Understanding the effect of soil acidity treatments on soil microbiology in the Mallee.

A report produced in August 2024

LANDSCAPE SOUTH AUSTRALIA MURRAYLANDS AND RIVERLAND

Lameroo Soil acidity site- Understanding the effect of soil acidity treatments on soil microbiology in the Mallee.

Introduction

Through the Murraylands and Riverland Landscape Board's Soil Extension Officer project, it was proposed to understand the impact that soil acidity treatments have on soil microbiology.

Several different treatments were trialled to understand how they may mitigate topsoil and sub-surface acidity in a minimum tillage cropping system in the South Australian Mallee region. The trial included comparing broadcasting and incorporated lime, deep ripping, biochar, and clay. Results were determined by comparing before and post-treatment soil pH measurements conducted by Brian Hughes from PIRSA and funded by GRDC. The trial began in 2020 and is due to finish in 2025.

During the 2023 growing season, Barrie Williams from the landscape board worked with Brian Hughes to access the site, take relevant microbiological soil samples, and interpret the data to ascertain how the trial treatments were affecting the soil microbiology on a microscopic level.

Trial design

	Treatment type	Treatment description
	Rip + Lime	Ripped to 30 cm
	Sulphur Cultivate	Elemental Sulphur 1t/ha
	High Lime Cultivate	Lime 6 t/ha
	Biochar + Lime	Biochar 3t/ha, Lime 5t/ha
	Medium Lime +Gypsum Surface	Lime 3 t/ha, Gypsum 5 t/ha
	Control Surface	No addition
	Clay Cultivate	(100 t/ha from onsite)

Table 1: Treatment plots where soil microbiological samples were taken

Soil sampling methods for microbes:

Each treatment plot is 2 X 4 metres in size.

- Take up to six samples of soil per treatment plot, to a depth of 10 cm,
- The soil is combined from each plot
- Then fill a small plastic (zip-lock bag) with approx. 250 grams
- Label the bag well and place it in a cooler bag.
- Post early in the week to avoid shipping delays over the weekend
- The samples were sent to the Soil Food Web Institute in South Australia

During the 2023 winter cropping season, soil samples were taken from the treatment plots listed in Table 1 for microbiological testing. The soil was sampled 3 times over the season which were before pre-planting, before harvest, and after harvest to show microbial activity over the growing season. During pre-planting the soil would be left undisturbed for several months; near harvest the plants would be green, growing and feeding the microbes; and after harvest (when there would be stubble mulch on top of the soil and soil settling down) and in a dry summer cycle.

Trial Results

Sample date 18/04/2023												
	Active Bacteria µg/g	Total Bacteria µg/g	Active Fungi µg/g	Total Fungi µg/g	Hyphal Diameter µm	Flagellates #/g	Amoebe #/g	Ciliates #/g	Nematodes #/g	VAM	Nitrogen kg/ha	Actino Bacteria
Range Low - High	**1-5**	**175-300**	**1-5**	**175-300**	**3**	**5000**	**5000**	**50-100**	**10-20**	**40-80%**	**-**	**-**
N# 1 Rip +Lime	1.37	146.00	3.23	39.70	3.00	576.00	5767.00	58.00	0.00	3.00	50-75	0.25
N# 2 Sulphur + cul	1.73	163.00	3.26	40.10	3.00	465.00	4663.00	28.00	0.24	0.00	25-50	0.25
N#3 High Lime	1.57	151.00	1.65	33.80	3.00	283.00	4709.00	0.00	0.00	3.00	<25	0.50
N#7 Biochar +Lime	0.53	167.00	0.00	36.50	3.50	14383.00	56969.00	441.00	0.04	3.00	112-168	0.00
N#9 Medium Lime	1.42	145.00	1.67	32.00	3.00	477.00	4783.00	0.00	0.12	7.00	25-50	25.00
N#11 Surface cult	1.37	126.00	1.62	35.40	3.00	462.00	5789.00	58.00	0.14	3.00	50-75	2.21
N# 14 Clay cult	1.57	138.00	3.29	38.20	3.00	289.00	4707.00	28.00	0.00	7.00	<25	1.12

Table 2: First microbiology sampling results start of the season.

The results highlighted in yellow represent a sample taken a month later in the season after planting on 30/05/2023.

Results Table 2:

General observations

Biochar + Lime has the highest total numbers of bacteria at 167 µg/g but lower active bacteria. Taking the sample a month later may have attributed to this. The Sulphur + Cultivate has the second highest total bacteria 163 µg/g and the highest total fungi 40.10 µg/g in the treatments.

Nearly all treatments have active fungi which is most likely due to low soil disturbance, whereas the Biochar + Lime treatment has zero active fungi. A possible reason for this is that planting may have disturbed the soil fungi and killed them.

The fungi across all treatments have a hyphal diameter of 3.00 µm or greater, indicating that the fungi are non-pathogenic, and they are beneficial fungi for the soil.

The vesicular arbuscular mycorrhiza (VAM) fungi appear at the highest level (7%) in the Clay Cultivate and Medium Lime, and 3 % in the rest. An optimal figure for VAM fungi is 40% to 80%. VAM helps to protect plants from different pathogenic fungi, allows for better nutrient uptake, improves plant growth, and can help plants survive drought. VAM can also improve the soil structure by secreting a glue-like substance called glomalin, produced in the fungi's hyphae, which is used to stick soil aggregates together like glue. There are two types of VAM - ecto (outside of roots) and endo (inside the root).

