SOiL CRC

Performance through collaboration

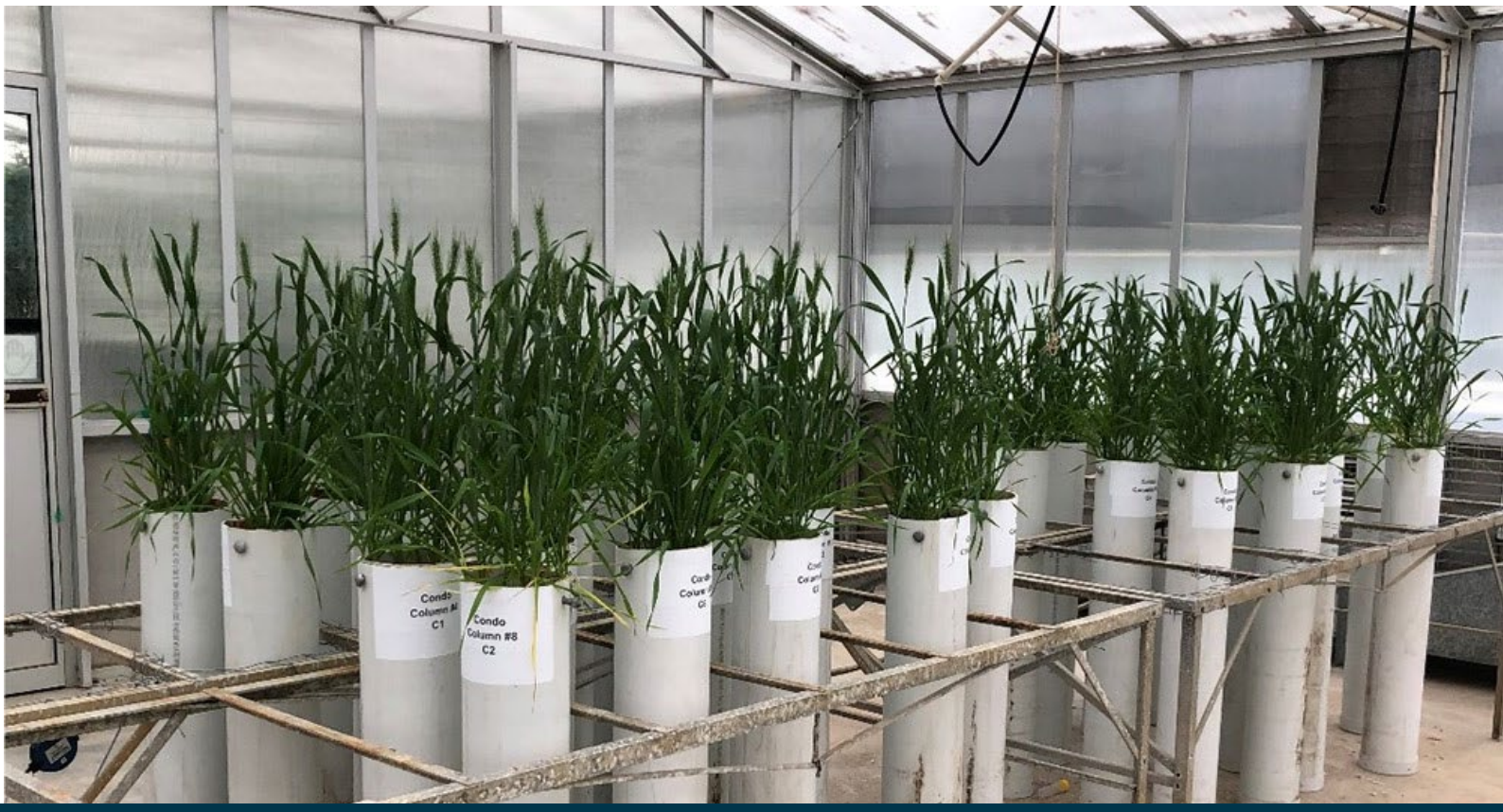
Mechanistic understanding of the mode of action of novel soil re-engineering methods for complex chemical and physical constraints

Addressing complex soil constraints

Final report for project 4.2.002

A/Prof Jason Condon, Prof. Roger Armstrong, Mark Whatmuff and Michael Weiss

September 2022



soilcrc.com.au

Australian Government
Department of Industry, Science and Resources

Cooperative Research Centres Program

Contents

Project participants

Charles Sturt University (CSU)
New South Wales Department of Primary Industries (NSW DPI)
Agriculture Victoria (DEDJTR)
Central West Farming Systems (CWFS)
Riverine Plains
FarmLink
Birchip Cropping Group (BCG)
HART Field-Site Group Inc. (HART)
Wimmera Catchment Management Authority (WCMA).

Disclaimer

Any opinions expressed in this document are those of the authors. They do not purport to reflect the opinions or views of the Soil CRC or its partners, agents or employees.

The Soil CRC gives no warranty or assurance and makes no representation as to the accuracy or reliability of any information or advice contained in this document, or that it is suitable for any intended use. The Soil CRC, its partners, agents and employees, disclaim any and all liability for any errors or omissions or in respect of anything or the consequences of anything done or omitted to be done in reliance upon the whole or any part of this document.

Peer review statement

The Soil CRC recognises the value of knowledge exchange and the importance of objective peer review. It is committed to encouraging and supporting its research teams in this regard.

The author(s) confirm(s) that this document has been submitted for review to the program leader. These reviewers evaluated its:

- originality
- methodology
- rigour
- compliance with ethical guidelines
- conclusions against results
- conformity with the principles of the Australian Code for the Responsible Conduct of Research (NHMRC 2018), and provided constructive feedback which was considered and addressed by the author(s).

Keywords

Amelioration, amendment, fertiliser, nutrition, constraint, soil structure.

Citation

Condon, J., Armstrong, R., Whatmuff, M. & Weiss, M. (2022). Mechanistic understanding of the mode of action of novel soil re-engineering methods for complex chemical and physical constraints: Addressing complex soil constraints. Final Report for Project 4.2.002. Cooperative Research Centre for High Performance Soils, Callaghan, NSW.

Executive summary

Australian soils commonly exhibit multiple constraints to plant productivity. Six farming system groups identified problem soils that exhibit multiple constraints to plant production. These soils were treated with amendments that address the identified constraints to quantify the benefit of amelioration and determine the mode of action of the observed benefit.

Dispersion associated with sodicity was common in most soils studied. The addition of organic amendments can influence microaggregate flocculation as measured by decreased turbidity however the effectiveness was much less than gypsum. Gypsum and S treatments also have the potential to increase soil EC above the thresholds for healthy plant growth.

Plant growth enhanced the effectiveness of subsoil organic matter amendment's influence on soil structure, with benefits increasing with time (>12 months). However structural benefits from organic matter alone did not translate into increased plant growth.

Organic amendments have the advantage of simultaneously increasing N, P, S and K nutrition with chicken manure pellets providing greater nutrition compared to wheat straw pellets.

Plant performance was improved in soils exhibiting multiple constraints with the addition of amendments that provided N and P to the subsoil. Nutrition, as N and/or P, from organic material or synthetic sources applied to the subsoil increased yield as much or more than manure alone. Evidence of plant/microbe interaction in P cycling existed. Available P in the soil at the end of experimentation was greater in the presence of plants compared to where no plants were grown. Surface applied manure and fertilising with N and/or P in the subsurface layer provided biological benefit by increasing abundance of genes associated with C, P and N cycling.

The improved nutrition in the subsurface layers provides biological benefit to the soil system that may carry over to subsequent crops. Field investigation of the longer-term benefit of adequate subsurface/subsoil nutrition to plant performance and plant/soil biology interaction is warranted.

Objectives

Aim 1: Assess the potential effectiveness of both current and novel soil reengineering methods to improve plant productivity on key soils.

Aim 2: Determine the mechanisms responsible for changes in soil properties following amelioration by novel methods.

Aim 3: Based on evidence from glasshouse studies, identify potential amelioration options for field evaluation in future projects.

Results

- Organic amendments were able to enhance plant performance on soils that had multiple soil constraints. Organic amendments have the advantage of simultaneously increasing N, P, S and K nutrition with chicken manure pellets providing greater nutrition compared to wheat straw pellets.
- Plant growth enhanced the effectiveness of subsoil organic matter amendment's influence on soil structure, with benefits increasing with time (>12 months).

However structural benefits from organic matter alone did not translate into increased plant growth.

- The plant performance provided by organic amendments was able to be matched or bettered by addition of nitrogen and/or phosphorus to the subsoil of soils with multiple constraints.

Next steps

- Field investigation of the longer-term benefit of adequate subsurface/subsoil nutrition to plant performance and plant/soil biology interaction is warranted.
- Field evaluation, over different seasons, of the influence of N and/or P fertiliser in subsurface layers is warranted. This should be compared with organic amendments providing the same quantity of nutrient applied.

1. Introduction

Australian soils commonly exhibit multiple constraints to plant productivity. Soil sodicity, acidity, nutrient deficiencies or toxicities and poor structure limit root growth. Such limitations may be driven by soil constraints that limit root exploration of the soil or may be the result of interactions between plant and the soil solution, including biological interaction and processes. Whilst plant species and variety may influence the impact of constraints, this project has aimed to investigate the effectiveness of inorganic and organic amendment to soils to overcome yield restrictions induced by the soil.

Six collaborating farming system groups (grower groups) identified their priority soil constraints and soils to be included in this project. The six groups were the Birchip Cropping Group (BCG), FarmLink, Riverine Plains, Central West Farming systems (CWFS), Hart Field-Site Group Inc. (HART) and Wimmera Catchment Management Authority (WCMA). Soils were collected from each collaborating grower group from three layers within their profiles which were then used in a series of experiments that aimed to quantify the benefit to the soil of amendments to plant performance and create an understanding of the mechanisms responsible for observed effects.

1.1 Project objectives

1. Assess the potential effectiveness of both current and novel soil re-engineering methods to improve plant productivity on key soils.

2. Determine the mechanisms responsible for changes in soil properties following amelioration by novel methods.

3. Based on evidence from glasshouse studies, identify potential amelioration options for field evaluation in future projects.

1.2 Project strategy

The research was based on a series of glasshouse studies using key soils identified as having multiple constraints by six grower groups. The research comprised three phases of experiments:

Phase 1: Initial column studies were conducted testing the “best knowledge” treatments to overcome the identified constraints of each soil. An untreated control was used as a comparison to quantify the impact on plant performance of best knowledge treatments and in doing so, quantified the magnitude of the constraint on plant production.

Phase 2: A series of laboratory-based incubations were conducted to quantify product by rate impacts on chemical and physical properties of individual layers from each soil. Where appropriate, interactions of products were also included. This phase of experiments enables rate response data to be obtained for wider use beyond the project and for enhanced understanding of the mechanism responsible for changes in soil properties following amendment.

Phase 3: Based on the findings of Phase 1 and 2, a short list of treatments was selected for experiments on responsive soils to determine the mechanism for observed responses relating to amendment application.

2. Identification of soil constraints and soil characterisation

2.1 Introduction

The project aimed to provide value to grower groups and the land managers they represent. The prioritisation of soils by each collaborating grower group was an important process. Grower groups were asked to prioritise soil/soil constraints to be utilised in project experiments. This was conducted by consultation with group members, group staff and key consultants operating in areas of the grower groups. Observations of how the soils behaved in years of differing weather events, perceived constraint and how plant growth is influenced were considered. Often, the selected sites represented “problem soils”, those that have not been able to be improved via amendment in the past.

2.2 Method

Soil locations were identified by each grower group (Table 2.1). Soils were then collected using an excavator to remove soil in layers. Sampling took pace in August and September 2019; the Wimmera soil was collected in April 2020. Soil layers were briefly stored separately in 1 tonne field bins prior to air drying. Soils were then sieved to pass 5 mm. Initial chemical properties were analysed from 500 g subsamples taken from each layer.

2.3 Sites and soils

The location and site name for sites exhibiting prioritised soils are listed in Table 2.1.

Table 2.1 Soil locations

Group	FarmLink	CWFS	RivPlains	BCG	HART	WCMA
Coordinates	-34.206500, 147.588000	-32.977966, 147.167302	-35.411561, 146.049193	-35.966914, 142.823780	-34.163692, 138.878899	-36.879421, 142.195764
Site name	Trungley Hall	Condobolin	Oaklands	Birchip	Marrabel	Wonwondah

BCG - Birchip Cropping Group
CWFS - Central West Farming systems
HART - Hart Field-Site Group Inc.
WCMA - Wimmera Catchment Management Authority.

2.3.1 Condobolin

CWFS selected a site north of Condobolin which had been under Lucerne for several years. The soil was thought to have an acidic topsoil and suffered from compaction in the subsurface soil. The soil was also likely to possess low chemical fertility due to low fertiliser input; standard fertiliser use would be 50 kg MAP /ha at sowing.

At the time of sampling a severe drought had impacted the landscape. Evidence of wind erosion was apparent as Lucerne crowns were approximately 3 to 4 cm above the sounding soil.

Profile description

0-15 cm – dark reddish brown, fine sandy clay loam, weak platy over subangular blocky, roots present, clear to,

15-50 cm – red, light sandy clay, weak angular blocky to massive, few roots, clear to,

50-100 cm – red, heavy clay, weak angular blocky to massive, 5-10% coarse rock fragments (shale), no roots.

