinc, iron, and copper.
- Notably total carbon was higher between plants than under in 2020 which was counterintuitive. It suggested the tilling prior to planting may have induced a decline in carbon rather than the anticipated increase with plant growth. By 2021 there was no longer a consistent total carbon % trend the 2 more western plots had lower content between compared to under and the 2 eastern plots had opposite. Note that total carbon percentage was still significantly below 2019 sample levels. Given this observation the tilling induced decline in carbon would appear confirmed. This potential alternate impact/variable, beyond the analysis of the role the plant presence has on soil carbon, highlights the caution required in drawing conclusions within a short term project timeframe. Total nitrogen results were also inconsistent. (See below graph)



Total Carbon %

Total Nitrogen %

with inconsistent presentation within the 4 plots and compared to the 2019 Plantation Site mean. 2020 Vs 2021

# 3. Plantation Site Soil Bacteria DNA Diversity Profiling -2020/2021

**ALPHA DIVERSITY** - the mean diversity of species in different sites or habitats within a local scale - in 2021, showed an increase for samples extracted under relative to between plants.

**BETA DIVERSITY -** measuring similarity or dissimilarity of two communities - showed differences of community composition within sample location (under Vs between) and soil depth (0-10 Vs 10-30cm) in both 2020 and 2021. Phylum level (broad scale) relative abundance for 2021 displayed increases in Actinobacteria, Firmicutes and Proteobacteria in the 0-10cm soil profile.

**CARBON CYCLING GENES** – (1) Catalase - bacteria with potential to mineralise harder carbon compounds (e.g. lignin) - in 2020 and 2021 identified an increase within the 0 to 10cm soil profile. (2) Beta-galactosidase - bacteria with potential to mineralise easily available carbon - In 2021, 0-10cm depth, trend increasing irrespective of sample location (under/between), (3) Beta-glucosidase degrading capacity (e.g. ability degrade plant or microbe derived cellulose) increasing trend for under the plants, for both soil profiles.

**NITROGEN CYCLING GENES** – N fixation and both nitrification (amoB, HaO) in 2020 was greater at soil depth 10 to 30cm. By 2021 Minor increases in N fixation (nifD) and a decrease in denitrification (nirK) at the deeper soil depth 10 to 30cm were noted. Nitrogen cycling potential was not impacted by sample location (under or between plants).

All changes to soil microbiology at Plantation Site indicate minor positive improvements between 2020 and 2021 likely driven by the increased plant biomass above ground impacting both soil depths, with greater enhancement directly under individual saltbush plants as a function of time.

# 4. Production Well Data

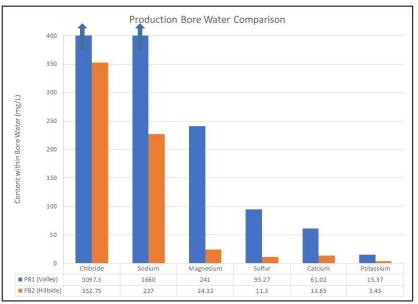
**WATER SAMPLES** - The two water production wells (Production Bore (Valley) 1 (PB1), Production Bore (Hill) 2 (PB2)) were installed adjacent to the Plantation Site in February 2020. The EC data of water samples and their passage through the greenhouse is presented in Table 4.2.6 and identifies the PB1 EC at ~14% that of sea water and classified as within the drinking tolerance for sheep (7.5-14.9 dS/m), but at the cusp of salinity for salt tolerant crops (8.1 dS/m) (Ref: Measuring salinity (publications.qld. gov.au)). From the data the Heart-leafed Ice-plant 1 and Slender Ice-plant have the lowest EC results within the growing medium and hence potentially show the highest salt extraction potential. Note: the comparative samples between Production Bore 1 and Production Bore 2 shows Bore 2 to have ~14% that of Production Bore 1 and more of a brackish water EC. (See below table).

r EC of Plant Growing Wealum Post Irrigation & Plant Gro						
Raw	Corrected					
0.3	0					
	55					
	9.03					
	1.34					
8.2	7.9					
0.6	0.3					
0.3	0					
Raw	Corrected					
7.9	7.6					
7.5	7.2					
7.4	7.1					
1.6	1.3					
4.2	3.9					
2.5	2.2					
	0.3 8.2 0.6 0.3 7.9 7.5 7.4 1.6 4.2					

Greenhouse Water EC o	f Plant Growing Me	dium Post Irrigation &	Plant Growth. (04/03/2021)

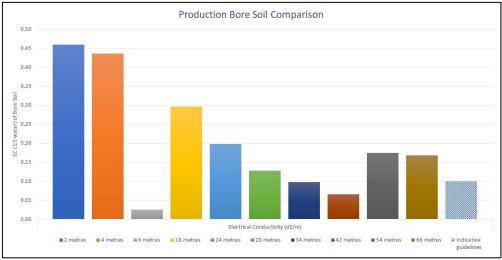
Ref\* - https://www.agric.wa.gov.au/soil-salinity/measuring-soil-salinity

Comparison of the bore water samples indicate that the mineral content and EC of PB1 (Valley) was substantially higher than that of PB2 (Hill) only 500 metres away with the exception of phosphorus. The pH of PB1 was slightly lower that than of PB2 (PB1 pH = 6.8, PB2 pH = 7.1). (See below graph).