Some strains of Actinobacteria can fix nitrogen and make soil-bound phosphorus and potassium more available to the plants. All treatments have Actinobacteria (varying levels from 0.25 µg/g to 25 µg/g) except for the Biochar + Lime treatment. The low number in Biochar + Lime is possibly due to the higher number of protozoa consuming the active bacteria.

Nematode type	N# 1 Rip +Lime	Total number #/g	N# 2 Sulphur + cul	Total number #/g	N#3 High Lime	Total number #/g	N# 7 Biochar +Lime	Total number #/g	N#9 Medium Lime	Total number #/g	N#11 Surface cult	Total number #/g	N# 14 Clay cult	Total number #/g
Bacterial feeders			Acrobeloides	0.06			Achromadora	0.006	Alaimus	0.07				
			Cephalobus	0.07			Acrobeloides	0.006	Acrobeloides	0.05				
							Cervidellus	0.004						
							Plectus	0.006						
							Cephalobus	0.004						
fungal feeders			Eudorylaimus	0.05			Epidorylamus	0.005						
							Thonus	0.001						
fungal/root feeders			Ditylenchus	0.03			Merlinius	0.004			Aglenchus	0.07		
											Filenchus	0.03		
Predatory			Coomansus	0.03			Coomansus	0.004			Clarkus	0.04		
Root Feeders							none							

Table 3: Total amount of nematodes for first sample 18/04/2023

In the Biochar + Lime treatment, the bacterial-eating nematodes were 65% of the total nematode population. Higher number of protozoa reduced the active bacteria in the treatment plot.

Sample date 9/11/2023												
	Active Bacteria µg/g	Total Bacteria µg/g	Active Fungi µg/g	Total Fungi µg/g	Hyphal Diameter µm	Flagellates #/g	Amoebe #/g	Ciliates #/g	Nematodes #/g	VAM	Nitrogen kg/ha	Actino Bacteria
Range Low - High	**1-5**	**175-300**	**1-5**	**175-300**	**3**	**5000**	**5000**	**50-100**	**10-20**	**40-80%**	-	-
N# 1 Rip +Lime	1.74	160.00	0.00	54.90	3.50	28224.00	141123.00	4340.00	37.00	6.00	200.00	0.25
N# 2 Sulphur + cul	1.75	129.00	0.00	31.60	3.00	284520.00	590428.00	5904.00	55.60	0.00	300+	0.25
N#3 High Lime	1.90	91.40	0.00	33.40	3.00	14034.00	140346.00	5825.00	16.80	0.03	200+	0.25
N#7 Biochar +Lime	2.00	152.00	0.00	47.80	3.50	45356.00	147479.00	8848.00	0.00	0.00	250+	0.26
N#9 Medium Lime	1.48	130.00	0.00	80.50	4.00	64408.00	499102.00	4620.00	20.60	0.00	300+	0.53
N#11 Surface cult	1.57	113.00	0.00	36.10	3.00	5891.00	47164.00	4366.00	33.30	7.00	100-150	0.25
N# 14 Clay cult	1.57	96.10	0.00	31.40	3.00	28270.00	58666.00	4347.00	24.20	0.00	200+	0.25

Table 4. Soil microbiology results for samples taken on 9 November 2023.

Levels of active bacteria increased in this testing period, possibly due to some early November rainfall events prior to sampling. September and October had low rainfall amounts of 13 mm and 8 mm respectively whereas November had 66 mm of rain.
The average active bacteria increased by 0.40 µg/g during the growing season.
The total bacteria decreased by 0.24 µg/g average during the growing season.

There were no active fungi in any of the treatment plots sampled during the growing season.
Total fungi increased by 8.5 µg/g over the growing season.
The hyphae size increased by 0.27µm making for a more disease-resistant soil.

The number of protozoa (Flagellates, Amoebae, and Ciliates) is very high and some of the highest numbers that the Soil Food Web Institute has seen in 30 years of operating. The Sulphur + Cultivate treatment had Flagellates 284,520 #/g, Amoebae 590,428 #/g Ciliates 5904 #/g. compared to the results in Table 2: Sulphur + Cultivate treatment Flagellates 465 #/g, Amoebae 4663 #/g, Ciliates 28 #/g