Table 2.2 Chemical properties of the profile layers of the Condobolin soil sampled in 2019

Property	Unit	0-15 cm	15-50 cm	50-100 cm
pH (1:5 Water)		5.9	5.8	5.4
pH (1:5 $CaCl_2$)		5.2	4.9	4.4
EC (1:5)	(dS/m)	0.13	0.05	0.03
Chloride	(mg/kg)	28	<10	<10
Nitrate Nitrogen	(mg/kg)	55	4.7	1.9
Ammonium Nitrogen	(mg/kg)	4	0.72	0.94
Phosphorus - Colwell	(mg/kg)	39	5.3	<5.0
Phosphorus Buffer Index - Colwell		58	110	140
Copper (DTPA)	(mg/kg)	0.89	0.32	0.22
Iron (DTPA)	(mg/kg)	14	5.4	4.5
Manganese (DTPA)	(mg/kg)	72	6.4	3.4
Zinc (DTPA)	(mg/kg)	0.69	0.028	<0.020
Boron	(mg/kg)	0.87	0.74	0.9
Sulfur (KCl40)	(mg/kg)	5.6	12	16
Organic Carbon	(%)	1.11	0.46	0.22
Organic Matter	(%)	1.9	0.79	0.38
Aluminium ($BaCl/NH_4Cl$)	(cmol +/kg)	<0.10	0.11	0.51
Calcium ($BaCl/NH_4Cl$)	(cmol +/kg)	8.5	6	3.7
Potassium ($BaCl/NH_4Cl$)	(cmol +/kg)	2.3	0.9	0.36
Magnesium ($BaCl/NH_4Cl$)	(cmol +/kg)	1.7	1.4	3.2
Sodium ($BaCl/NH_4Cl$)	(cmol +/kg)	0.038	0.052	0.074
CEC ($BaCl/NH_4Cl$)	(cmol +/kg)	13	8.5	7.8
Aluminium % of Cations	(%)	0.77	1.3	6.5
Na%	(%)	0.29	0.61	0.95
Ca:Mg		5.0	4.3	1.2

2.3.2 Oaklands

The Riverine Plain Inc. group selected a soil that exhibits poor water infiltration and reported poor root penetration. Waterlogging in wet years was common and haying off in dry springs also occurs. In 2019 the site was under canola.

Profile description

0-10 cm – Brown clay loam, granular over strong angular block, many roots, gradual to,

10-50 cm – Red medium clay, strong angular blocky, slickensides present, roots around aggregates, gradual to,

50-100 cm – Brown heavy clay, strong angular blocky, carbonate present.

Photo: Cassie Schefe

Table 2.3 Chemical properties of the profile layers of the Oaklands soil sampled in 2019

Property	Unit	0-10 cm	10-50 cm	50-100 cm
pH (1:5 Water)		5.9	8.2	9.2
pH (1:5 $CaCl_2$)		5.3	6.7	8.1
EC (1:5)	(dS/m)	0.3	0.1	0.215
Chloride	(mg/kg)	86	22.0	16.5
Nitrate Nitrogen	(mg/kg)	81	2.9	2.45
Ammonium Nitrogen	(mg/kg)	15	1.1	0.725
Phosphorus - Colwell	(mg/kg)	66	5.2	<5.0
Phosphorus Buffer Index - Colwell		58	96	120.0
Copper (DTPA)	(mg/kg)	2.6	2.3	1.55
Iron (DTPA)	(mg/kg)	100	30.0	13.5
Manganese (DTPA)	(mg/kg)	78	22.0	3.9
Zinc (DTPA)	(mg/kg)	0.97	0.1	0.099
Boron	(mg/kg)	1.4	2.9	5.3
Sulfur (KCl40)	(mg/kg)	36	11.7	14
Organic Carbon	(%)	1.44	0.4	0.24
Organic Matter	(%)	2.5	0.8	0.335
Aluminium ($BaCl/NH_4Cl$)	(cmol +/kg)	<0.10	<0.10	<0.10
Calcium ($BaCl/NH_4Cl$)	(cmol +/kg)	8.5	9.2	10
Potassium ($BaCl/NH_4Cl$)	(cmol +/kg)	1.7	0.7	0.755
Magnesium ($BaCl/NH_4Cl$)	(cmol +/kg)	6.2	11.7	13.5
Sodium ($BaCl/NH_4Cl$)	(cmol +/kg)	1.4	4.3	5
CEC ($BaCl/NH_4Cl$)	(cmol +/kg)	18	26.0	29.5
Aluminium % of Cations	(%)	0.56	0.4	0.34
Na%	(%)	7.8	16.3	17
Ca:Mg		1.4	0.8	0.7

2.3.3 Trungley Hall

The soil selected by FarmLink was observed to establish crops well and generally display good early vigour. Then, roots often fail to penetrate beyond 30 cm and cannot access moisture below depth. For this reason, these crops will fail in a dry spring. Conversely, when the season is wet (e.g. 2016), excessive rainfall will perch on the clay layer (10-40 cm) and the crop can become waterlogged.

Profile description

0-10 cm – Dark brown, fine sandy clay loam, moderate sub-angular blocky, many roots, clear to,

10-40 cm – Pale brown, medium clay, massive, gradual to,

40-100 cm – Greyish brown, medium clay, moderate sub-angular blocky, carbonate present, roots absent.

Table 2.4 Chemical properties of the profile layers of the Trungley Hall soil sampled in 2019.

Property	Unit	0-10 cm	10-40 cm	40-100 cm
pH (1:5 Water)		6.5	8.6	9.55
pH (1:5 $CaCl_2$)		5.6	7.2	8.6
EC (1:5)	(dS/m)	0.14	0.12	0.43
Chloride	(mg/kg)	27	30	205
Nitrate Nitrogen	(mg/kg)	20	8.25	2.1
Ammonium Nitrogen	(mg/kg)	5	1.55	0.92
Phosphorus - Colwell	(mg/kg)	50	9.25	7.3
Phosphorus Buffer Index - Colwell		58	47	65.5
Copper (DTPA)	(mg/kg)	0.48	0.63	0.835
Iron (DTPA)	(mg/kg)	77	25.5	10.65
Manganese (DTPA)	(mg/kg)	15	4.55	2.15
Zinc (DTPA)	(mg/kg)	1	0.625	0.13
Boron	(mg/kg)	0.53	1.215	1.85
Sulfur (KCl40)	(mg/kg)	34	9.7	51
Organic Carbon	(%)	0.76	0.275	<0.15
Organic Matter	(%)	1.3	0.47	0.26
Aluminium ($BaCl/NH_4Cl$)	(cmol +/kg)	<0.10	<0.10	<0.10
Calcium ($BaCl/NH_4Cl$)	(cmol +/kg)	6	6	5.75
Potassium ($BaCl/NH_4Cl$)	(cmol +/kg)	0.74	0.46	0.705
Magnesium ($BaCl/NH_4Cl$)	(cmol +/kg)	2.2	7.1	10.05
Sodium ($BaCl/NH_4Cl$)	(cmol +/kg)	1	3.15	7
CEC ($BaCl/NH_4Cl$)	(cmol +/kg)	10	16.5	23.5
Aluminium % of Cations	(%)	1	0.615	0.43
Na%	(%)	10	19	29.5
Ca:Mg		2.7	0.8	0.6

2.3.4 Marrabel

The HART trial site group selected a soil at an existing trial location. Observations from previous experiments suggest potassium deficiency may be expected. Sodicity is a constraint of the subsoil.

Profile description

0–10 cm – Dark reddish brown, fine sandy loam with weak, sub-angular blocky, clear to,

10–40 cm – Reddish yellow, clay loam, weak blocky to massive abrupt to,

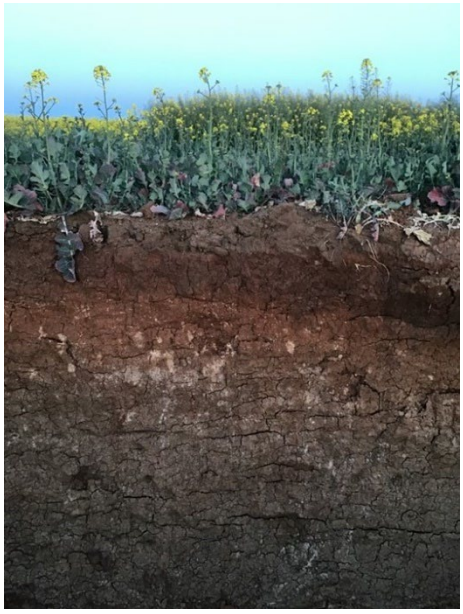
40–60 cm – Dark reddish brown, heavy clay, moderate angular blocky, gradual to,

60–100 cm – Red heavy clay, weak blocky to massive.

Table 2.5 Chemical properties of the profile layers of the Marrabel soil sampled in 2019

Property	Unit	0-20 cm	20-60 cm	60-100 cm
pH (1:5 Water)		6.7	6.7	9.5
pH (1:5 $CaCl_2$)		6.1	5.7	8.5
EC (1:5)	(dS/m)	0.19	0.12	0.34
Chloride	(mg/kg)	30	20	25
Nitrate Nitrogen	(mg/kg)	9.3	10	5.8
Ammonium Nitrogen	(mg/kg)	3.8	1.1	0.9
Phosphorus - Colwell	(mg/kg)	61	8.3	<5.0
Phosphorus Buffer Index - Colwell		58	50	80
Copper (DTPA)	(mg/kg)	0.72	0.86	0.7
Iron (DTPA)	(mg/kg)	100	30	7.3
Manganese (DTPA)	(mg/kg)	14	5.4	0.52
Zinc (DTPA)	(mg/kg)	3.1	0.5	0.34
Boron	(mg/kg)	0.92	1.6	8.8
Sulfur (KCl40)	(mg/kg)	110	38	44
Organic Carbon	(%)	1.14	0.27	<0.15
Organic Matter	(%)	2	0.46	0.26
Aluminium ($BaCl/NH_4Cl$)	(cmol +/kg)	<0.10	<0.10	<0.10
Calcium ($BaCl/NH_4Cl$)	(cmol +/kg)	5.5	3.8	7.5
Potassium ($BaCl/NH_4Cl$)	(cmol +/kg)	0.24	0.28	0.84
Magnesium ($BaCl/NH_4Cl$)	(cmol +/kg)	0.3	2.4	11
Sodium ($BaCl/NH_4Cl$)	(cmol +/kg)	0.26	0.87	5.7
CEC ($BaCl/NH_4Cl$)	(cmol +/kg)	6.4	7.5	25
Aluminium % of Cations	(%)	1.6	1.3	0.4
Na%	(%)	4.1	12	23
Ca:Mg		18.3	1.6	0.7

2.3.5 Birchip



The Birchip soil is alkaline, sodic, with boron toxicity. It also exhibits waterlogging and is saline in the deep subsoil.

Profile description

0-10 cm – Reddish brown, light clay, soft surface condition, slightly calcareous; clear to,

10-25 cm – Dark reddish brown, light medium clay; strong medium to fine sub-angular blocky, slightly calcareous, clear to,

25-40 cm – Reddish brown, light medium clay, well developed sub-angular blocky, carbonates present (5-10%), clear to,

40-100 cm – Brown grey, light medium clay, moderate blocky structure; carbonates present (5-10%).

Table 2.6 Chemical properties of the profile layers of the Birchip soil sampled in 2019

Property	Unit	0-20 cm	20-60 cm	60-100 cm
pH (1:5 Water)		8.1	9.7	9.3
pH (1:5 $CaCl_2$)		7	8.7	8.7
EC (1:5)	(dS/m)	0.1	0.44	1.35
Chloride	(mg/kg)	14	150	770
Nitrate Nitrogen	(mg/kg)	11	4.9	4.7
Ammonium Nitrogen	(mg/kg)	2.2	0.98	0.81
Phosphorus - Colwell	(mg/kg)	20	5.6	5.8
Phosphorus Buffer Index – Colwell		58	67	120
Copper (DTPA)	(mg/kg)	0.73	0.82	0.84
Iron (DTPA)	(mg/kg)	7	5.8	6.6
Manganese (DTPA)	(mg/kg)	10	2.2	2.8
Zinc (DTPA)	(mg/kg)	1.1	0.37	0.51
Boron	(mg/kg)	2.8	21	23
Sulfur (KCl40)	(mg/kg)	7.7	43	430
Organic Carbon	(%)	0.89	0.32	0.19
Organic Matter	(%)	1.5	0.55	0.33
Aluminium ($BaCl/NH_4Cl$)	(cmol +/kg)	<0.10	<0.10	<0.10
Calcium ($BaCl/NH_4Cl$)	(cmol +/kg)	15	10	9.5
Potassium ($BaCl/NH_4Cl$)	(cmol +/kg)	1.6	1.1	1.2
Magnesium ($BaCl/NH_4Cl$)	(cmol +/kg)	4.4	11	9.9
Sodium ($BaCl/NH_4Cl$)	(cmol +/kg)	1.5	9.1	14
CEC ($BaCl/NH_4Cl$)	(cmol +/kg)	23	31	35
Aluminium % of Cations	(%)	0.43	0.32	0.29
Na%	(%)	6.5	29	40
Ca:Mg		3.4	0.9	1.0

2.3.6 Wonwondah

The Wonwondah soil was selected due to potassium deficiency and exhibiting poor general plant growth.

Profile description

0-5 cm – Light brown, sandy loam, granular, many roots, clear to,

5-30 cm – Pale brown, sandy loam, blocky, roots present clear to,

30-100 cm – Yellow brown, medium clay, weak blocky to massive.

Table 2.7 Chemical properties of the profile layers of the Wonwondah soil sampled in 2019

Property	Unit	0-20 cm	20-60 cm	60-100 cm
pH (1:5 Water)		5.9	8.2	9.6
pH (1:5 $CaCl_2$)		4.7	6.8	8.7
EC (1:5)	(dS/m)	0.07	0.15	0.56
Chloride	(mg/kg)	27	58	320
Nitrate Nitrogen	(mg/kg)	5.1	1.1	1.1
Ammonium Nitrogen	(mg/kg)	2.4	1.5	0.96
Phosphorus - Colwell	(mg/kg)	46	9.6	6.5
Phosphorus Buffer Index - Colwell		58	39	35
Copper (DTPA)	(mg/kg)	0.13	0.039	0.092
Iron (DTPA)	(mg/kg)	200	26	8.9
Manganese (DTPA)	(mg/kg)	4.9	1.2	0.34
Zinc (DTPA)	(mg/kg)	1.2	0.45	0.26
Boron	(mg/kg)	0.62	2.5	5.1
Sulfur (KCl40)	(mg/kg)	4.9	16	72
Organic Carbon	(%)	1.05	0.22	<0.15
Organic Matter	(%)	1.8	0.38	0.26
Aluminium ($BaCl/NH_4Cl$)	(cmol +/kg)	0.23	<0.10	<0.10
Calcium ($BaCl/NH_4Cl$)	(cmol +/kg)	3.1	4.1	9.5
Potassium ($BaCl/NH_4Cl$)	(cmol +/kg)	0.25	0.27	0.34
Magnesium ($BaCl/NH_4Cl$)	(cmol +/kg)	2.1	6.5	8.6
Sodium ($BaCl/NH_4Cl$)	(cmol +/kg)	0.51	2.4	5.4
CEC (BaCl/NH4Cl)	(cmol +/kg)	6.15	13.3	23.8
Aluminium % of Cations	(%)	3.8	<1	<1
Na%	(%)	8.3	18	23
Ca:Mg		1.5	0.63	1.1

3. Phase 1: Glasshouse trial - reconstructed soil columns

3.1 Methods

Soils profiles were reconstituted in 15 cm diameter x 105 cm long PVC pipes based on depths from which they were collected from the field. The bottom two layers of soil were gradually moistened and mixed to create moist soil to which amendments were added prior to mixing. This moistened soil was then gradually packed into the PVC columns using a plunger to achieve consistent bulk densities for each soil type of around 1.5 g cm^{-3} for subsoil and 1.3 g cm^{-3} for surface soils. The top layer of soil was reconstituted with air dry soil then gradually moistened by surface watering until 70% Plant Available Water (PAW) was achieved. A basal application of DAP fertiliser (11 kg P/ha; 10 kg N/ha) was applied to the topsoil.