Plantation Site Production Bore Water Sampling Results.

**SOIL SAMPLES** - During the production bore drilling process, soils were extracted and deposited on the surface representing soil characteristics encountered every two meters. The soil texture transitions from a sandy loam soil at 2 metres depth through a sandy clay loam (6, 24 metres) to a loamy sand at 54 meters. The EC had a moderately consistent decreasing trend to 42 m suggesting the impact of salinity within run-off/surface waters infiltrating – i.e. the delivery of ions to depth reducing due to reducing bulk penetration depending on rainfall and attraction/filtration of ions as the water moves down through the soil. Alternately this may be an impact of agricultural practices similarly washed down through the soil profile. The step change at 54m reinforced by the 66m analysis suggests the potential capillary action of ions from the ground water on contact or from fluctuating level / rainwater over time to the present day. Note: Production Bore (Valley) 1 (PB1) has a pumped water EC of 9.03 dS/m thus the wicking impact of the capillary action was seen at these depths. (See below graph).This salinity profile was similar to the effective cation exchange capacity (ECEC) data.

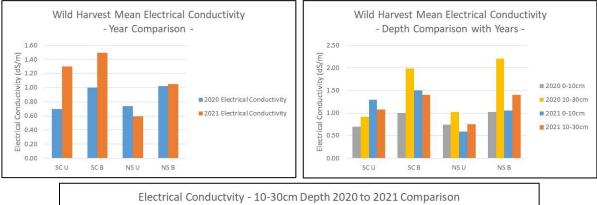


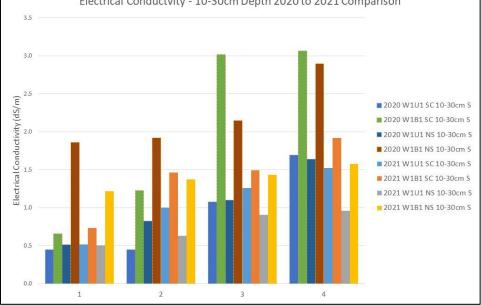
Plantation Site Production Bore Soil Sampling Electrical Conductivity Results to Depth.

# 5. Wild Harvest Site Results - 2020/2021

**SALINITY** – All results, irrespective of depth, plant proximity or scarification indicated elevated salinity compared to the laboratory recommended guideline. There was a persistent presentation of higher salinity indicators within the scarified compared to non-scarified areas. The indicators of salinity (EC and sodium contents) were significantly higher between the plants compared to under them at both depths. This provides an indication of the natural state in the undisturbed environment. Whilst the ECEC (reflected in the exchangeable sodium content) demonstrated a rise from 2020 to 2021 within both the under and between locations of the scarified area, whilst the non-scarified remained relatively consistent. (See graphs over page).

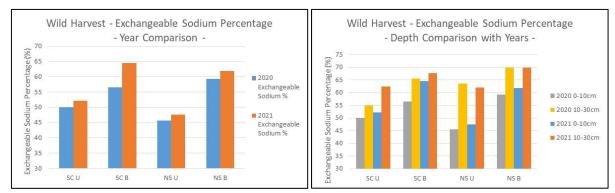
For the under plant subset, the 0-10cm depth the electrical conductivity were all higher within the scarified area. The higher exchangeable sodium and ECEC in the scarified compared to non-scarified area was replicated in the 10-30cm depth. Within the 2021 sampling regime there was a statistically significant difference identified between the electrical conductivity (EC) of the scarified and non-scarified soil samples taken from the shallow depth soil under plants (1.30dS/m and 0.59dS/m respectively). Additionally both in the 0-10cm depth and the 10-30cm depth, there was a significantly higher EC identified between the plants as opposed to under them (0.59dS/m to 1.05dS/m and 0.75dS/m to 1.40dS/m respectively). Between the 2020 and 2021 sampling, there was an overall increase in the EC for the total data set and for the scarified data set (0.79dS/m to 1.03dS/m and 0.78dS/m to 1.4dS/m respectively. 10-30cm samples had a lower EC in 2021 compared to 2020 for the scarified area, whereas the inverse was true for the non-scarified area.



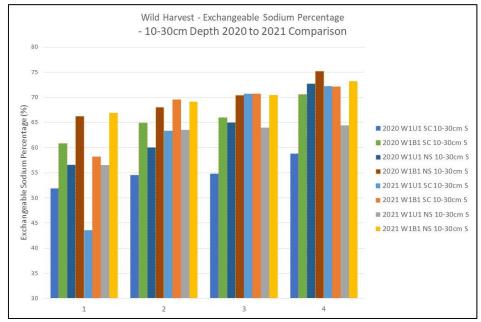


Electrical Conductivity Comparison between Scarified and Non-Scarified.

The exchangeable sodium identified the total data and the scarified under plant data to have a significant increase from 2020 to 2021 (867 to 1080mg/kg and 743 to 1289 mg/kg respectively). The ESP identified the scarified data, the between plant data and the scarified between plant data to have increased between 2020 and 2021 (53 to 60%, 56 to 64% and 56 to 65% respectively). All results, irrespective of depth, plant proximity or scarification indicated elevated salinity compared to the laboratory recommended guideline of 23.5mg/kg and 3.3% dS/m sandy soils). (See graphs below and over page).