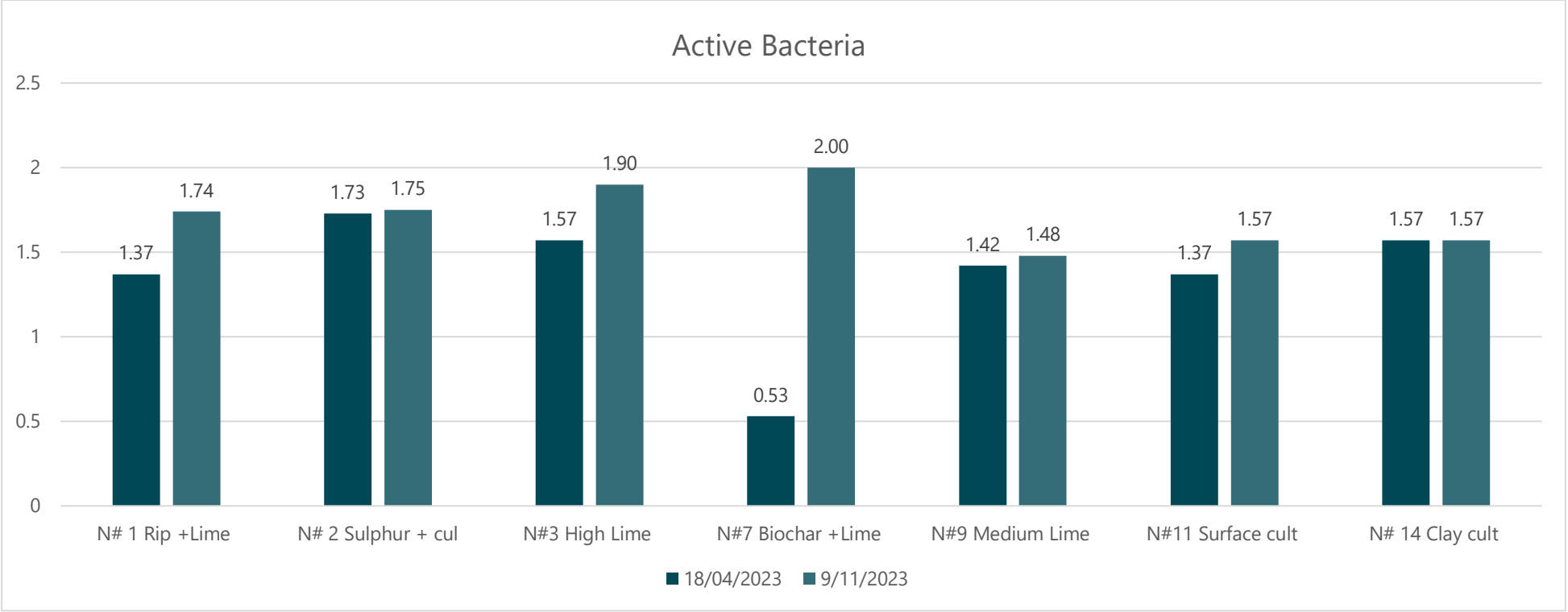

Table 5. A comparison of active bacteria µg/g between the pre-seeding and growing season soil tests.

Active bacteria levels are higher in this sampling compared to the first sampling, possibly due to the green and photosynthesising plants producing plant exudates (sugars). Exudates are a food source for the bacteria, enabling them to grow in numbers. These exudates are used as food source by soil microbes in exchange for nutrients that the plant needs.

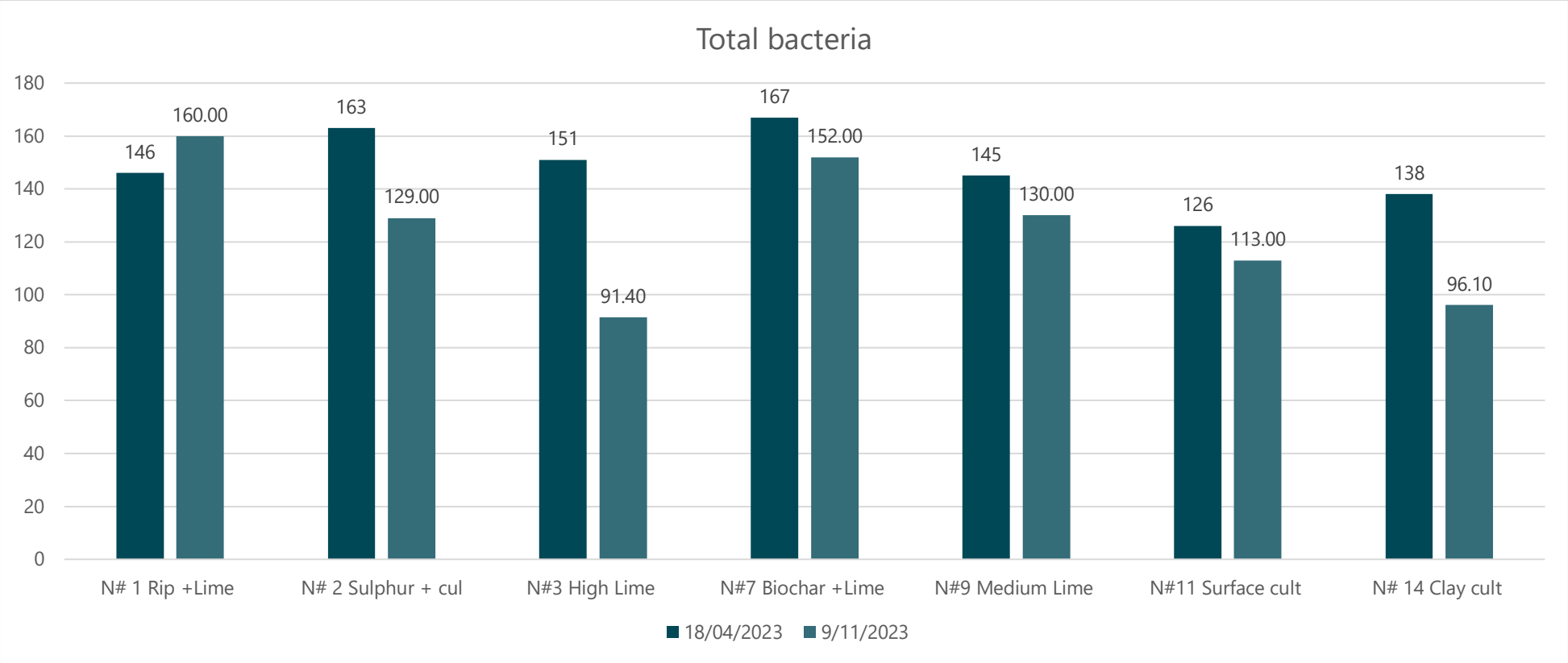

Table 6. A comparison of total bacteria µg/g between pre-seeding and growing season soil tests.

Total bacteria in most of the treatment plots decreased except for the Rip + Lime which increased in total bacteria.