Six pre-germinated seeds of wheat (*Triticum aestivum* cv. Vixen were sown on NSW component soils and cv. Schomburgk grown on Victorian component soils) were sown per column and thinned to three plants after emergence. All treatments of the Birchip soil were sown with a boron tolerant near isogenic line of cv. Schomburgk as that soil was the only one to contain boron in potentially toxic concentrations. Columns were kept moist while seedlings established then watered to weight twice a week (70 % PAW) from growth stage Z14-47 after which time watering was stopped to encourage root growth in the subsoil. Ammonium nitrate (equivalent to 30 kg/ha N) was applied to all columns at 24 and 38 days.

The mass of water applied to columns during the experiment was recorded. Plant height, tiller and head number were measured at maturity. Above ground plant material was then cut and dry biomass recorded prior to grain harvest by manual threshing. The soil columns were then split vertically for destructively sampling. Subsamples for gravimetric water content and chemical analyses were taken prior to roots being washed from the soil in layers. Roots were then oven dried at 65 degrees for 48 hours and weighed.

Treatments were customised for each soil layer at each site depending on the results of soil analysis. Deficiencies, toxicities, sodicity and pH values varied between sites however, in general, sodicity was treated with gypsum, alkaline layers with sulfur, low pH with lime, poor structure with organic matter and poor general fertility with manure. Specific elemental deficiencies were addressed with direct application of plant available forms of the nutrient required. Table 3.1 summarises individual soil treatments for each site and depth.

Table 3.1: Treatment type and application rate for each layer allocated to Victorian and NSW component soils

Victorian soils:

Depth (cm)	Birchip	(t/ha)	Marrabel	(t/ha)	Wonwondah	(t/ha)
0-20	Manure Pellets	1	Manure Pellets	1	Manure Pellets	1
			Potassium Sulfate	0.05 K	Potassium Sulfate	0.05 K
					Lime	3
					Copper Sulfate	0.005 Cu
20-50	Manure Pellets	5	Manure Pellets	5	Manure Pellets	5
	Gypsum	4	Gypsum	4	Gypsum	4
	Sulfur	0.4	Potassium Sulfate	0.05 K	Potassium Sulfate	0.05 K
	Zinc Sulfate	0.005 Zn	Zinc Sulfate	0.005 Zn		
50-100	Manure Pellets	14	Manure Pellets	14	Manure Pellets	14
	Gypsum	4	Gypsum	4	Gypsum	4
	Sulfur	1.2	Sulfur	1.2	Sulfur	1.2
	Zinc Sulfate	**0.005 Zn**	**Zinc Sulfate**	**0.005 Zn**		

NSW soils:

Depth (cm)	Oaklands	(t/ha)	Depth (cm)	Trungley Hall	(t/ha)	Depth (cm)	Condobolin	(t/ha)
0-10	Manure Pellets	5	0-10	Manure Pellets	5	0-15	Manure Pellets	5
							Lime	5
10-50	Manure Pellets	10	10-40	Manure Pellets	5	15-50	Manure Pellets	5
	Gypsum	5		Sulfur	1.6		Lime	5
	Sulfur	1.6						
50-100	Manure Pellets	10	40-100	Manure Pellets	5	15-80	OM Pellets	10
	Gypsum	5		OM Pellets	10		Lime	5
	Sulfur	**1.6**		**Sulfur**	**1.6**			

3.2 Results

The effect of amendments varied with soil type. Amendments caused significant decreases in plant root growth (mass) to a depth of 50 cm in the Birchip soil, the 0-20 and 50-100 cm layers of the Wonwondah soil and the 50-100 cm layer of the Marrabel soil (Figure 3.1). However, amendment significantly improved root mass in the 20-50 cm layer of the Wonwondah soil and the 50 cm in the Marrabel soil and from 20-60 cm in the Trungley Hall soil. Small but statistically significant increases in root mass were also recorded in the Condobolin soil in 10-20 and 30-40 cm layers. All other soil layers in Condobolin, Trungley Hall and all layers of the Oaklands soil resulted in no significant difference between root mass of plants grown in amended and control treated soil.

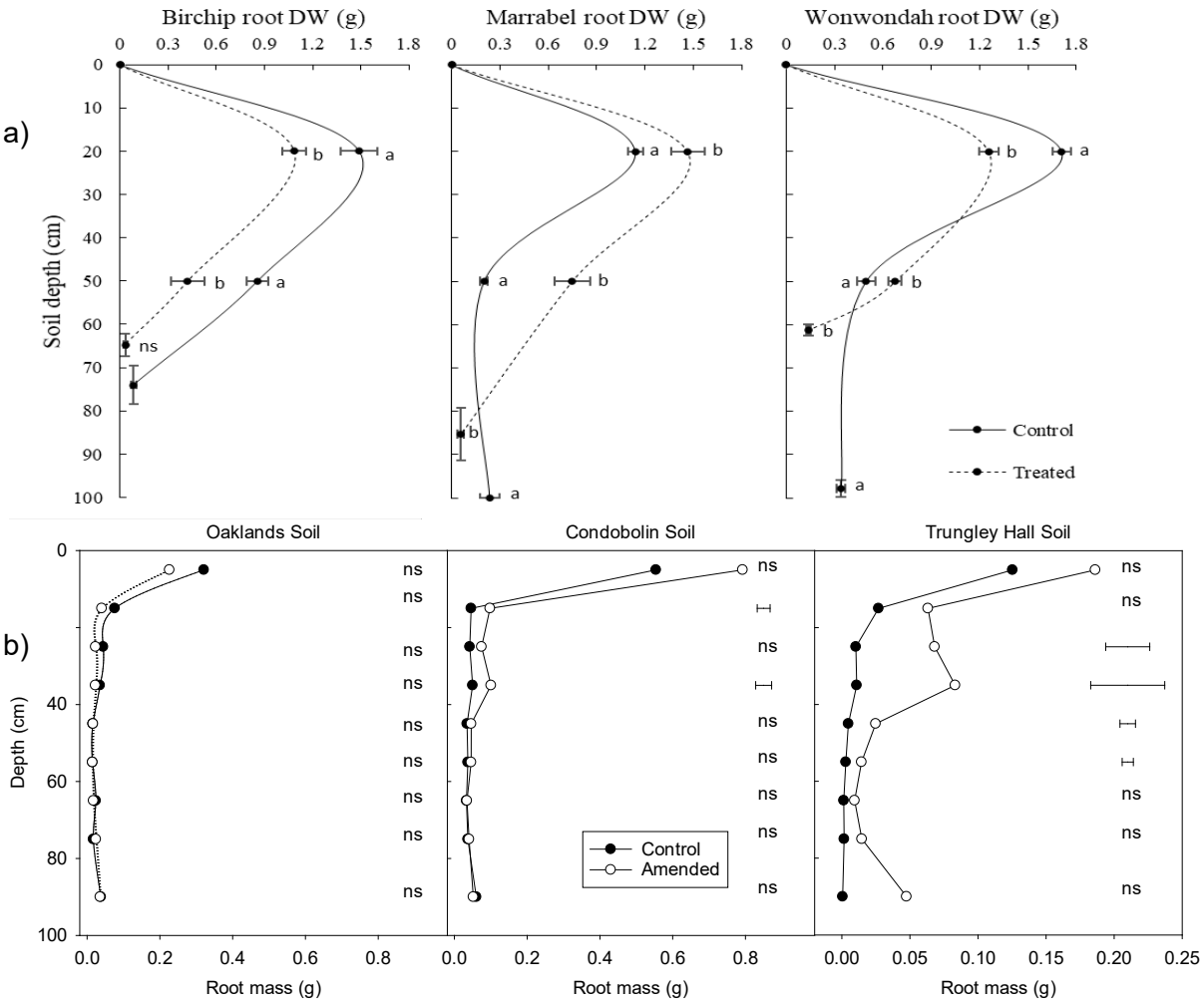


Figure 3.1 Root mass of control and amended soils in sampled depth increments for soils of the Victorian (a) and NSW (b) components. Data of individual soil layers marked with different letters indicates statistical different p<0.05 for Figure 3.1a, horizontal bars are standard error of the mean, vertical bars indicate standard error of the mean of maximum rooting depth

Applying amendment produced significant (P<0.05) decreases in above ground biomass and grain yield compared to the untreated control in the Birchip and Wonwondah soil columns (Table 3.2). Amendment produced no growth effect in the Marrabel and Oakland soils, whilst significant increases in yield due to amendment resulted in the Trungley Hall and Condobolin soils.

Significantly greater tiller numbers due to amendment were recorded ay Marrabel, Trungley and Condobolin although this did not always relate to increased grain yield.

*Table 3.2: Average shoot dry weight, grain yield, Harvest Index (HI), tiller number, number of heads and seed weight for each soil type and treatment (control and treated) (ns=not significant, *** denoted statistical significance (P<0.05) where lsd not given)*

Treatment	Shoot Dry Weight (g/core)	Grain Yield (g/core)	Harvest Index	Tiller No.	Head No.	Total water use (L)
Birchip						
Control	31.82	13.97	0.44	22	13	5.48
Treated	28.86	11.94	0.42	20	12	4.61
l.s.d. (0.05)	2.44	1.78	ns	ns	ns	***
Marrabel						
Control	39.53	19.08	0.48	19	18	5.98
Treated	36.67	16.73	0.45	24	17	5.91
l.s.d. (0.05)	ns	ns	0.02	1.9	ns	ns
Wonwondah						
Control	30.42	12.55	0.41	14	11	5.33
Treated	22.13	7.88	0.36	12	9	4.29
l.s.d. (0.05)	2.07	1.03	0.04	ns	Ns	***
Oaklands						
Control	9.07	4.13	0.46	5.75	5.75	1.87
Treated	11.85	4.69	0.40	7.00	7.00	1.68
l.s.d. (0.05)	ns	ns	ns	ns	ns	ns
Trungley Hall						
Control	1.67	0.79	0.45	2.75	2.75	1.13
Treated	9.12	4.11	0.45	6.00	6.00	1.04
l.s.d. (0.05)	2.36	0.81	ns	2.0	2.0	ns
Condobolin						
Control	12.63	4.34	0.34	9.75	9.75	1.98
Treated	17.58	7.53	0.43	13.5	13.5	2.20
l.s.d. (0.05)	2.64	2.45	ns	2.72	2.72	ns

The addition of amendments (both organic and inorganic) caused significant increases in salinity, measured as electrical conductivity, in deeper layers of most soils (Table 3.3). Salinity recordings that may have been deleterious to plant growth (>0.4 dS/m) existed in the amended treated layers of Birchip (20-100 cm), Marrabel (50-100 cm), Wonwondah (50-100 cm), Oaklands (0-10) and Trungley Hall (0-10, 40-100 cm).

Adding lime to the Condobolin soil increased soil pH in all layers. The use of elemental sulfur to decrease soil pH however seems largely ineffective on most soils treated with S (Birchip, Marrabel, Wonwondah, Oaklands and Trungley Hall). However significant acidification was measured in the surface layer at Wonwondah.

*Table 3.3: Soil salinity (dS/m) and soil pH_{Ca} measured after harvest for control and amendment treated soils. P values marked with ns denoted no significant difference, *ns denotes significance at p<0.10*

		Soil salinity (dS/m)			Soil pH (1:5 $CaCl_2$)		
	depth (cm)	control	amended	P value	control	amended	P value
Birchip	0-20	0.09	0.14	0.059	6.96	7.01	ns
	20-50	0.39	0.63	0.052	7.96	7.91	ns
	50-100	1.37	1.51	0.001	8.36	8.28	<0.001
Marrabel	0-20	0.21	0.16	ns	6.35	6.44	ns
	20-50	0.13	0.38	0.010	6.01	6.29	0.009
	50-100	0.36	0.44	<0.001	8.13	8.12	ns
Wonwondah	0-20	0.10	0.09	0.042	5.82	4.71	0.013
	20-50	0.20	0.31	<0.001	6.57	6.79	*ns
	50-100	0.55	0.65	0.001	8.56	8.51	*ns
Oaklands	0-10	0.42	0.61	ns	5.45	5.85	0.034
	10-50	0.16	0.21	ns	7.02	6.79	*ns
	50-100	0.25	0.34	ns	7.94	7.92	ns
Trungley Hall	0-10	0.30	0.98	ns	5.32	5.45	ns
	10-40	0.08	0.18	0.048	6.58	6.76	ns
	40-100	0.29	0.43	ns	8.18	7.90	*ns
Condobolin	0-15	0.04	0.03	ns	5.04	5.25	0.02
	15-50	0.04	0.05	<0.001	4.87	5.54	<0.001
	50-100	0.04	0.05	0.058	4.34	5.25	0.003

There was no significant difference in soil water content between amended and control treatments in the surface layer of any soils examined (Figure 3.2). Similarly, there were also no differences in soil water concentration in treatments applied to the Oakland and Condobolin soils. Soil water concentration in the Birchip, Marrabel and Wonwondah soils was less in the subsoil layers of control treated soil than the amended columns at harvest. The amended soil resulted in significantly drier soil in the subsoil of only the Trungley Hall soil.