Exchangeable Sodium Potential Comparison between Scarified and Non-Scarified – Part 1.



Exchangeable Sodium Potential Comparison between Scarified and Non-Scarified – Part 2

**GENERAL** – The Wild Harvest Site had typically a lower available and total soil nutrient content than the Plantation Site. If it is assumed that the 2020 to 2021 trajectory best represents the recovery of the Plantation Site system, then this snapshot indicates (See graphs over page): Total Data Sets:

- 2021 higher than 2020
  - Scarified electrical conductivity (EC) (0.78 to 1.40dS/m), total nitrogen % (0.035 to 0.053), effective cation exchange capacity (ECEC, 7.1 to 10.2 cmol+/kg), sodium ESP % (53% to 60%), and silicon (19mg/kg to 25mg/kg).
  - Non-Scarified total nitrogen % (3.3% to 5.1%), aluminium % (0.13 to 0.31%), Manganese (0.7mg/kg to 1.6mg/kg), Boron (0.7mg/kg to 1.1mg/kg) and silicon (14mg/kg to 30mg/kg).
- 2020 higher than 2021
  - Scarified carbon nitrogen ratio (20.4 to 15.6), nitrate nitrogen (4.06mg/kg, to 0.94mg/kg), and potassium % (2.0% to 1.4%).
  - Non-Scarified Nil
- 2021 higher than 2020
  - Under total nitrogen % (0.042 to 0.056), aluminium % (0.14 to 0.28%), Manganese (0.96mg/kg to 2.1mg/kg), Boron (0.95mg/kg to 1.5mg/kg), and silicon (18mg/kg to 33mg/kg).
  - Between total nitrogen % (0.027 to 0.046), sodium ESP% (56% to 64%) and silicon (15mg/kg to 25mg/kg).
- o 2020 higher than 2021
  - Under carbon nitrogen ratio (20.1 to 14.8), and plant available phosphorus (11.8mg/kg to 6.5mg/kg).
  - Between carbon nitrogen ratio (20.5 to 15.7), Nitrate Nitrogen (2.6mg/kg to 0.95mg/kg.), calcium % (13.1% to 9.3%), and magnesium % (27% to 65%).

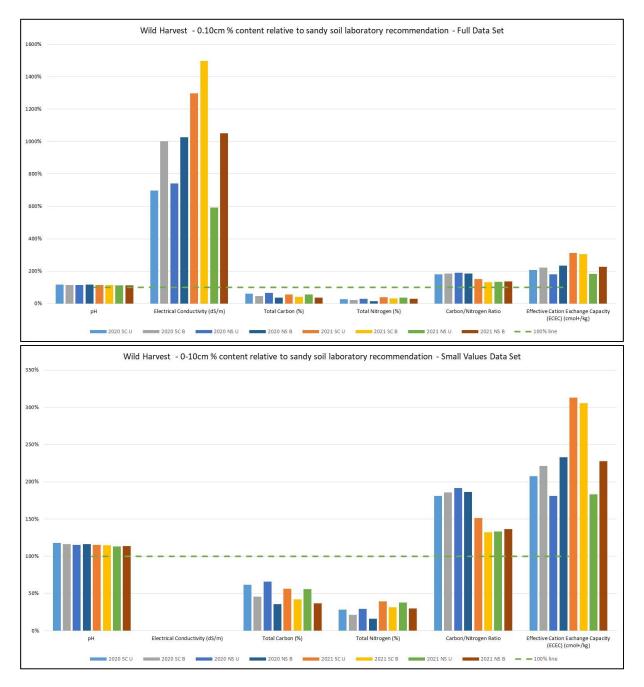
#### Scarified data set:

- $\circ$  2021 higher than 2020
  - Under Exch. magnesium (214mg/kg to 313mg/kg), exch. sodium (743mg/kg to 1289mg/kg), and effective cation exchange capacity (ECEC, 6.9 to 10.3 cmol+/kg).
  - Between sodium ESP % (56% to 65%), and silicon (16mg/kg to 26mg/kg).
- $\circ$  2020 higher than 2021
  - Under nitrate nitrogen (5.27mg/kg, to 0.92mg/kg).

Between - carbon nitrogen ratio (20.4 to 14.5), and nitrate nitrogen (2.9 mg/kg, to 0.96mg/kg).

Non-Scarified data set:

- o 0-10cm data 2021 higher
  - Under for total carbon %, sol. calcium, exch. calcium, calcium %, magnesium%, potassium %, aluminium %, calcium/magnesium ratio, boron content and silicon content
  - Between electrical conductivity (EC), sulfur content, exch. sodium, the effective cation exchange capacity (ECEC) and exch. sodium % (ESP).
- 2020 higher than 2021
  - Under nitrate nitrogen (5.27mg/kg, to 0.92mg/kg).
  - Between carbon nitrogen ratio (20.4 to 14.5), and nitrate nitrogen (2.9 mg/kg, to 0.96mg/kg).