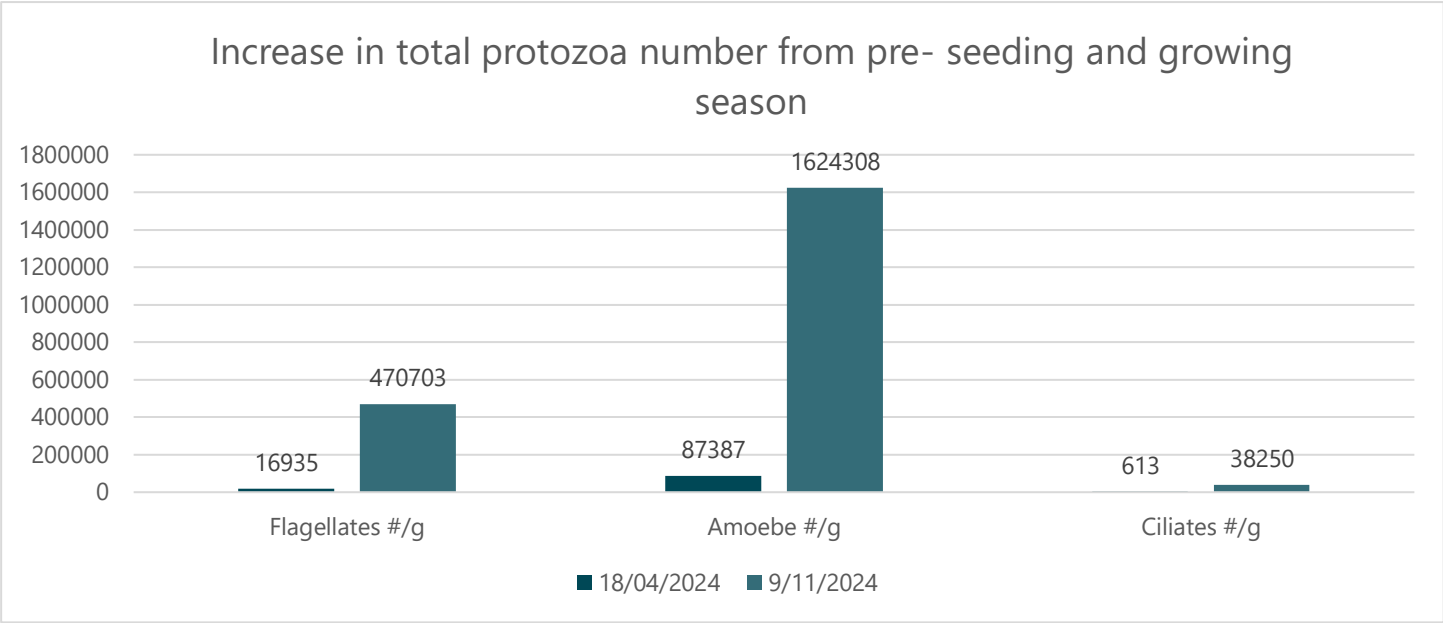

Table 7: The change in total protozoa numbers between pre-seeding and growing season soil tests

There has been a large increase in protozoa numbers since the first sampling due to the increased exudates (sugars) being released into the soil.

Nematode type	N# 1 Rip +Lime	Total number #/g	N# 2 Sulphur + cul	Total number #/g	N#3 High Lime	Total number #/g	N#7 Biochar +Lime	Total number #/g	N#9 Medium Lime	Total number #/g	N#11 Surface cult	Total number #/g	N# 14 Clay cult	Total number #/g
	Achromadora	7.95	Achromadora	3.78	Acrobeloides	4.16	none	0	Ironus	3.17	Acrobeles	6.1	Acrobeloides	3.21
Bacterial feeders	Acrobeloides	10.22	Acrobeloides	23.21	Cephalobus	4.99		0	Plectus	2.11	Acrobeloides	5.08	Acrobeloides	3.85
											Caenorhabditis	6.1	Zeldia	5.15
fungal feeders			Eudorylaimus	5.8			none		Epidorylaimus	3.69				
fungal/root feeders	Tylenchus	4.54	Filenchus	9.67			none		Filenchus	3.17	Cephalenchus	5.08	Tylenchus	3.21
									Merlinius	3.17	Coslenchus	3.05		
Predatory	Clarkus	7.95	Mononchus	7.74	Clarkus	2.49	none		Clarkus	2.11	Clarkus	4.07	Mononchus	3.21
			Mylonchulus	3.87					Coomansus	1.58	Coomansus	3.05	Mylonchulus	2.57
Root Feeders	Longidorus	5.68			Pratylenchus	4.99	none						Pratylenchus	2.57

Table 8: Nematodes from the 9/11/2023 sample

There has also been an increase in the number of nematodes in all tests except the Biochar+ Lime, which has none.

Bacterial-eating nematode levels were found to be higher at this time of sampling, with up to 4 different species of nematodes in some treatments. The presence of a high level of bacteria-eating nematodes and an increased level of protozoa has more than likely led to a decrease in total bacteria. The

highest number of nematodes were found in the Sulphur + Cultivate treatment with 55.60 #/g compared to the Biochar+ Lime with 0 #/g. Other species of nematodes found in the plots were fungal feeders, fungal root feeders, and predatory nematodes.

The second sample had most of the nematodes in the double-digit figures compared to decimal points in the first sample.

The VAM in this test is lower compared to the first test, with only three treatments having VAM associations.

The Actinobacteria had also decreased in this sample.

Increased soil moisture from the rain events may have led to increased levels of microbes. Taking the sample from around growing plants' roots would have also increased the number of microbes as they congregated around the roots of growing plants. This would be the biggest difference in testing between tests taken prior to seeding, and the one taken during the growing season.