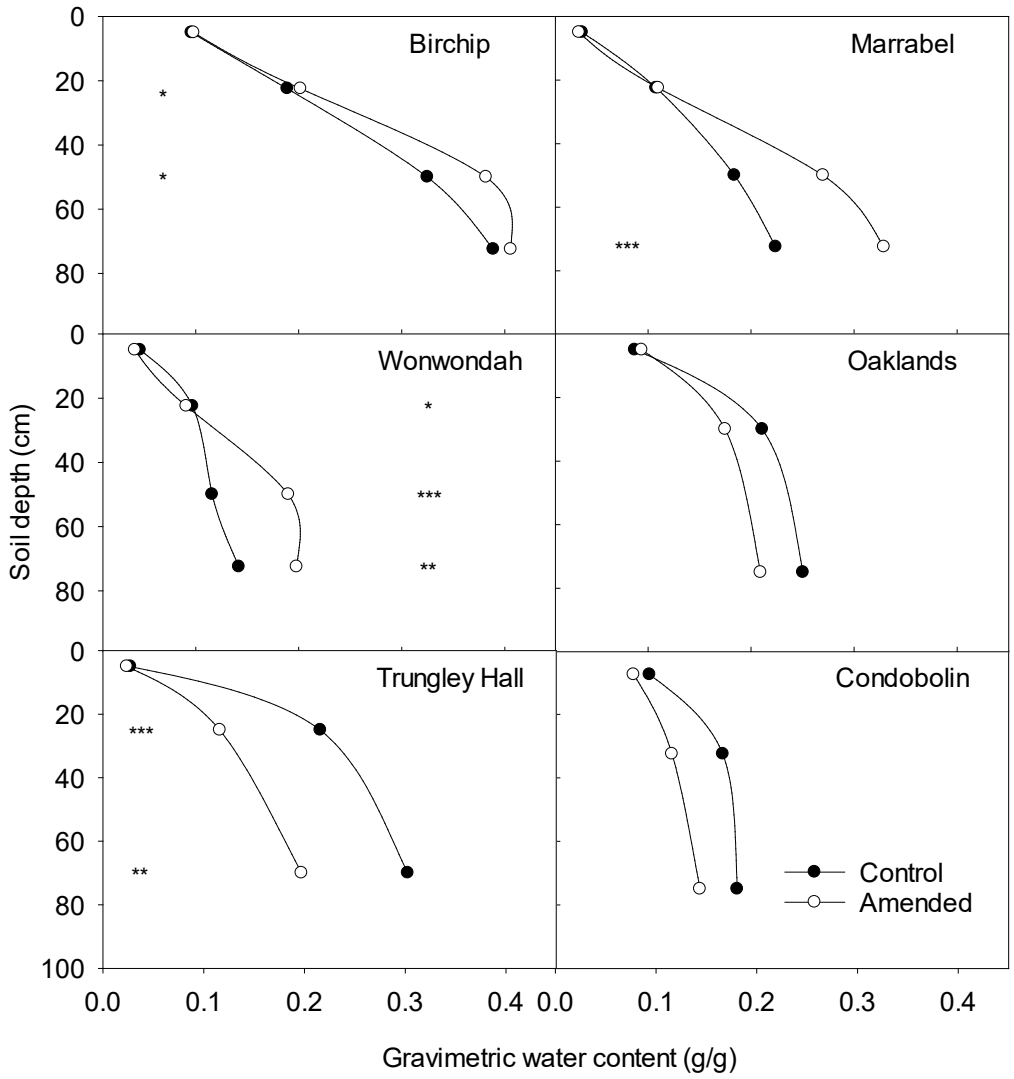


*Figure 3.2. Soil gravimetric water content (g/g) of control and amended soil columns after harvest of wheat for each of the six soils examined. Layers marked with *, ** and *** denotes statistical difference at P<0.05, 0.05<P>0.001, and P<0.001, respectively.*

3.3 Discussion

Organic amendments caused increased root growth compared to the control in some but not all subsurface layers of Marrabel, Wonwondah, Condobolin and Trungley Hall soils. That these positive root effects did not always result in increased above ground plant performance indicates that other limitations may have impacted on grain yield. Organic amendment to soils with poor structure associated with sodicity (Birchip, Marrabel, Wonwondah, Oaklands) tended to either have no response or negative response to amendment in terms of plant responses (biomass or grain yield). Notably, Birchip and Wonwondah suffered decreased yield following amendment. These soils initially exhibited sodicity in all layers, including the surface layer, which may have limited aeration when soils dispersed on wetting. The resultant poor aeration may have been exacerbated with the addition of organic amendments which create a high microbial oxygen demand during decomposition. Soils that were non-sodic in the topsoil may provide adequate aeration to lower layers preventing anoxic conditions thereby allowing improvements to root growth (e.g. for Marrabel, Trungley Hall and Condobolin).

It is noteworthy that soils where amendment addition resulted in significant decreased yield (Birchip and Wonwondah) also experienced significant increases in salinity of the deepest soil layers where electrical conductivities greater than 0.5 dS/m were recorded. Such salinities would be sufficient to impact yield by limiting root growth (Figure 3.1) and water uptake (Figure 3.2, Table 3.2).

Changes in soil water generally corresponded with plant response to applied treatment. In the Birchip and Wonwondah soils where the control produced more plant growth than the amended treatment, the soils were drier in the control columns. However, in soils where amendment resulted in improved growth, only the Trungley Hall soil had drier soil in the amended treatment, suggesting that the increased yield of the Condobolin soil was not due to greater water uptake, an observation supported by a lack of increased root growth to depth for the Condobolin amended soil. Therefore, it is likely that the plant yield response to added amendment in the Condobolin soil was due to increased nutrition rather than overcoming a physical constraint, allowing greater root access to subsoil water. Alternatively, the performance of the Trungley Hall soil when amended can be attributed largely to increased root growth to a depth of 60 cm. This may be a response to nutrition but given the initial properties it would be probable that the plant response was due to physical improvement of the second layer of the subsoil (10-50 cm).

This experiment has highlighted that organic amendments to soils exhibiting structurally challenged topsoils and subsoils soil can be problematic in the short term. Amendments that may improve surface and upper subsoil performance but that add to salinity of the deeper subsoil may result in a net negative effect on plants. The impact of this response may be contextually specific to varying patterns in the amount and timing of rainfall in the field.

It was possible to amend surface and upper subsoil layers to improve root growth but not deep subsoil layers. This is an important finding as application of amendment is commercially possible to the surface and upper subsoil but currently impractical to deep subsoils. Therefore, these column studies would suggest that efforts to ameliorate deep subsoils will not be rewarded by improved soil performance and plant growth.

4. Phase 2: Lab incubation studies

4.1 Introduction

In parallel to the experiments of phase 1, laboratory incubation experiments were conducted to gain data on amendment type and rate response on the selected soils.

4.2 Methods

The soil from each site was divided according to morphological horizon and treated separately as it was not intended to study the interaction between horizons for the incubation experiment. The soils were treated with multiple rate-response treatment formulations; applied either in isolation, or in combination, depending on the identified constraints present.

The ameliorants used for the incubation included;

- chicken manure pellets (CMP) – nutrient, C (structure) and pH amendment
- lime (commercial grade) – pH amendment
- organic matter – pea hay pellets (ground) – C (soil structure) and pH amendment
- elemental S – pH amendment
- gypsum (commercial grade) (soil structure)
- MgSi (olivine, ground), pH amendment, P release from soil.
- Reactive Rock Phosphate (RPR) (pH amendment, P addition).

Treatment rates are summarised below in Table 4.1a and Table 4.1b for Victorian and NSW components respectively. Soil batches were air dried and sieved to 2 mm then field capacity, permanent wilting point and gravimetric water content were obtained. Air dry soil (100 g) was placed in 120 mL polypropylene (PP) containers and treated on an area basis and once added, these were mixed end over end for one hour.

Lime, elemental S and MgSi rates were determined based on an estimate of pH buffering capacity from similar soils. Gypsum rates were estimated to be high enough to overcome sodicity but not cause salt effects deleterious to plant growth. Reactive rock phosphate (RPR) was based on a neutralising value of 50% as determined in previous incubations on similar soils. Rates of manure and organic matter addition were based on previous studies conducted by DEDJTR. The range of ameliorant application rates covered 0, 50, 100 and 200% of the recommended application rates. These rates were intended to provide a rate response for each treatment combination or material.

De-ionised water was added to soil samples until 70% plant available water was achieved and incubated at 22^{o}C in the dark for three months. Containers were opened and closed weekly for aeration. At the termination of the incubations, soil was sampled for analysis of soil pH (in water and $CaCl_2$), electrical conductivity (1:5 soil:water), Colwell P, mineral N, exchangeable cations allowing Ca:Mg and exchangeable sodium percentage (ESP) to be calculated, and available sulfate concentration.

Table 4.1a Treatments and rates (t/ha) for phase 2 laboratory incubations - Victorian component

Birchip

Topsoil (0-20 cm)	*Subsoil (20-60 cm) - individual*	*Subsoil (20-60 cm) - combinations*
Chicken manure pellets (CMP) 0, 5, 10, 20	Gypsum 0, 2, 4, 8	CMP:Gypsum 10:2, 10:4, 10:8
Straw 0, 5, 10, 20	CMP 0, 5, 10, 20	Gypsum:S 2:0.2, 2:0.4, 8:0.8
Straw with nutrients (straw:CMP equivalents) 10:5, 10:10, 10:20	Sulfur (S) 0, 0.2, 0.4, 0.8	Straw:nutrients (CMP equiv.) 10:5, 10:10, 10:20
	Straw 0, 5, 10, 20	

Marrabel

Topsoil (0-20 cm)	*Subsoil (20-60 cm) - individual*	*Subsoil (20-60 cm) - combinations*
CMP 0, 5, 10, 20	Gypsum 0, 2, 4, 8	CMP:Gypsum 10:2, 10:4, 10:8
Straw 0, 5, 10, 20	CMP 0, 5, 10 ,20	Straw:nutrients (CMP equiv.) 10:5, 10:10, 10:20
Straw with nutrients (straw:CMP equivalents) 10:5, 10:10, 10:20	Straw 0, 5, 10, 20	

Wonwondah

Topsoil (0-20 cm)	*Subsoil (20-60 cm) - individual*	*Subsoil (20-60 cm) - combinations*
CMP 0, 5, 10, 20	Gypsum 0, 2, 4, 8	CMP:Gypsum 10:2, 10:4, 10:8
Straw 0, 5, 10, 20	CMP 0, 5, 10, 20	Straw:nutrients (CMP equiv.) 10:5, 10:10, 10:20
Straw with nutrients (straw:CMP equivalents) 10:5, 10:10, 10:20	Straw 0, 5, 10, 20	

Table 4.1b Treatments and rates (t/ha) for phase 2 laboratory incubations - NSW component

Oaklands

Topsoil (0-10 cm)	*Subsoil 1 (10-50 cm)*	*Subsoil 2 (50-100 cm)*
Gypsum: 0, 2.5, 5, 10	Gypsum; 0, 2.5, 5, 10	Gypsum; 0, 2.5, 5, 10
	Sulfur (S) 0, 0.2, 0.4, 0.8	Sulfur 0, 0.3, 0.6, 1.2
	Gypsum:Sulfur G =0; S = 0, 0.2, 0.4, 0.8 G = 2.5; S = 0, 0.2, 0.4, 0.8 G = 5; S = 0, 0.2, 0.4, 0.8 G = 10; S = 0, 0.2, 0.4, 0.8	Gypsum plus Sulfur G =0; S = 0, 0.3, 0.6, 1.2 G = 2.5; S = 0, 0.3, 0.6, 1.2 G = 5; S = 0, 0.3, 0.6, 1.2 G = 10; S = 0, 0.3, 0.6, 1.2

Trungley Hall

Topsoil (0-10 cm)	*Subsoil 1 (10-40 cm)*	*Subsoil 2 (40-100 cm)*
Gypsum: 0, 2.5, 5, 10	Organic matter (OM - pea hay) 0, 5, 10, 15	Organic matter (OM - pea hay) 0, 5, 10, 15
Organic matter (OM - pea hay) 5, 10 15	Sulfur; 0.15, 0.3, 0.6	Sulfur; 0.6, 1.2, 2.4
Gypsum plus OM - pea hay: G = 2.5; OM = 5 G = 10; OM = 15	OM - pea hay plus S: OM = 5; S = 0.15 OM = 15; S = 0.6 S plus OM (wheat straw pellets): S = 0.15; OM = 5 S = 0.3; OM = 5 S = 0.6; OM = 5	OM - pea hay plus S: OM = 5; S = 0.6 OM = 15; S = 2.4 S plus OM (wheat straw pellets): S = 0.6; OM = 5 S = 1.2; OM = 5 S = 2.4; OM = 5

Condobolin

Topsoil (0-15 cm)	*Subsoil 1 (15-50 cm)*	*Subsoil 2 (50-100 cm)*
Chicken manure pellets: 0, 5, 10, 15	MgSi: 0, 0.05, 0.25, 1, 3	MgSi: 0, 0.05, 0.25, 1, 3
Organic matter (pea hay pellets): 5, 10, 15	Lime: 2.5, 5, 10	Lime: 2.5, 5,10
Lime: 1, 2, 3	Reactive Rock Phosphate (RPR) 1, 2, 4	

4.3 Results

4.3.1 Birchip

Amendment to the surface layer of the Birchip soil with CMP increased soil electrical conductivity (EC) (Figure 4.1), with increases in available P and nitrate concentrations however it had no effect on flocculation as measured by turbidity. Available S and K also increased with CMP addition in line with the nutrient content of the amendment. The addition of synthetic nutrients with WSP increased EC and available S, K and nitrate concentrations. The addition of WSP alone significantly decreased nitrate concentration relative to the control. Interestingly, WSP alone decreased turbidity but only at the highest rate of application.