**CARBON** – The under plant total carbon was higher than between plant for 0-10cm depth in the non-scarified area in 2020 - highlighting the benefit of root systems in promoting and protecting soil flora and fauna (represented by carbon measurement). This indicates that the plant coverage was developing and protecting carbon stores pre-scarification. In 2021, where no significant statistical difference was determined between the scarified and non-scarified samples, it suggests that with coverage, the system continues this recovery. However it is noted that for the non-scarified area, a higher carbon content was identified under the plants compared to between in 2021 suggesting that the more plants that are available in the long term, the higher the system's total carbon. However, the scarified area had minimal plants compared to the non-scarified area due to the scarification process suggesting a far lower average mass of soil carbon per unit area. The bush layer as opposed to the current, fine leaved ground cover / individual small plants is anticipated to take many years to return to pre-scarification levels.

#### UNDER VS BETWEEN -

For the comparison of total data set for the 2021 sampling (See graphs over page):

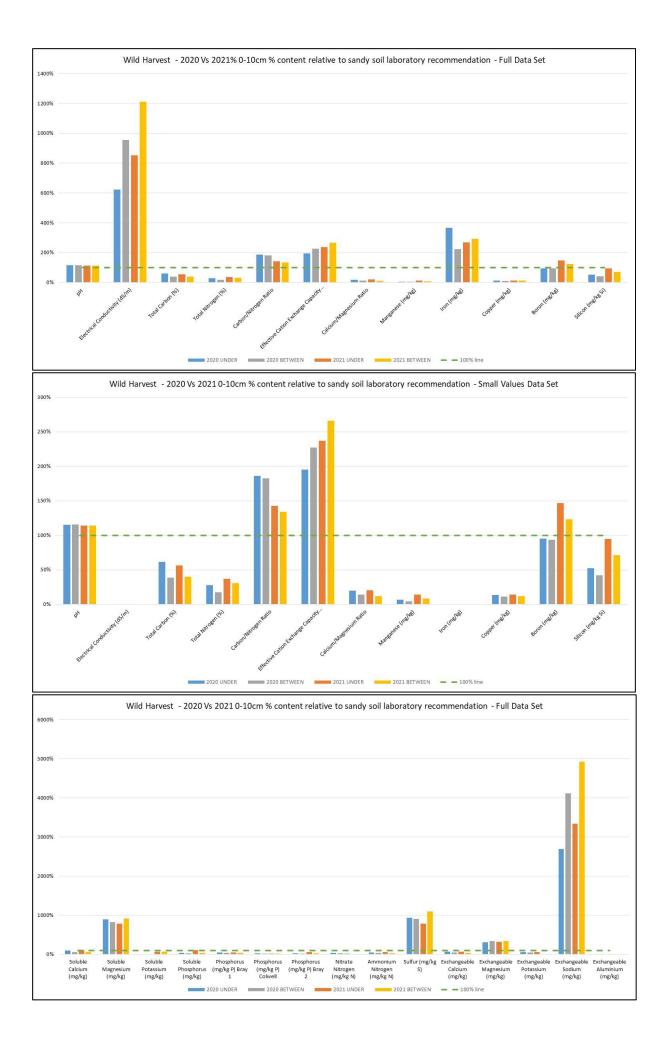
- Under higher than between:
  - 0-10cm total carbon (0.79% to 0.56%), sol. calcium (197mg/kg to 99mg/kg), exch. calcium (270mg/kg to 160mg/kg), Magnesium % (28% to 25%), Potassium % (2.2% to 1.1%), calcium/magnesium ratio (0.64 to 0.37) and manganese content.
  - 10-30cm Electrical Conductivity (EC, 0.91dS/m to 1.40dS/m), aluminium % (0.23% to 0.16%), and calcium magnesium ratio (0.45 to 0.26).
- Between higher than under:
  - 0-10cm Exch. sodium (913mg/kg to 1247mg/kg), and ESP % (50% to 64%).
  - 10-30cm Sol. magnesium (172mg/kg to 229mg/kg), sulfur (61mg/kg to 95mg/kg), exch.magnesium (174mg/kg to 271mg/kg), exch. sodium (863mg/kg to 1245mg/kg), effective cation exchange capacity (ECEC, 6.5cmol/kg to 9.5cmol/kg), and sodium ESP% (65% to 70%).

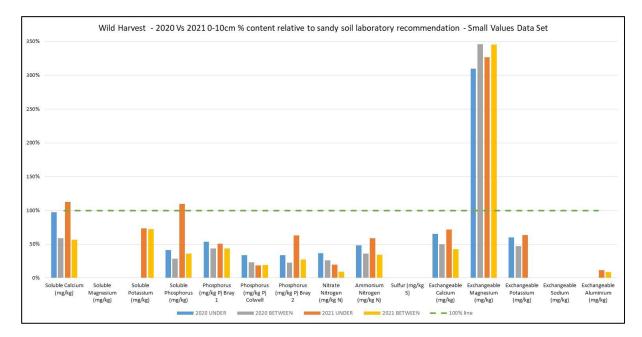
For the comparison of under plant data set for the 2021 sampling:

- Scarified higher than Non-Scarified / Control:
  - 0-10cm EC (1.3dS/m to 06dS/m), exch. magnesium (313mg/kg to 220mg/kg), exch. sodium (1290mg/kg to 670mg/kg), and ECEC (10.3cmol/kg to 6.1cmol/kg).
  - 10-30cm pH (7.7 to 7.0), exch. sodium (1105mg/kg to 755mg/kg), and ECEC (7.7cmol/kg to 5.3cmol/kg).
  - Non-Scarified higher than Scarified / Control:
    - 0-10cm Potassium % (1.7% to 2.7%) only.
    - 10-30cm Aluminium % (0.19% to 0.31%).