Plant-available nitrogen has increased from <25 kg/ha in the pre-seeding sample test compared to 200-300+ kg/ha of plant-available nitrogen during the growing season sampling. The high readings are from the protozoa and nematodes eating the bacteria and excreting out nitrogen in the process and stimulating other nitrogen-fixing bacteria.

8/02/2024												
	Active Bacteria µg/g	Total Bacteria µg/g	Active Fungi µg/g	Total Fungi µg/g	Hyphal Diameter µm	Flagellates #/g	Amoebae #/g	Ciliates #/g	Nematodes #/g	VAM	Nitrogen kg/ha	Actino Bacteria
N# 1 Rip +Lime	2.09	130	0	42.8	3.5	586	4698	59	0	3	50-75	0.5
N# 2 Sulphur + cul	1.57	168	0	26.9	3	282	1412	141	0	3	50-75	0.25
N#3 High Lime	1.24	97.9	0	34.3	3	477	7228	579	0	3	50-75	0.25
N#7 Biochar +Lime	1.39	139	0	57.7	3.5	4670	5834	466	0.12	3	75-100	0.5
N#9 Medium Lime	1.74	138	0	31.4	3	585	5858	141	0	0	50-75	0.25
N#11 Surface cult	1.33	145	0	32.1	3	2886	5988	60	0.1	7	50-75	0.51
N# 14 Clay cult	2.55	149	3.05	75.4	4	1477	4908	2281	0.15	6	50-75	0.52

Table 9. Soil microbiology results for samples taken on 8 February 2024.

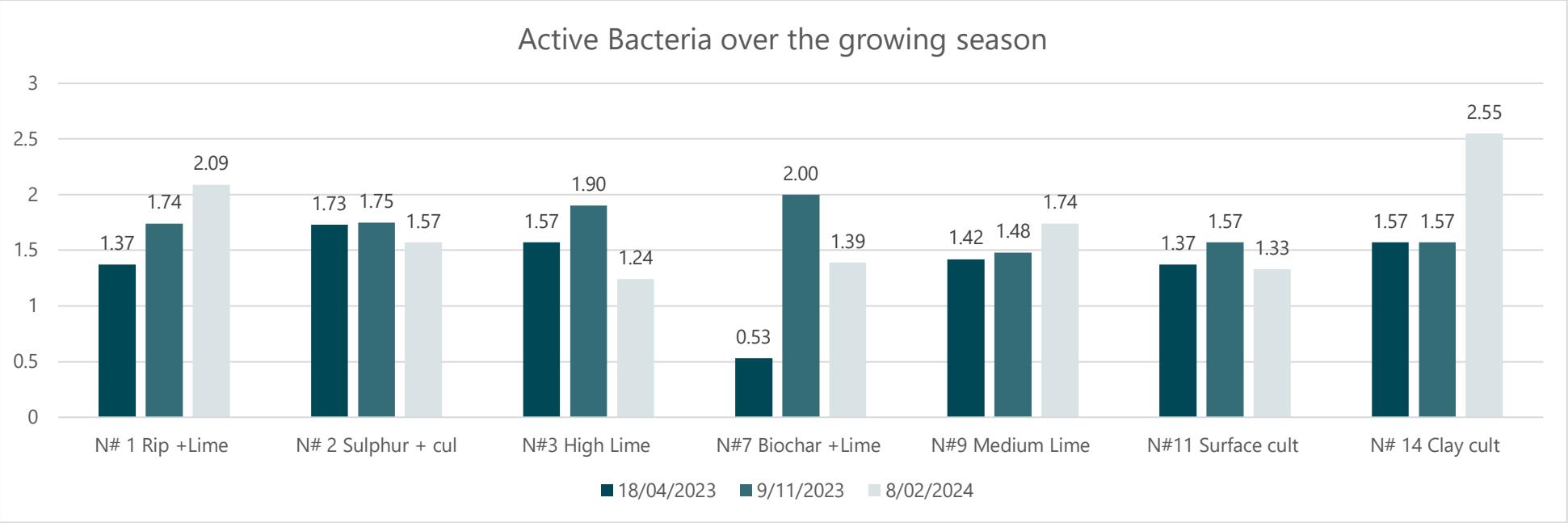

Table 10. A comparison of active bacteria levels throughout the growing season by treatment type.

Table 10 shows levels of active bacteria which varied significantly over the season and across the treatment types.