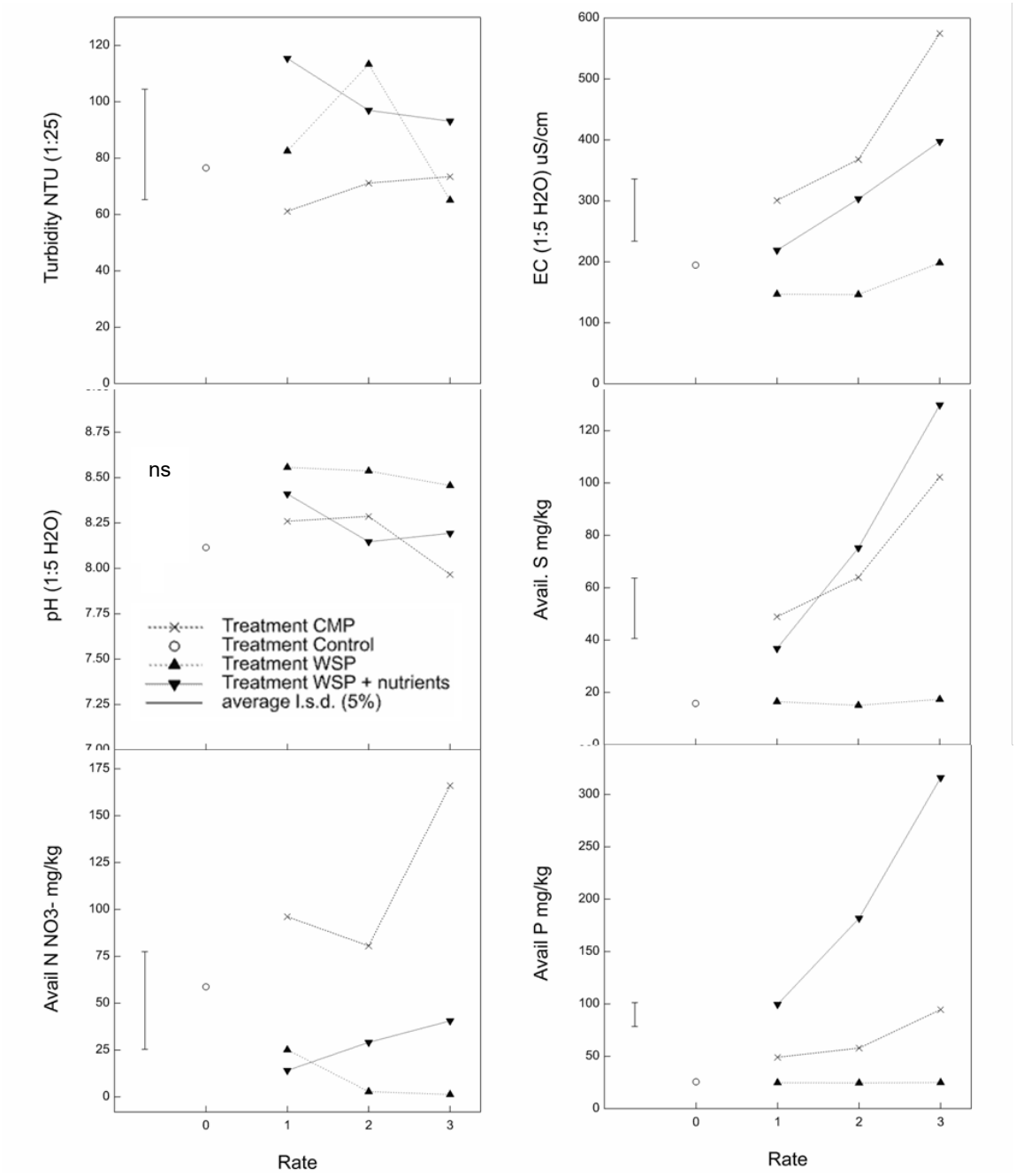


Figure 4.1. Soil chemistry changes in the Birchip topsoil (0-20 cm) due to amendment with chicken manure pellets (CMP), wheat shoot pellets (WSP) (rate 0, 1, 2, 3, represent 0, 5, 10, 20 t/ha) and WSP+nutrients. Vertical bars represent lsd p=0.05, ns = not significant.

In the subsoil (Figure 4.2) treatment addition increased EC, gypsum treatments increased EC more than other treatments. Sulfur treatments were effective in decreasing soil pH. The addition of WSP increased measured turbidity.

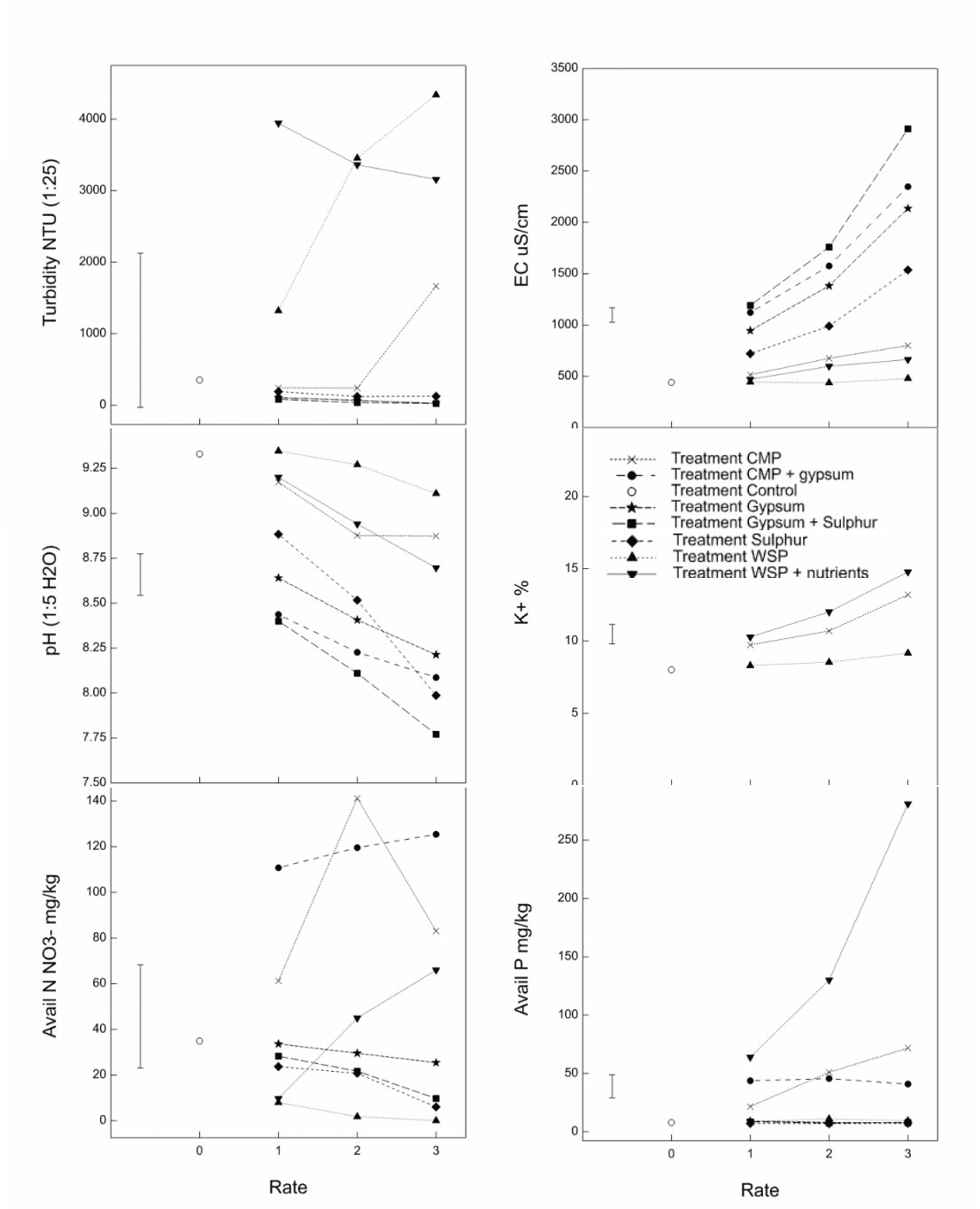


Figure 4.2. Soil chemistry changes in the Birchip subsoil (20-60 cm) due to amendment with chicken manure pellets (CMP), wheat straw pellets (WSP) (rate 0, 1, 2, 3, represent 0, 5, 10, 20 t/ha) and WSP+nutrients, Gypsum (rate 0, 1, 2, 3 represents 0, 2, 4, 8 t/ha) and sulfur (rate 0, 1, 2, 3 represents 0, 0.2, 0.4, 0.8 t/ha). Vertical bars represent lsd p=0.05, ns = not significant.

4.3.2 Marrabel

Turbidity of the Marrabel topsoil increased at the highest application rate of WSP+nutrients but decreased when only WSP was added (Figure 4.3). A relatively large decrease in nitrate concentration corresponded to an increase in pH at the highest application rate of CMP suggesting immobilisation of nitrate or denitrification occurred in response to that treatment. Added P in the nutrient solution was significantly greater than that supplied in CMP.

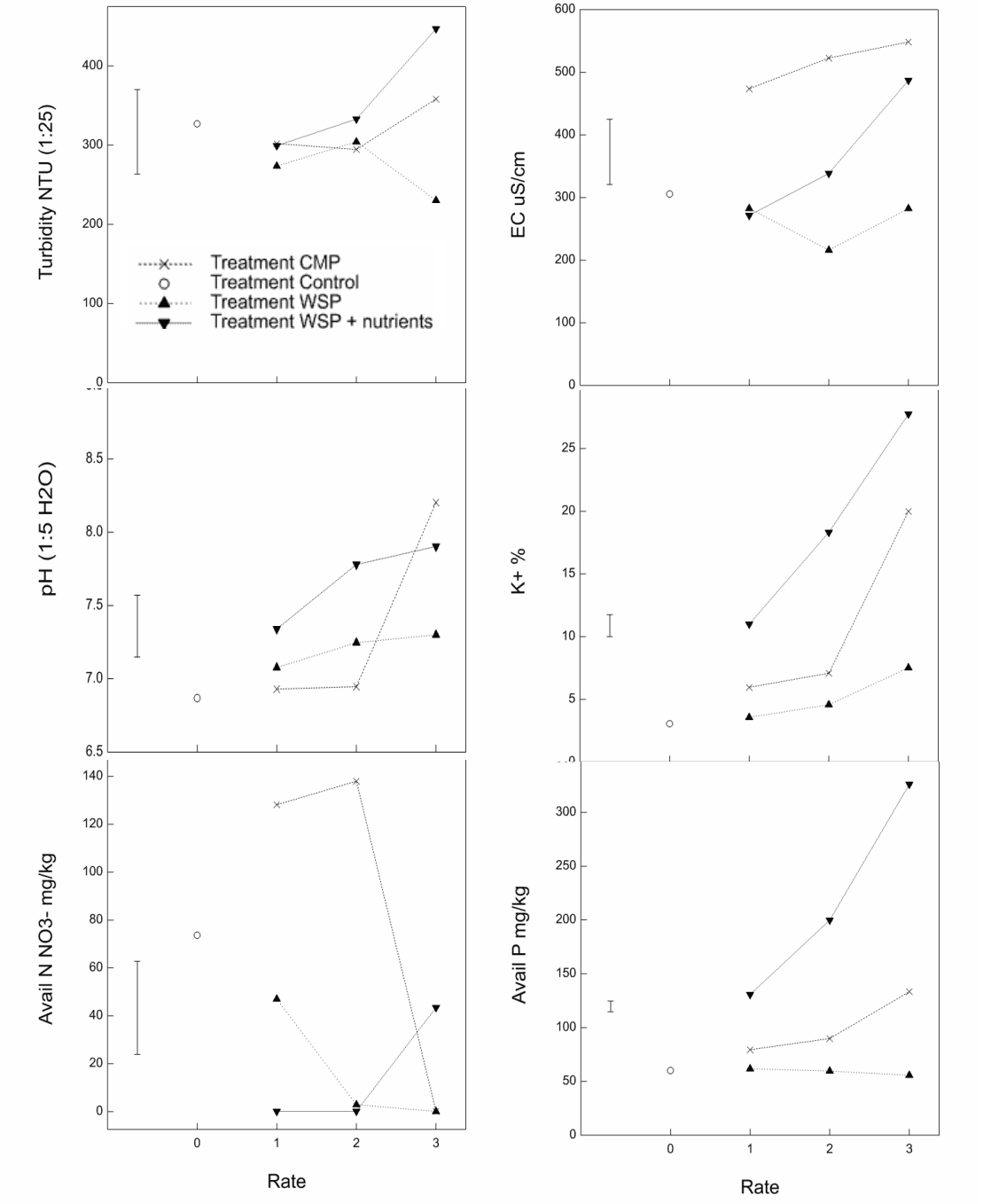


Figure 4.3. Soil chemistry changes in the Marrabel topsoil (0-20 cm) due to amendment with chicken manure pellets (CMP), wheat shoot pellets (WSP) (rate 0, 1, 2, 3, represent 0, 5, 10, 20 t/ha) and WSP+nutrients. Vertical bars represent lsd p=0.05, ns = not significant.

Gypsum application to the Marrabel subsoil was effective in flocculating the dispersive clay based on a measured decrease in turbidity as the rate of gypsum application increased (Figure 4.4). The WSP was also effective in decreasing turbidity. Gypsum decreased soil pH but this effect was not evident when added with CMP. Nitrate concentrations were greatest in CMP treatments. Available P increased with increased rate of CMP but not in the presence of gypsum.