For the comparison of between plant data set for the 2021 sampling:

- Scarified higher than Non-Scarified / Control:
  - 0-10cm exch. sodium (1523mg/kg to 1074mg/kg), and ECEC (10.1cmol/kg to 7.5cmol/kg).
  - 10-30cm sol. calcium (114mg/kg to 81mg/kg), exch. calcium (144mg/kg to 106mg/kg), and silicon content (22mg/kg to 16mg/kg).
- Non-Scarified higher than Scarified / Control:
  - 0-10cm Nil.
  - 10-30cm Aluminium % (0.19% to 0.31%).

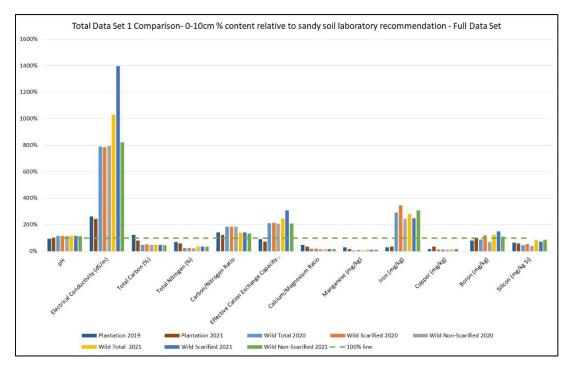


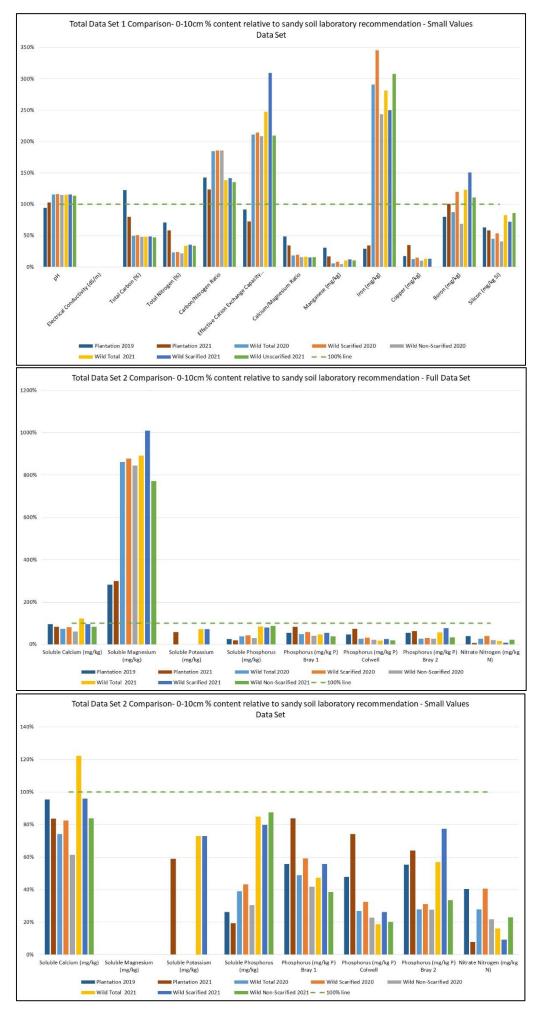


#### PLANTATION SITE TO WILD HARVEST SITE COMPARISON -

The Plantation and Wild Harvest Site soils were very different (See below and over page graphs):

- pH Plantation Site increased in pH from 2019 to 2021 (95% to 103%), but remained less than the Wild Harvest Site (114-116%) which was relatively consistent from 2020 to 2021.
- Electrical Conductivity (EC) Plantation Site decreased from 2019 to 2021 (263% to 243%), but was always below the Wild Harvest Site which increased from 2020 (783%-795%) to 2021 (822-1397%).
- Total Carbon Plantation Site decreased from 2019 to 2021 (123% to 80%), but was above the Wild Harvest Site (47-51%) which was relatively consistent from 2020 to 2021.
- Total Nitrogen Plantation Site decreased from 2019 to 2021 (71% to 58%), but was above the Wild Harvest Site which increased from 2020 (22-24%) to 2021 (34-36%).
- Effective cation exchange capacity (ECEC) Plantation Site decreased from 2019 to 2021 (92% to 72%), but was below the Wild Harvest Site which had a dominant increase in the scarified area from 2020 (214%) to 2021 (309%) with the non-scarified remaining similar.





# 6. Wild Harvest Site Soil Bacteria DNA Diversity Profiling -2020/2021

**ALPHA DIVERSITY** - the mean diversity of species in different sites or habitats within a local scale – in 2021, showed no major between in scarified and non scarified soils in either the 0 to 10 cm or 10 to 30cm soil profiles. This indicates no negative impact of such a soil disturbance on the mean diversity of species present. However, deeper soil profiles (10 to 30 cm) saw less diversity between than under plants, indicating the plant species above ground impacting the deeper soils with a positive influence on soil bacteria diversity.