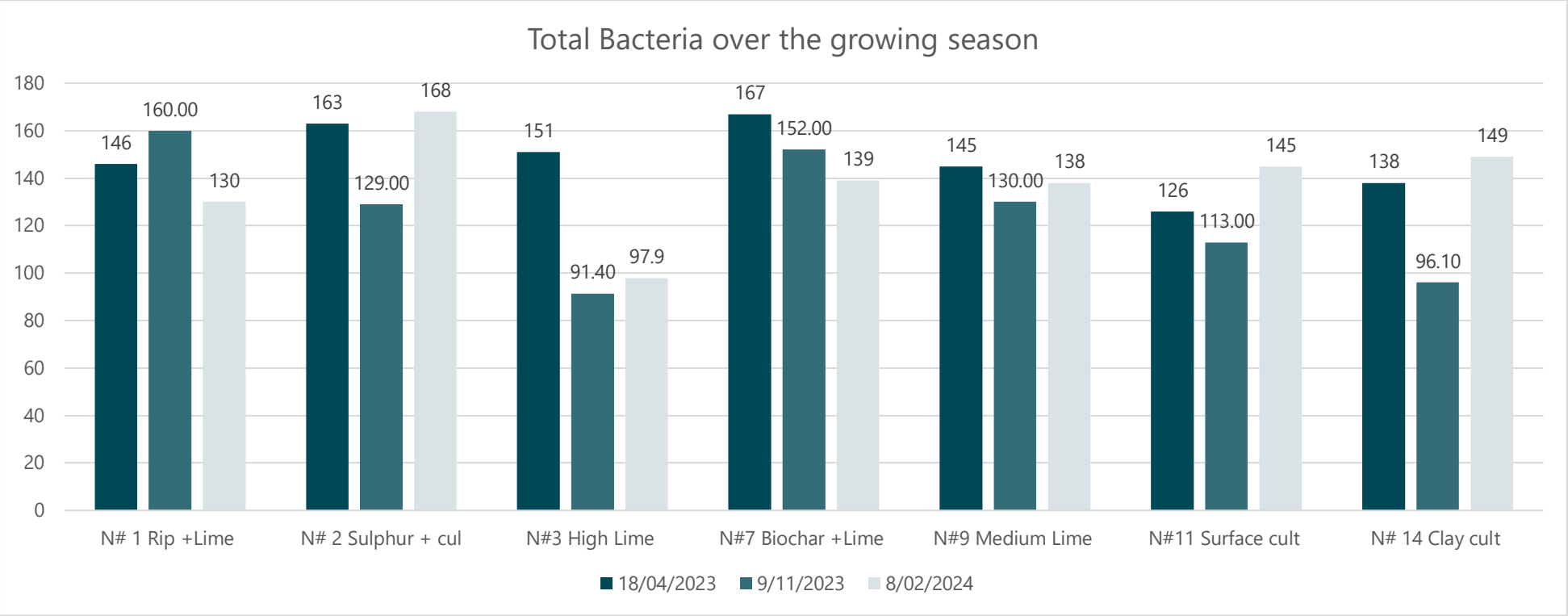

Table 11. A comparison of total bacteria over the growing season by treatment type.

The total bacteria have in some cases recovered a small amount since the pre-harvest sample. Some of the treatment plots have not returned to the pre-planting sample levels.

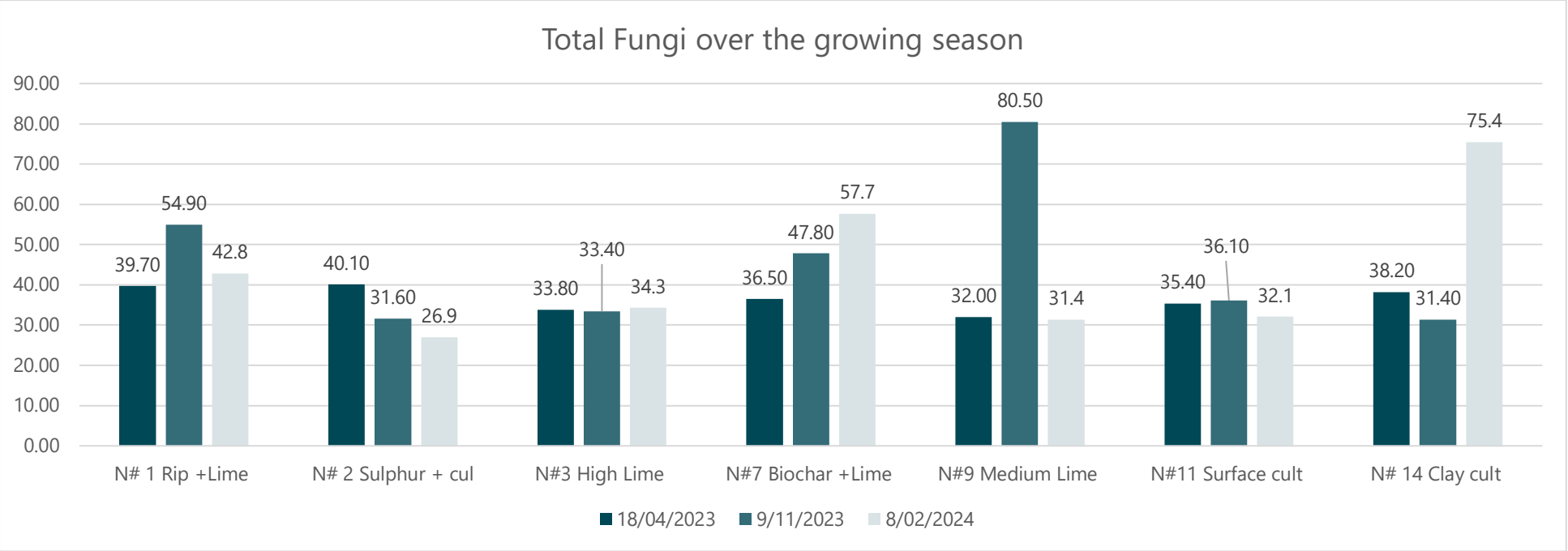

Table 12. A comparison of total fungi over the growing season by treatment type.

The total fungi levels fluctuate over the growing season - some are lower than the pre-harvest sampling and a couple of the total fungi levels are better.

The Clay Cultivation treatment plot recorded the highest level of total fungi after harvest as well as being the only one to have active fungi appear. It is possible that the clay is holding on to some moisture which has benefited the fungi.

There has been no change in the range of fungal hyphal diameter between the second and third soil samplings.