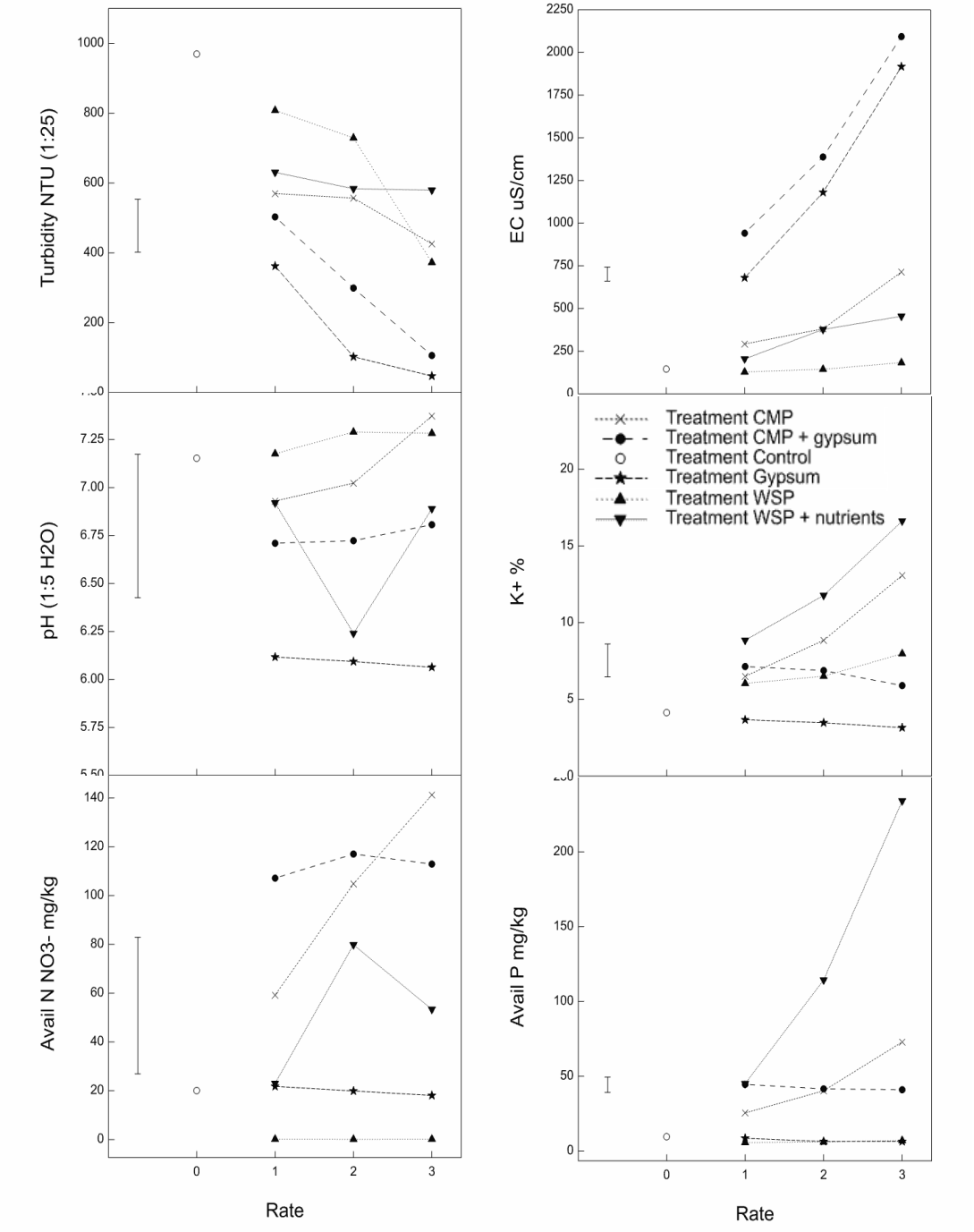


Figure 4.4. Soil chemistry changes in the Marrabel subsoil (20-60 cm) due to amendment with chicken manure pellets (CMP), wheat straw pellets (WSP) (rate 0, 1, 2, 3, represent 0, 5, 10, 20 t/ha) and WSP+nutrients, and Gypsum (rate 0, 1, 2, 3 represents 0, 2, 4, 8 t/ha). Vertical bars represent lsd p=0.05, ns = not significant.

4.3.3 Wonwondah

The application of WSP aided flocculation (decreased turbidity) but turbidity increased with WSP+nutrients when these were added to the Wonwondah topsoil (Figure 4.5). Soil EC increased with increased rate of application with CMP resulting in the highest EC relative to other treatments. CMP also resulted in high nitrate concentrations but decreased at the highest application rate, which was also associated with an increase is soil pH. This effect was also evident in the WSP+nutrient treatment.

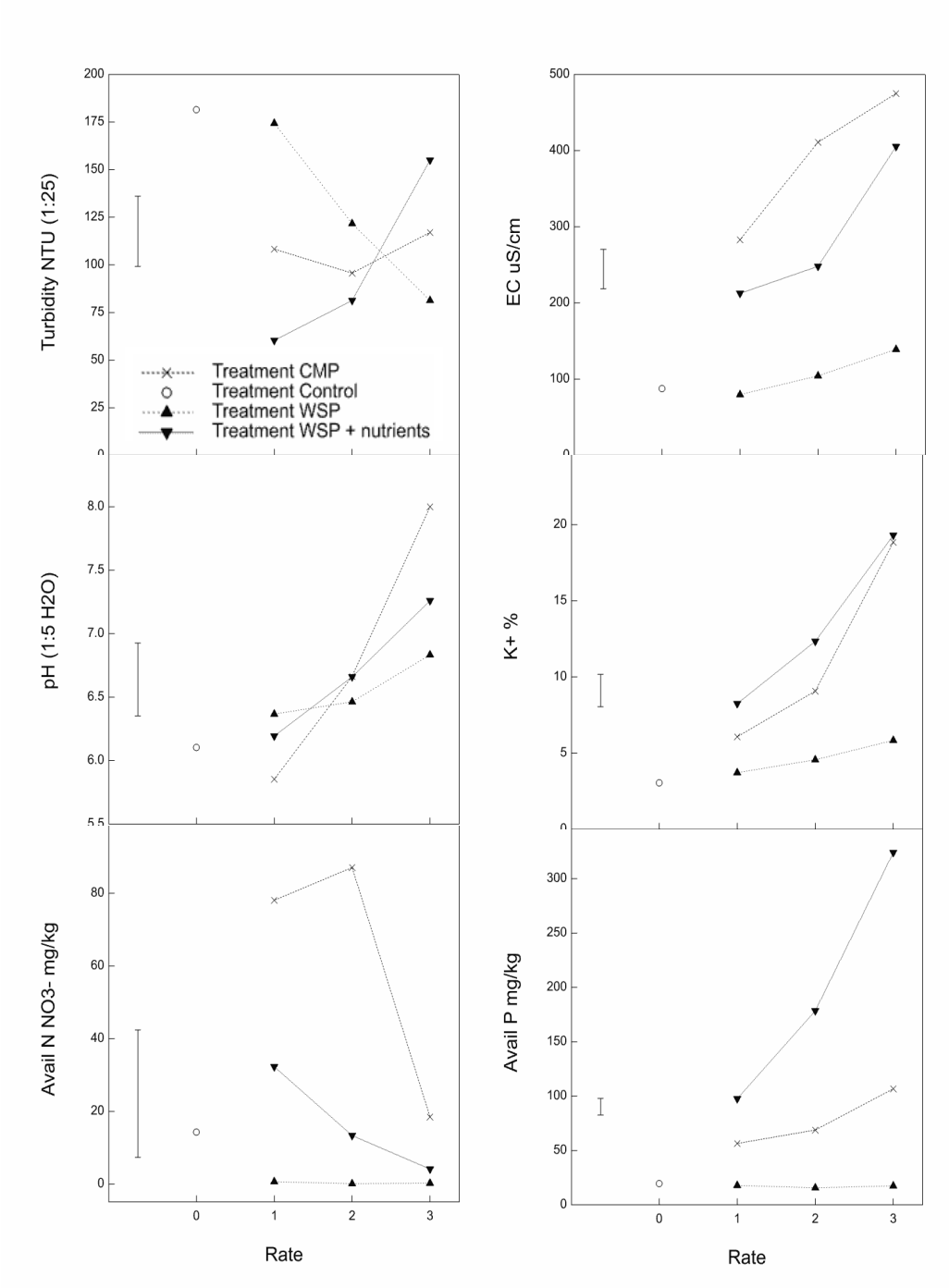


Figure 4.5. Soil chemistry changes in the Wonwondah topsoil (0-20 cm) due to amendment with chicken manure pellets (CMP), wheat shoot pellets (WSP) (rate 0, 1, 2, 3, represent 0, 5, 10, 20 t/ha) and WSP+nutrients. Vertical bars represent lsd p=0.05, ns = not significant.

Gypsum decreased turbidity when applied to the Wonwondah subsoil (Figure 4.6). However, this was also associated with significant increases in EC. There appears no benefit to flocculation from gypsum rates greater than 2 t/ha. Gypsum alone decreased soil pH significantly but this effect was not in response to the rate of application. Nitrate concentration at the highest CMP rate decreased and was associated with an increase in soil pH.

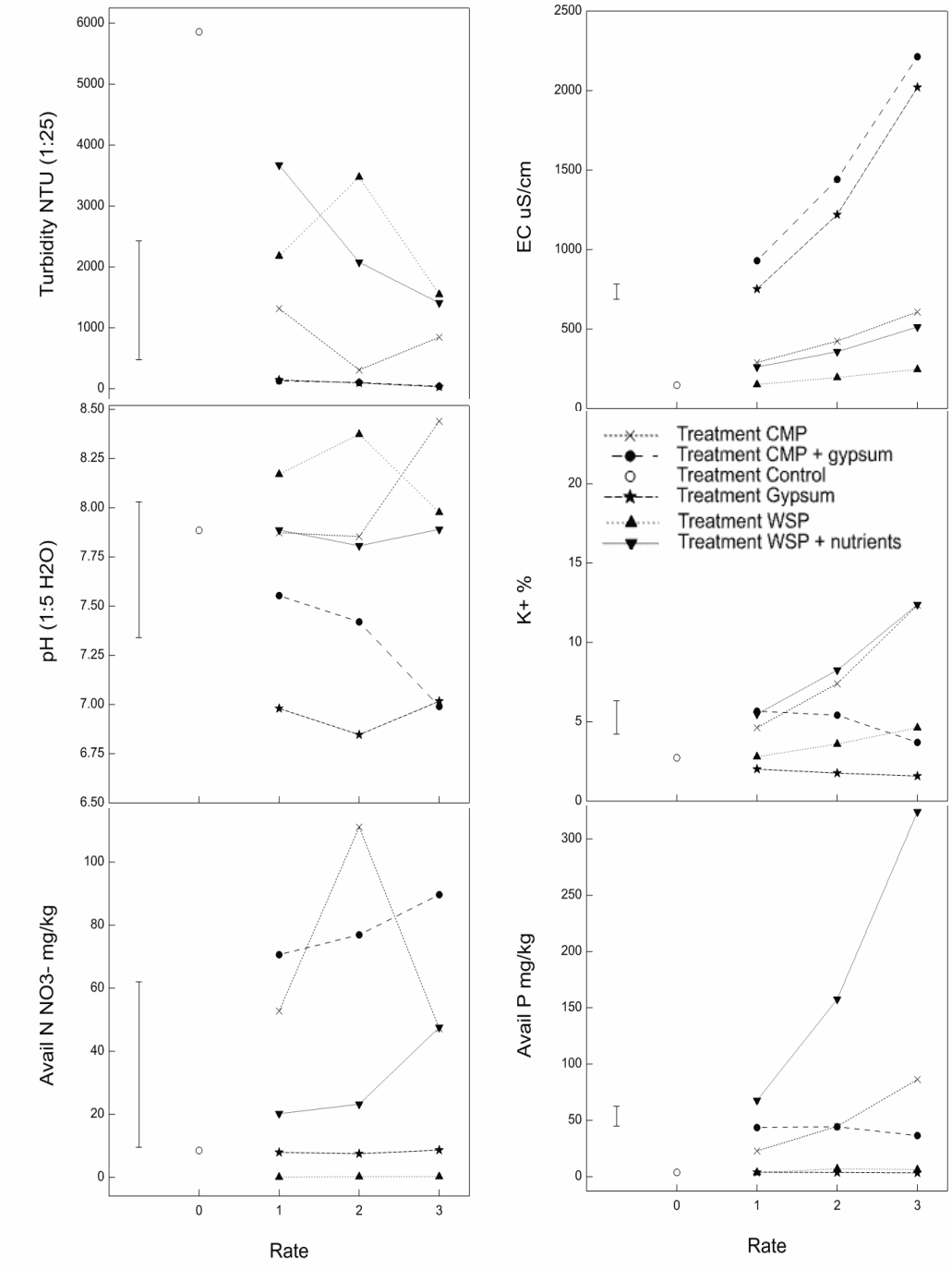


Figure 4.6. Soil chemistry changes in the Wonwondah subsoil (20-60 cm) due to amendment with chicken manure pellets (CMP), wheat straw pellets (WSP) (rate 0, 1, 2, 3, represent 0, 5, 10, 20 t/ha) and WSP+nutrients, and Gypsum (rate 0, 1, 2, 3 represents 0, 2, 4, 8 t/ha). Vertical bars represent lsd p=0.05, ns = not significant.

4.3.4 Oaklands

The application of gypsum to the topsoil (0-10 cm) of the Oaklands soil decreased ESP from 8.5 to 6.5% for the control to 10 t/ha gypsum, respectively (Figure 4.7). This change was also associated with a slight increasing trend in Ca:Mg however the maximum Ca:Mg remained less than 2 which would suggest that aggregate stability may still be compromised. However, the EC also increased with increasing rate of gypsum application. The combined influence of decreased ESP and increasing EC was a decreasing trend in turbidity representing improved microaggregate stability as gypsum rate increased.

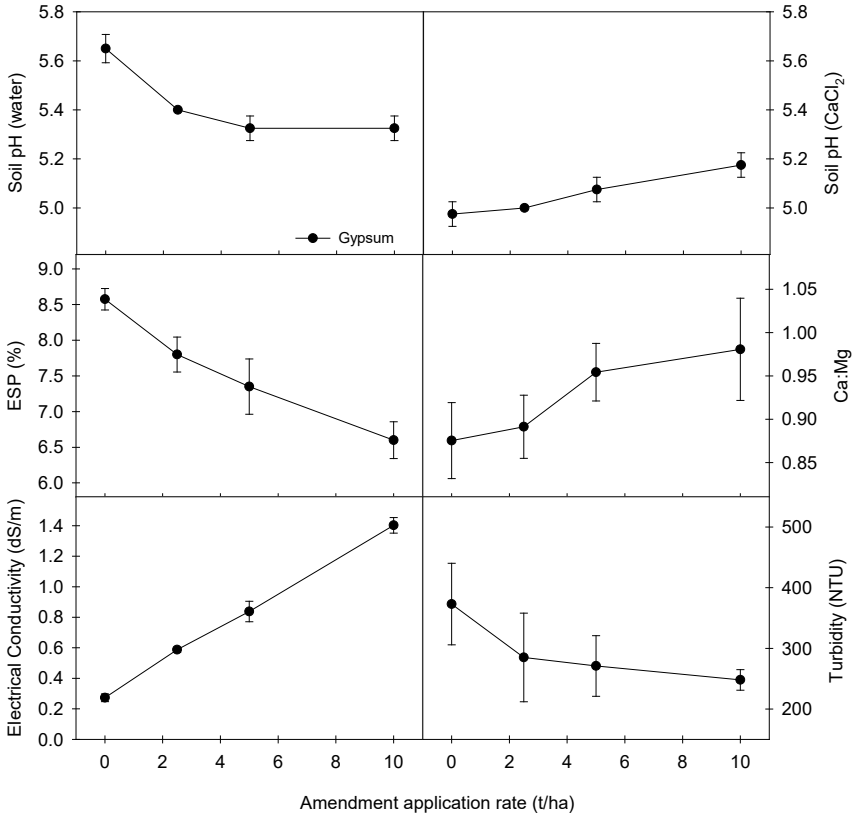


Figure 4.7. Soil chemistry changes in the Oaklands topsoil (0-10 cm) due to amendment with gypsum (0, 2.5, 5, 10 t/ha). Vertical bars are standard deviation of the mean (n=4).