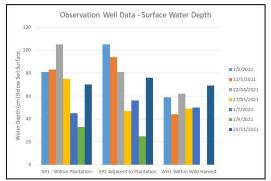
**BETA DIVERSITY** - measuring similarity or dissimilarity of two communities - showed a dynamic impact of scarification on Actinobacteria for both under and between plants, and also that diversity was higher with scarification in 2020, but was decreasing with scarification in 2021. The initial increase is likely driven by greater exposure of organic matter and water availability in 2020 as this key phyla plays fundamental roles in organic matter decomposition. The 2021 decrease may be due to the system stabilising. Examining broad scale communities between compared to under the plants within the 0 to 10 cm soil profile. No difference was seen between scarified and non-scarified comparisons in the deeper soils.

**CARBON CYCLING GENES** – (1) In the 2020 shallow and deep soil profiles no impacte of scarification was seen, although in the shallow soil profile there was increases in most carbon cycling genes under the plant compared to between. (2) Glucoamylase – bacteria with the potential to mineralise the easily digestible carbon cycling capacity starch - In the 2021 shallow soil profile (0 to 10cm) there was a net increase under the plant. (3) Endoglucanase produced a similar trend of reduction of hemicellulose for the same treatment (0 to 10cm, under plant). (4) Betaglucosidase - bacteria able to degrade cellulose - was also seen to increase. Whilst there was increasing potential carbon cycling capacity in the soil through the analysis of these bacteria, the alterations were not impacted by scarification and were only minor fluctuations, hence having little overall impact to soil biological processes. (5) A minor impact for soil carbon cycling process occurred at the deeper soil profile (10 to 30cm) within the sample location in relation the plants. Indeed, only one significant result was observed at this depth and it was an increase in the ability to degrade more recalcitrant carbon (e.g. lignin). There was no impact at to any other carbon cycling potential, and the impact of the land management practice of scarification showed little effect.

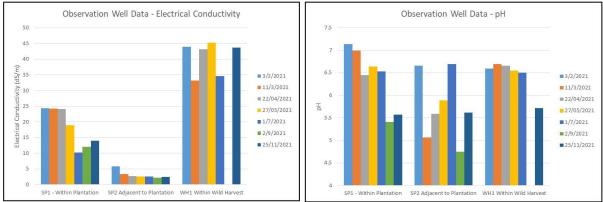
**NITROGEN CYCLING GENES** – The full suite of nitrogen cycling capacity was observed, and is indicative of a perfectly functional soil able to perform all levels of nitrogen cycling. The predicted nitrogen cycling capacity was marginally impacted in both 2020 and 2021 between the scarified and non-scarified locations, with the denitrification (narG, nirK) showing both increases and decreases for sample location for both under and between plant location. Overall, there appears to be no adverse impact over the sampling time period to soil scarification, however the analysis of the ratio of the total area under plants compared to between in the scarified versus non-scarified locations – i.e. adding weighting to the conflicting results - may have rendered a tangible impact.

# 7. Observation Well Water Data

Seven water sampling regimes were completed throughout 2021. As only one year's data is available, the data is provided as baseline information only. (See below and over page graphs).



Observation Well Data: Surface Water Depth Comparison Over 2021.



Observation Well Data: Electrical Conductivity (dS/m) and pH Comparison Over 2021.

#### 8. Plant Tissue Analysis

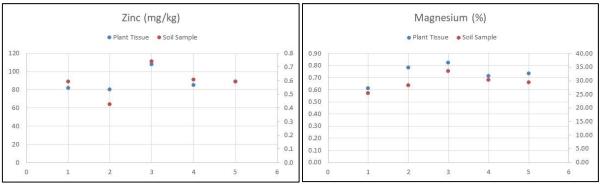
Samples of Plantation Site Saltbush were collected in August 2021 at the same time as the final soil sampling regime and plant coverage study. Salinity within the Wheatbelt Region is typically associated with sodium chloride. The sodium content of the leaves had a mean of 6.23% (low 4.90% Plot 1 to high 6.99% plot 4) and had a consistently increasing trend from west to east across the Plantation Site. Saltbush can have a leaf salt content of up to 28% (AWI & CRC Salinity, 2006). The Chloride content mean was 64,588mg/kg (6.46%, low 4.87% Plot 1, high 7.33% Plot 3). The comparative study (Norman et al., 2004) had a sodium content range of 6.89 - 7.25% and 5.93 - 7.04%; and a chloride range of 11.6-11.8% and 10.3-12.35% in the Old Man and River Saltbush respectively (See below table).