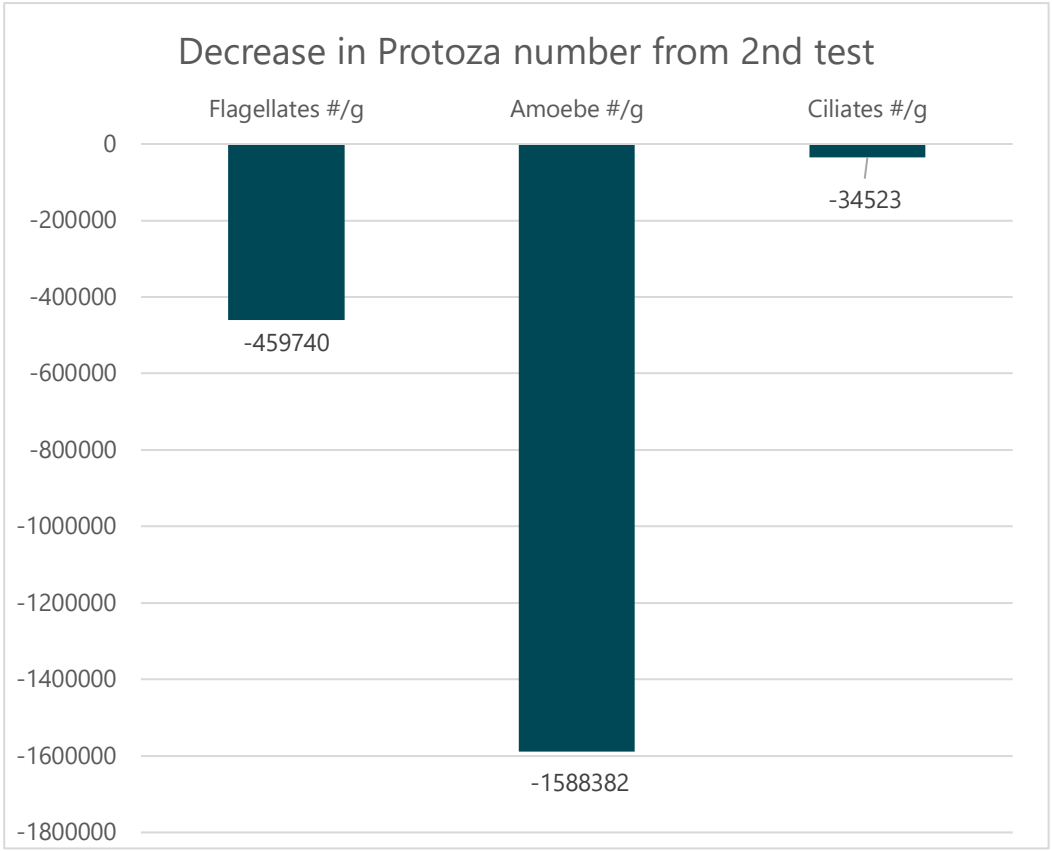

Table 13. A comparison between protozoa numbers between the second and third soil tests.

When comparing the results from the pre-harvest and after-harvest microbe tests, the greatest change was among the protozoa, which decreased significantly across all treatments.

The reduction would likely be due to several factors including the lack of live plant roots in the soil profile, a dry soil, and the bare soil.

Nematode type	N# 1 Rip +Lime	Total number #/g	N# 2 Sulphur + cul	Total number #/g	N#3 High Lime	Total number #/g	N#7 Biochar +Lime	Total number #/g	N#9 Medium Lime	Total number #/g	N#11 Surface cult	Total number #/g	N# 14 Clay cult	Total number #/g
							Alaimus	0.05			Acrobeloides	0.07	Achromadora	0.05
Bacterial feeders													Acrobeloides	0.05
fungal feeders											Thornia	0.03	Thonus	0.02
fungal/root feeders							Aphelenchoides	0.05					Bitylenchus	0.02
Predatory							Coomansus	0.05						
Root Feeders							none							

Table 14 Nematodes results for samples taken on 8 February 2024

Nematode numbers also decreased over this period. This is expected at this time of year due to the drier soil conditions and lack of live plant roots that provide a microbial food source.

There were no nematodes in the Biochar + Lime in the last sampling but there are in this sampling. The Rip + Lime, Sulphur + Cultivate, High Lime, and Medium Lime had no nematodes. The Surface Cultivated; Clay Cultivate had reduced species of nematodes compared to the last sampling.

The vesicular-arbuscular mycorrhiza (VAM) was present in all plots except the medium lime. The high number of fungal nematodes in the previous sampling period may have eaten the fungi. The highest VAM (7%) for the treatment plots was the surface cultivated which had VAM levels of 7%, and this was the same level for the pre-harvest sample.

The amount of nitrogen decreased in all treatments to 50-75 kg/ha except for the Biochar + Lime where it increased to 75-100 kg/ha. The higher nitrogen reading in the Biochar + Lime treatment is likely to be due to the comparatively higher number of protozoa. Protozoa consume bacteria that are rich in nitrogen. When e more bacteria are consumed then more nitrogen is excreted back into the soil by the protozoa. If bacteria are plentiful, it can cause protozoa numbers to increase and bring the bacteria into balance. The protozoa also stimulate atmospheric nitrogen-fixing bacteria.

The Actinobacteria has increased slightly by 0.7 µg/g, compared to the second sample of 2.04 µg/g, but still not as high as the first test, which was 29.33 µg/g.

Discussion

It seems that moisture has a big effect on microbe population and growth as seen in Table 1, with the Biochar + Lime sample taken a month later with having a higher number of microbes. The pre-harvest had some rains early in the month which led to an increase in protozoa, nematodes, and some total fungi numbers. The increased soil moisture breaks the dormancy and everything springs to life. Anything that can be done to increase the water-holding capacity of the soil will greatly benefit the soil microbial life and enhance the soil and plant life.