Within the subsoil layers of the Oaklands soil, application of gypsum had a greater influence on measured parameters than the addition of S on all but the soil pH measured in calcium chloride for the 10-50 cm layer (Figure 4.8) and all measured properties in the 50-100 cm layer (Figure 4.9). Application of S decreased soil pH_{Ca} whereas gypsum did not (Figure 4.8).

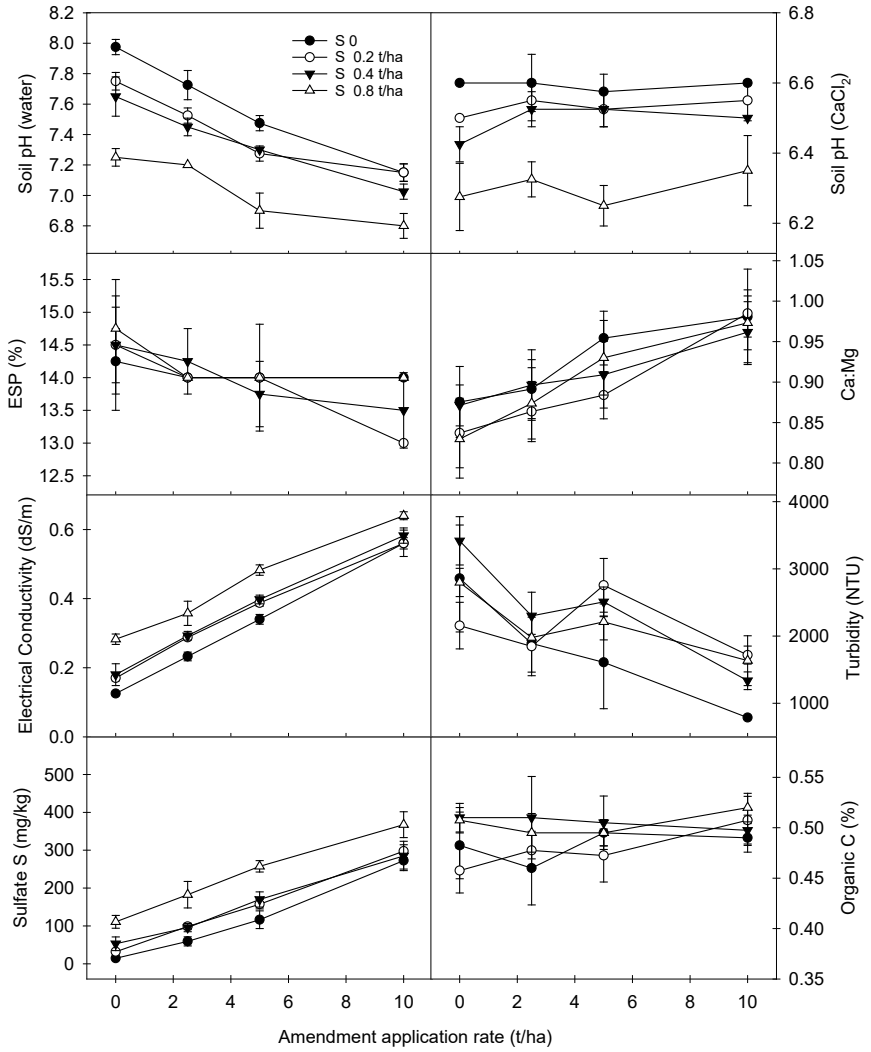


Figure 4.8. Soil chemistry changes in the Oaklands subsoil (10-50 cm) due to amendment with gypsum (0, 2.5, 5, 10 t/ha) with rates of elemental S (0, 0.2, 0.4, 0.8 t/ha, ●, ○, ▼, △, respectively). Vertical bars are standard deviation of the mean (n=4).

Rate responses to the addition of S in the 10-50 cm layer of the Oaklands soil was evident in the sulfur concentration, soil pH (water and $CaCl_2$) indicating that microbial oxidation of S had occurred. However, the application of S to the deepest subsoil layer of the Oaklands soil did not change soil properties (Figure 4.9) and may possibly be caused by a lack of *Thiobacillus* sp. in that layer.

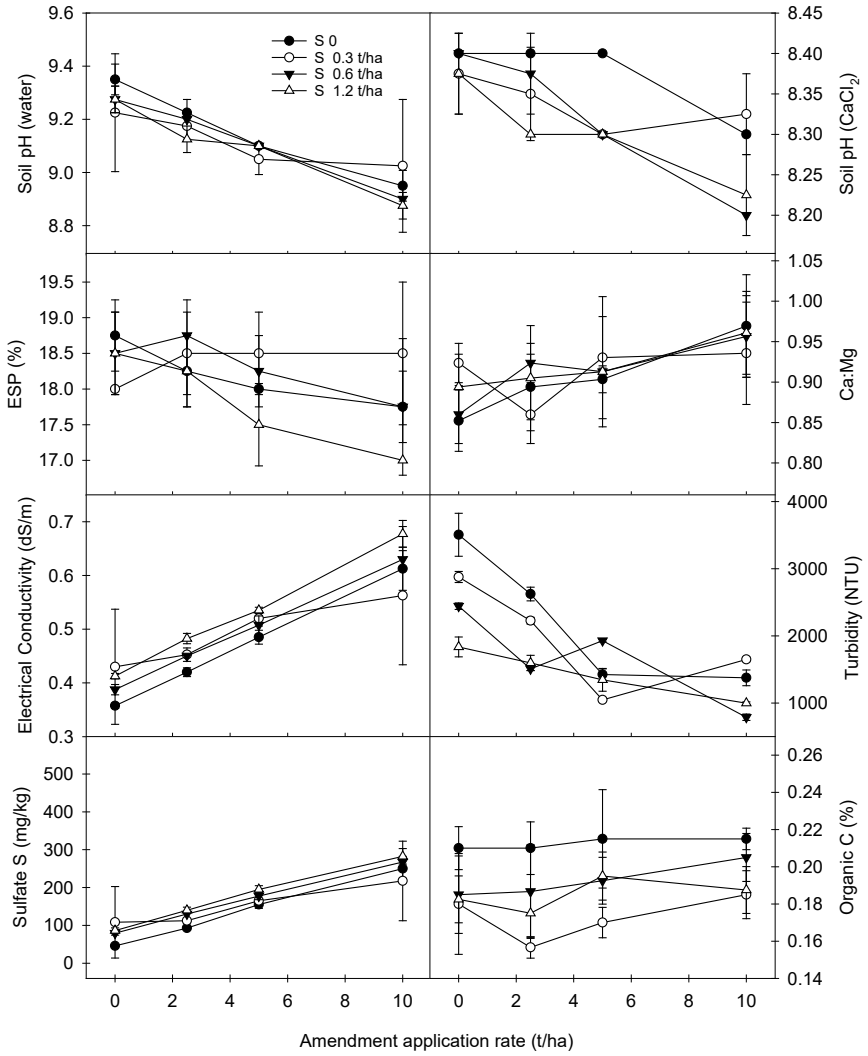


Figure 4.9. Soil chemistry changes in the Oaklands subsoil (50-100 cm) due to amendment with gypsum (0, 2.5, 5, 10 t/ha) with rates of elemental S (0, 0.3, 0.6, 1.2 t/ha, ●, ○, ▼, △, respectively). Vertical bars are standard deviation of the mean (n=4).

4.3.5 Trungley Hall

The application of gypsum to the 0-10 cm layer of the Trungley Hall soil increased salinity and as this layer was not sodic, and the Ca:Mg greater than 2, the gypsum treatment had only minor influence on turbidity (Figure 4.10) which was a much lower value than the subsoil layers of this soil.

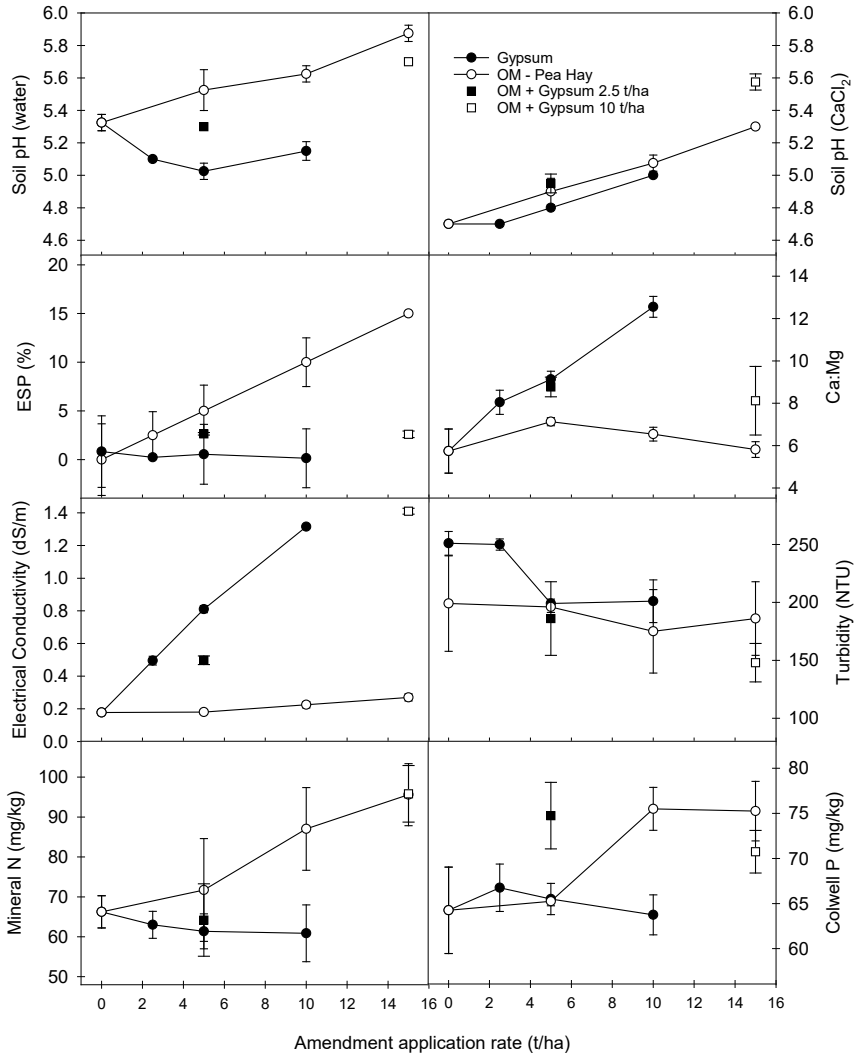


Figure 4.10. Soil chemistry changes in the Trungley Hall topsoil (0-10 cm) due to amendment with gypsum (0, 2.5, 5, 10 t/ha, ●), pea hay organic matter (0, 5, 10, 15 t/ha, ○), and pea hay with gypsum at 2.5 and 10 t/ha, ■ and □, respectively). Vertical bars are standard deviation of the mean (n=4).

The application of pea hay organic matter increased mineral N, Colwell P and soil pH due to microbial mineralisation of the organic matter (Figure 4.10). These changes may be beneficial to plant growth. However, the organic matter also released sodium resulting in an increase in ESP but this did not have deleterious impact on stability of microaggregates as measured by turbidity.

In the 10-40 cm layer of the Trungley Hall soil, the application of pea hay organic matter increased soil pH and mineral N due to mineralisation (Figure 4.11). The magnitude of mineral N increases was less than the topsoil possibly reflecting a difference in microbial activity associated with the initial organic matter concentration of the soil. The pea hay also increased electrical conductivity with increasing rate of application.

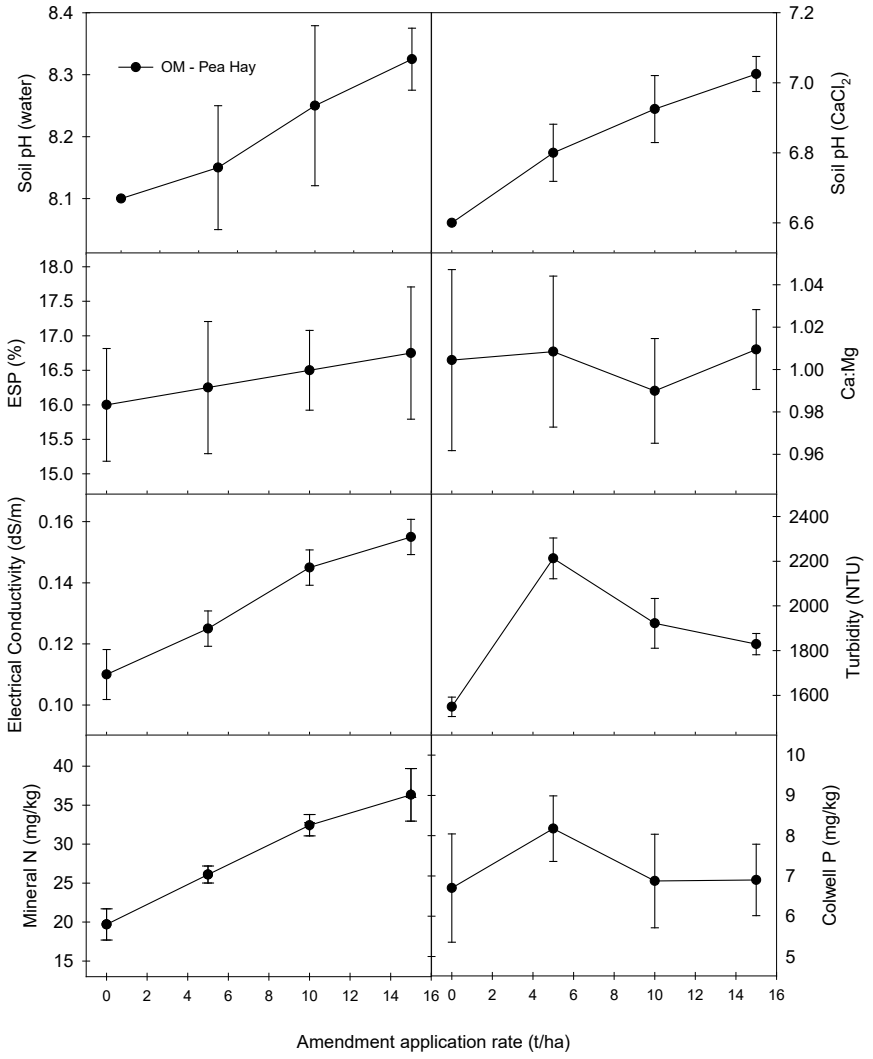


Figure 4.11. Soil chemistry changes in the Trungley Hall subsoil (10-40 cm) due to amendment with pea hay organic matter (0, 5, 10, 15 t/ha, ●). Vertical bars are standard deviation of the mean (n=4).