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Parameter	Plantation Plot 1	Plantation Plot 2	Plantation Plot 3	Plantation Plot 4	Mean
Nitrogen (%)	4.60	4.87	4.60	4.55	4.66
Phosphorus (%)	0.66	0.55	0.85	0.83	0.72
Potassium (%)	2.46	2.62	2.42	1.93	2.36
Sulfur (%)	0.41	0.45	0.42	0.42	0.43
Carbon (%)	42.3	40.8	39.3	40.5	40.7
Calcium (%)	0.67	0.71	0.57	0.59	0.63
Magnesium (%)	0.61	0.78	0.82	0.72	0.73
Sodium (%)	4.90	6.15	6.88	6.99	6.23
Copper (mg/kg)	5.8	7.2	7.4	7.1	6.88
Zinc (mg/kg)	82	80	108	85	88.9
Manganese (mg/kg)	220	143	152	181	174
lron (mg/kg)	58	57	53	61	57.2
Boron (mg/kg)	30	33	38	36	34.4
Molybdenum (mg/kg)	3.2	3.4	4.0	2.9	3.36
Cobalt (mg/kg)	0.83	0.50	0.63	0.85	0.70
Silicon (mg/kg)	394	370	383	385	383
Nitrogen : Sulfur Ratio	11.1	10.9	10.8	10.9	11.0
Nitrogen : Phosphorus Ratio	7.0	8.9	5.4	5.5	6.69
Nitrogen : Potassium Ratio	1.9	1.9	1.9	2.4	2.00
Carbon : Nitrogen Ratio	9.2	8.4	8.5	8.9	8.75
Crude Protein (%)	28.8	30.4	28.8	28.4	29.1
Chloride (mg/kg)	48,698	65,226	73,329	71,101	64588

Table 4.13: Plantation Site Saltbush Leaf Tissue Sample Analysis

Other key minerals – Calcium mean was 0.63% (0.73 to 0.85%, Norman et al., 2004), Magnesium was 0.73% (0.77-1.17%, Norman et al., 2004) and Potassium was 2.36% (2.66 to 3.83%, Norman et al., 2004). The mean nitrogen content was generally consisted across the Plantation Site with a mean of 4.66%. Plot 2 had the highest value of 4.87%. Comparative data had Old man at 2.03 to 2.46% and River saltbush at 1.55 to 1.62. The mean carbon content was also generally consisted across the Plantation Site with a mean of 40.7%. Plot 1 had the highest value of 42.3%.

It was of interest to see that some plant tissue mineralogy mimicked the soil sample content across the plots (See graphs over page).

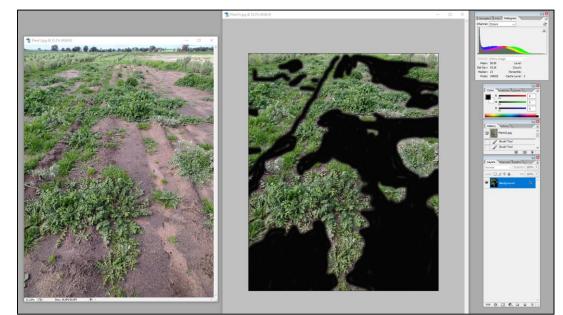


Zinc content (mg/kg) Magnesium content (%) Comparison of Plant (left axis) and Soil (right axis) for Plots 1 to 4 and the Mean (5).

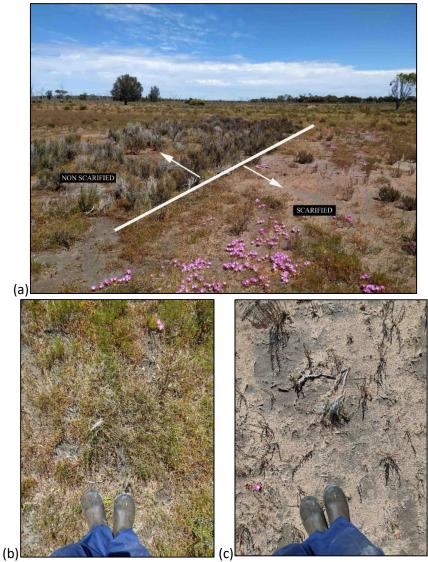
# 9. Soil Coverage and Plant Growth

**MEAN PLANT COVERAGE** - Mean plant coverage for Plantation Site soil increased from 70% in 2020 to 87% in 2021. Note: 0% is estimated at completion of Plantation Site preparation and 10-20% at completion of initial planting. However as no pre-preparation data is available, the total ecosystem impact cannot be determined. In 2020, Non-Scarified plots in Wild Harvest Site had greater coverage than the scarified (76% and 64% for Non-Scar Plot 1 and 2 respectively compared to 24% for Scarified). In 2021, the soil coverage differences between the scarified versus individual non-scarified plots were no longer significant, however the combined non-scarified areas demonstrated a higher coverage (78% versus 64%). In the 2021 data, the Plantation Site had a higher plant coverage than the Wild Harvest Site (87% compared to 74% respectively). Note: the examination of coverage does not account for the quality/ longevity/height of coverage which contributes to defining of environmental benefit and habitat/ micro-climate creation.

**MEAN TREE HEIGHT** - The Plantation Site mean tree height increased from 76cm in 2020 to 124cm in 2021. This indicated that even with harvesting occurring at various times in between the two sampling regimes, a generally larger vertical habitat was evident in 2021. With both a greater soil coverage and tree height, the increase in the habitat available which was visually evident has been reinforced through an objective analysis. Not surprisingly, in 2020 (4 months post scarification), the Wild Harvest plant heights for the Non-Scarified areas were ~ double that identified within the very limited number of plants in the Scarified area (51cm and 25cm height respectively). A similar relative presentation was recorded for 2021 (63cm Non-Scarified and 28cm for the Scarified areas). Plant height considered in conjunction with soil coverage demonstrates the significance of the reduced potential habitat immediately post the Plantation Site preparation or the Wild Harvest Site scarification (See below and over page figures).