The trial has shown that having green living photosynthesising plants will increase in microbe numbers as seen in the preharvest sampling.

The increased level of microbes in the pre-harvest sampling possibly led to the increase in plant available nitrogen as shown in Table 4. Ciliates and other protozoa eat up to 10,000 bacteria a day and excrete nitrogen and other nutrients in a plant-available form. This can be seen in Table 1, under the Biochar + Lime treatment, the active bacteria are in low numbers whereas the ciliates and other protozoa are in higher numbers compared to the other treatments.

The increased protozoa numbers encourage the growth of nitrogen-fixing bacteria. This could attract earthworms to the area if the soil has the right moisture level, correct pH, and plenty of food (protozoa) and organic matter.

The vesicular-arbuscular mycorrhiza (VAM) has been found in all the treatments over the project. Endo-mycorrhizae, which means that the fungi colonise inside the roots of the plants, was the most prevalent in the treatments compared to no Ecto-mycorrhiza found on the outside of the roots in these treatments,

Plants have a symbiotic relationship with VAM as VAM can help the plant search for water and nutrients in exchange for sugars (exudates). The hyphae act like an extension of the roots of the plants allowing the plant to explore the depths of the soil. The hyphae are thinner than plant roots which makes it easier for them to search out water and nutrients in the soil pores. The surfaces of these hyphae have a substance called glomalin on them which can glue soil particles together to form soil aggregates. This helps to increase soil health by increasing water holding capacity, holding on to organic matter, and reducing soil erosion and nutrient cycling.

Phosphorous that is bound in the soil can be released by VAM via a chemical reaction. Higher levels of VAM in the soil will help the plants to be healthier and less susceptible to disease pressure and attack from insects.

Soil disturbance can harm the active and total fungi hyphae and VAM. Ploughing can decrease the number of fungi in the soil and lead to a higher bacterial-dominated soil.

Some plant species, like canola and lupins, do not form symbiotic relationships with VAM. This can lead to reduced VAM levels in the paddock if these crops are grown as successive crops. After crops like canola and lupins, it may be beneficial to plant crops with a symbiotic relationship with VAM, like vetch, peas, beans, oats, barley, and wheat. This will help to restore the VAM levels in the soil.

In all samples, numbers of both total and active bacteria and fungi are low and should be encouraged to increase by feeding them with bacteria and fungi-friendly food. Application of a compost tea or extract may help to increase the numbers if lacking. Food sources like kelp, molasses and fish hydrolysates sprayed out three times a year will feed the microbes a more regularly ad will help to keep a green growing crop on the paddocks.

Actinobacteria are important as they help to break down stubbles, can fix nitrogen, and can make phosphorus available to plants. This nitrogen-fixing bacteria can fix atmospheric nitrogen by breaking the triple bonds that hold atmospheric nitrogen together. These bacteria can suppress diseases and pathogens that can attack plants.

The presence of many different nematode types in the treatments is a very positive thing to see, as they can keep one another in check and are a valuable feed source for macro arthropods. There are four main classes of nematodes: bacterial feeders, fungal feeders, fungal and root feeders, and predatory nematodes. Some treatments have up to three different species in each treatment.

The ciliates consume higher amounts of bacteria compared to amoebae and flagellates.

This trial has provided a good foundation to understanding microbiology in a growing cropping season. It would be beneficial to do some frequent testing to get more data and see what changes occur over a few more seasons.

Acknowledgments

This project was supported by the Murraylands and Riverland Landscape Board through funding from the Australian Government's National Landcare Program and landscape levies.

Thanks to Brian Hughes for allowing Barrie Williams to undertake this project in conjunction with his trial, to Garry Flohr the landowner, and the PIRSA team for their help to sample the trial plots.

This project worked in collaboration with an existing trial undertaken by PIRSA, New knowledge, and practices to address topsoil and sub-surface acidity under minimum tillage cropping systems of SA' (2019-2022) to compare and evaluate lime sources; to assess the impact of broadcasting lime vs incorporation; and investigate deep ripping, biochar and clay impacts on acidity.

Appendices

Plains Lameroo South weather station

Month	J	F	M	A	M	J	J	A	S	O	N	D	Annual
2023	8.2	9.4	14.0	35.8	30.0	86.2	26.8	43.6	13.0	8.8	66.2	78.8	420.8
2024	21.6	0	1.8										

Table 1: Rainfall mm (Austin

Month	J	F	M	A	M	J	J	A	S	O	N	D
2023			15.8	15.5	11.7	10.1	8.6	10.1	14.7	14.0	16.0	18.8
2024	20.6	20.9	18.3									

Table 2: Temperature Avg: 0C (Austin Plains Lameroo South weather station)

Summary of the Discussion for Implementation:

- Reduced tillage keeps soil fungi together and does not break hyphae or destroy soil aggregation.
- Keep green growing plants year-long to feed microbes and keep soil functioning.
- A diversity of plants feeds different microbes that are beneficial to the soil.
- Increase soil carbon to increase soil water holding capacity and keep more water in the soil profile.
- Vesicular arbuscular mycorrhiza (VAM) has symbiotic relationships with most plants except for some brassica and chenopods. Examples are canola and lupins. If possible, plant a crop with a high (VAM) relationship with wheat, barley, and oats.
- Use a compost tea or extract to increase microbe numbers in the soil.
- A diverse range of microbes is good for healthy soil and plants
- Nematodes are good for the soil, there are very few bad ones.