Example of Plant Coverage Assessment



(a) Comparative Scarified to Non-Scarified coverage, (b) Example of Good Scarified Area Soil Coverage (c) Example of Poor Scarified Area Soil Coverage

# 10. Project Objectives Addressed

The outcome of this study has highlighted the conflicting interest within the short term of a saltbush regenerative program coupled with a saltbush harvesting program on ecological systems. The detrimental impact on the soil health as a result of Plantation Site preparation (tilling / soil exposure rather than rehabilitation-typical individual plant holes) had not been recovered from by the conclusion of the trial where soil health is measured in terms of soil carbon. The salinity indicators, the EC and sodium content of the 2021 analysed soils both under and between the plants was approximately equivalent to the 2019. Long-term however it is anticipated that salinity indicators will reduce and, with light harvesting, the soil carbon and available mineral content as well as the above ground coverage and vertical habitat creation will increase. In turn this will provide ecological and environmental benefits potentially in excess of that present prior to the project. It was noted that in the Plantation Site where water logging occurred, hampered plants growth in 2021 and where heavy pruning was implemented, this benefit was set back.

In the Plantation Site by 2021 there were increases in bacterial species richness for samples collected directly under the saltbush plants which is a positive outcome. This represents an increased biodiversity, and a greater functional capacity of the soil was inferred. Increases in major Phyla of Actinobacteria, Firmicutes, and Proteobacteria represent major groups of bacteria involved in carbon and nitrogen cycling processes. The key take home message is that across the Plantation Site, there was a positive impact on the soil biology between 2020 and 2021, evident in the enhance

bacterial diversity underneath the plants, as well increased carbon and nitrogen cycling capacity. This effect was duplicated at both soil depths. No pre-impact DNA data is available.

Within the Wild Harvest Site, scarification to enhance bush food plant growth demonstrated that, in the short term (and under the weather conditions of the project period), the ecological cost was significant with exposed soil subject to weathering and micro climate / habitat removal. This impact was marked by an overall increase in EC from 2020 to 2021 for the total data set which was dominated by increases in the scarified data set (0.79dS/m to 1.03dS/m and 0.78dS/m to 1.4dS/m respectively). The effective cation exchange capacity (ECEC, reflected in the exchangeable sodium content) as a salinity indicator also demonstrated a rise from 2020 to 2021 within both the under and between locations of the scarified area, whilst the non-scarified remained relatively consistent. The sodium content as a percentage (ESP) of the ECEC was lowest under plants, in the non-scarified soils and within the 0-10cm depth. With the larger area scarified and with the majority of plants within this area removed, the average salinity in the Wild Harvest Site has been significantly increased and the habitat markedly depleted within the short to medium term.

With respect to the DNA analysis, dynamic results of alpha and beta diversity for the Wild Harvest Site, and these data showed great variation between and within the sampling time periods. However, there was a clear outcome of scarification by 2021 having no detrimental impact to the soil bacterial community over the time frame of this trial. All changes to higher order phylum relative abundance appeared to be only minor as a result of scarification. This was reflected in the carbon and nitrogen cycling capacity, and may be due to the carbon and nitrogen cycling genes being detected in relatively high proportions within the soil bacterial community. Overall, the key message is that the management practice of scarification can be continued with no detriment to the soil biological health as assessed by the bacterial community as quantified through DNA sequencing and subsequent analysis of functional genes pertaining to carbon and nitrogen cycling potential.

# Ground water sampling was only available for 2021 and therefore this single year's data is presented as a high rainfall year example for comparison purposes in the future.

By the conclusion, the total carbon within the Plantation Site had been generally re-established following the degradation incurred in the planting preparation process. Future carbon behaviour is anticipated to continue this increasing trajectory. Salt Bush harvesting, excluding the incident of compaction as a risk of increased traffic within the area, should serve to promote soil carbon through regular shedding of root systems as the plant foliage is reduced and the promotion of new root systems during regrowth. Soil coverage within the Plantation Site bodes well for the preservation and growth of the soil flora and fauna community, further increasing both the carbon and the system's resilience to extreme events impacting plant and soil health.

Within the Wild Harvest Site, the under plant total carbon was higher than the between plant for the shallow soil depth in the non-scarified area in 2020, highlighting the benefit of root systems in promoting and protecting soil flora and fauna (represented by carbon measurement). This indicates that plant coverage was developing and protecting carbon stores pre-scarification. With 2021 samples having no significant statistical difference between the scarified and non-scarified samples, it suggests that with coverage, the system is recovering. However it is noted that for the non-scarified area, a higher carbon content was identified under the plants compared to between in 2021. This implies that the more plants that are available in the long term, the higher the system's total carbon. The scarified area had minimal plants compared to the non-scarified area due to the treatment. The elevated bush layer as opposed to the current, fine leaved ground cover / individual tiny plants is anticipated to take many years to return to pre-scarification levels.

The 2021 total nitrogen within the Plantation Site demonstrated a similar trend to the carbon, with the 2021 data sets all higher than those from 2020 and approaching the 2019 original levels. Such information highlights the importance of minimum tillage and keeping soil coverage in place to minimize the impact of topsoil (and its ecosystem's) exposure to temperature swings, wind and sunlight.

### **11.REFERENCES**

Please refer to Environmental Reporting Document.