The oxidation of elemental S added to the 10-40 cm layer decreased soil pH, increased sulfate concentration and the electrical conductivity of the soil (Figure 4.12). These changes were also associated with a decrease in turbidity when S was applied at 0.3 t/ha. The addition of straw or pea hay in combination of S had no effect in the 10-50 cm layer.

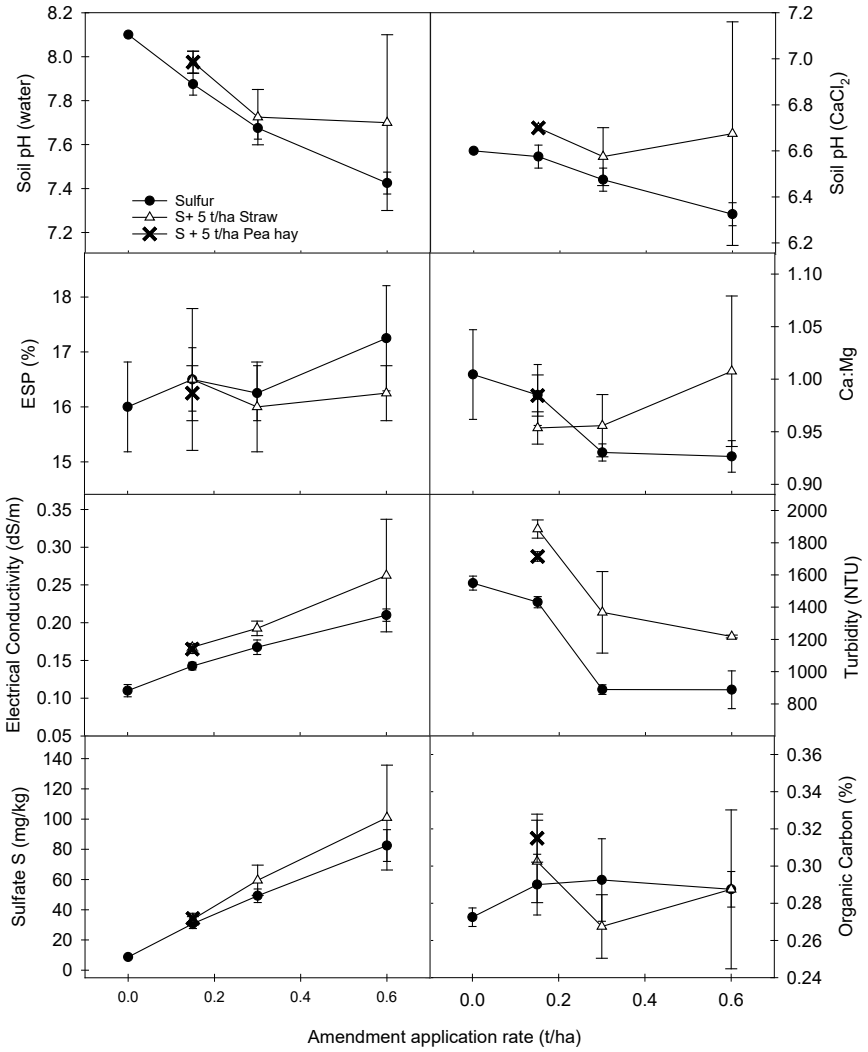


Figure 4.12. Soil chemistry changes in the Trungley Hall subsoil (10-40 cm) due to amendment with elemental sulfur (0, 0.15, 0.3, 0.6 t/ha) only, ●, elemental sulfur with straw (5 t/ha), △ and pea hay, X. Vertical bars are standard deviation of the mean (n=4).

When pea hay organic matter was applied to the deep subsoil layer of the Trungley Hall soil measured soil properties were largely unchanged (Figure 4.13). This may be the product of low biological activity in the initial soil.

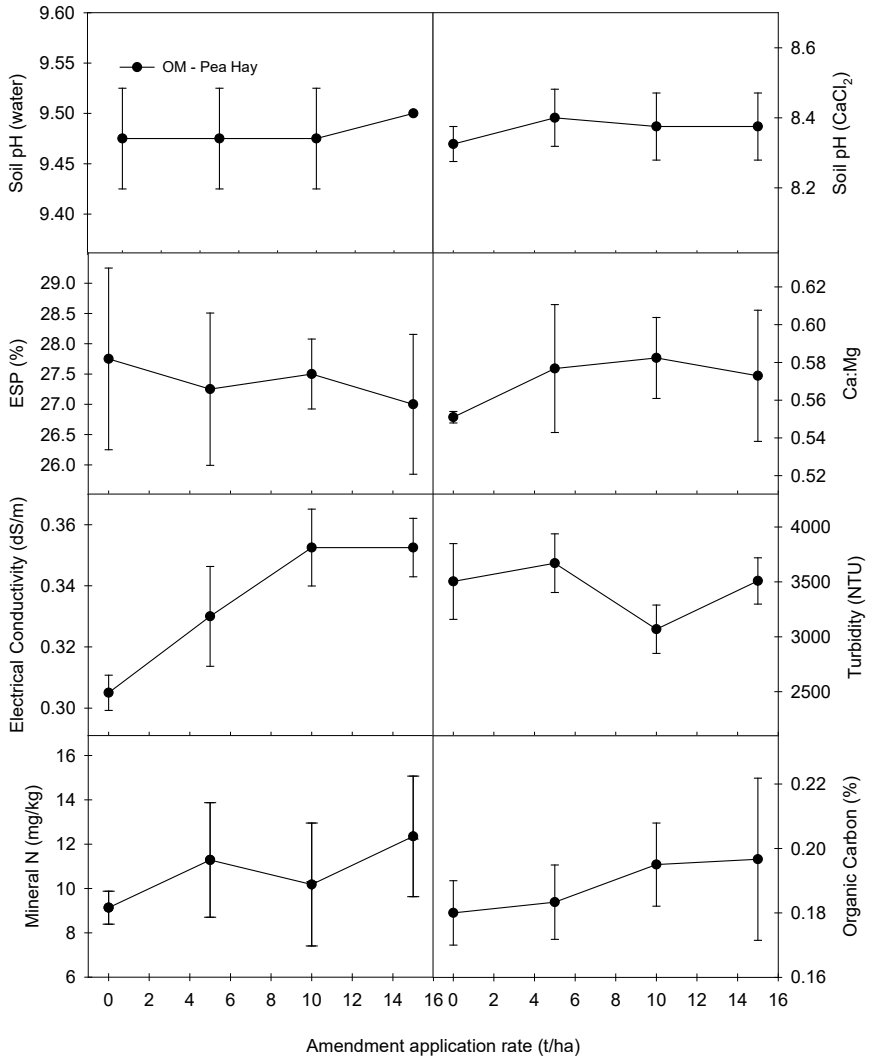


Figure 4.13. Soil chemistry changes in the Trungley Hall subsoil (40-100 cm) due to amendment with pea hay organic matter (0, 5, 10, 15 t/ha, ●). Vertical bars are standard deviation of the mean (n=4).

As for the upper subsoil layer, the application of S to the 50-100 cm deep subsoil layer decreased pH, increased EC and sulfate concentration (Figure 4.14). These results confirm microbial oxidation of elemental S and provide evidence of *Thiobacillus* in all subsoil layers of the Trungley Hall soil. Co-application of S with organic matter had little influence on measured properties.

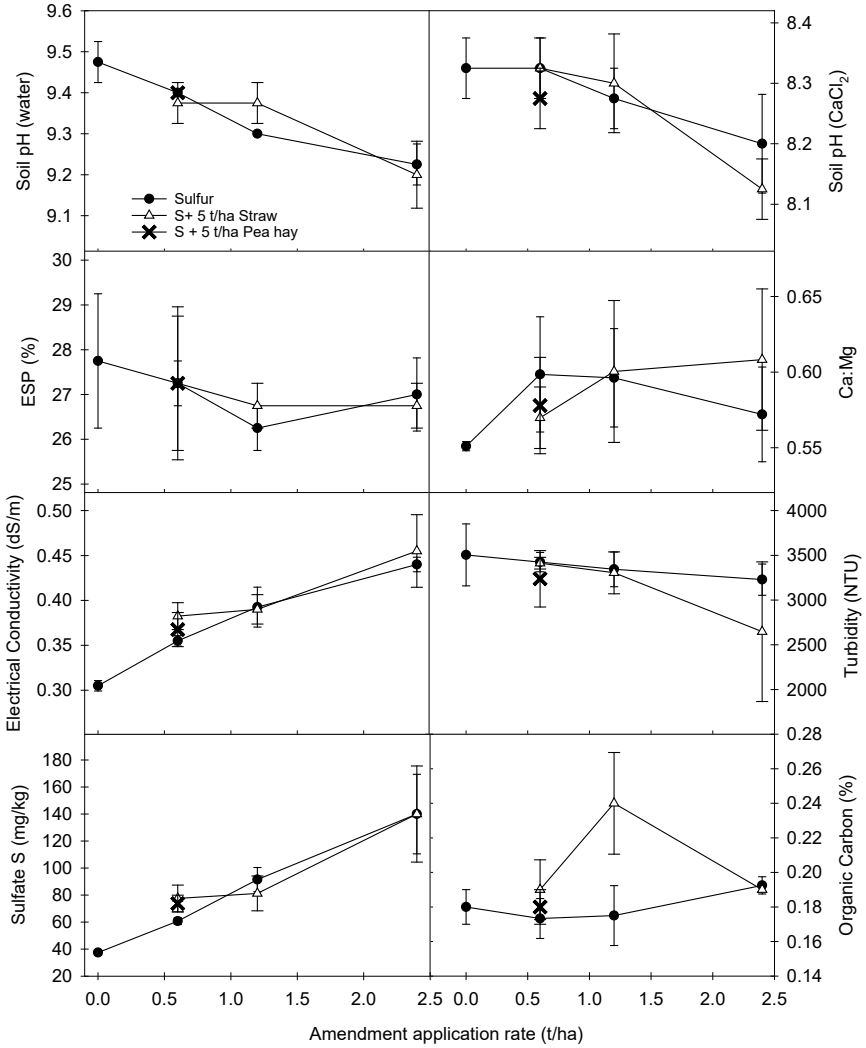


Figure 4.14. Soil chemistry changes in the Trungley Hall subsoil (40-100 cm) due to amendment with elemental sulfur (0, 0.15, 0.3, 0.6 t/ha) only, ●, elemental sulfur with wheat straw pellets (5 t/ha), △ and pea hay, X. Vertical bars are standard deviation of the mean (n=4).

4.3.6 Condobolin

The individual application of organic matter as pea hay or chicken manure increased soil pH and electrical conductivity (Figure 4.15). The scale of pH increase is small compared to that resulting from lime addition. The chicken manure organic matter contained greater concentrations of nutrients and this resulted in increased mineral N, Colwell P. However, the chicken manure also increased NaCl concentration with increasing rates of application which caused increased ESP% and electrical conductivity, though both remained below the critical values associated with poor plant growth.

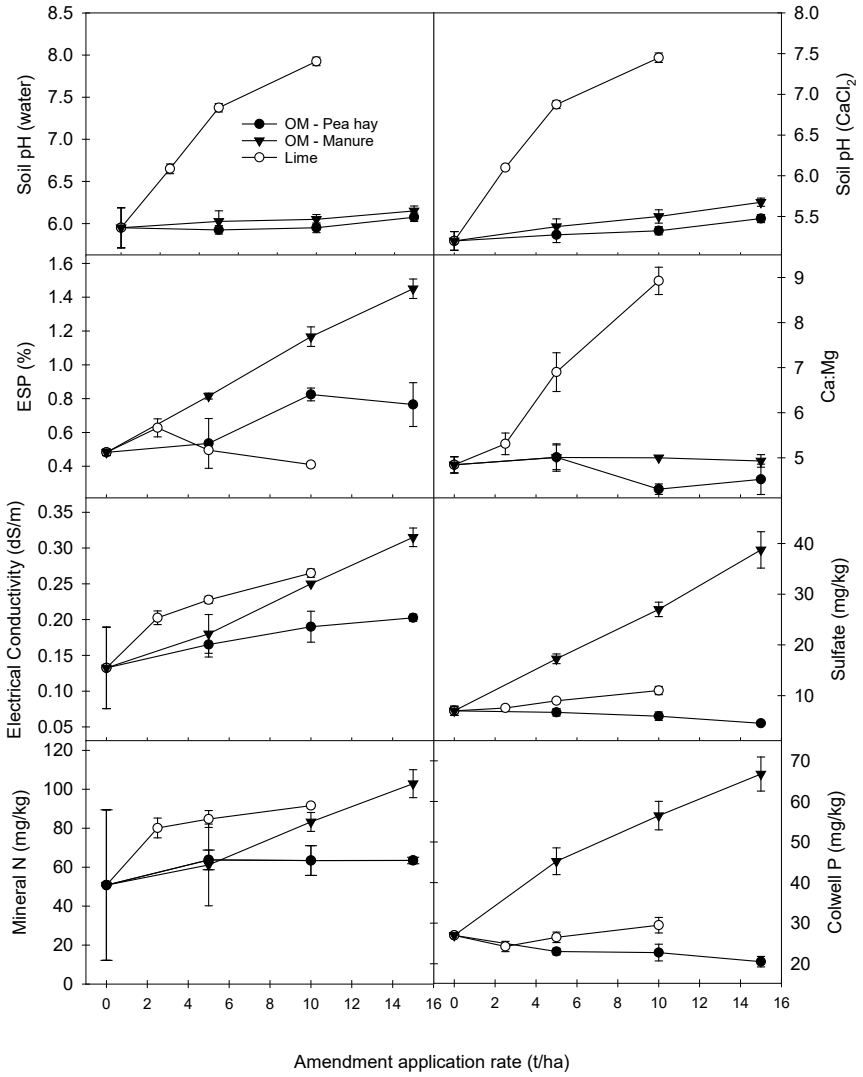


Figure 4.15. Soil chemistry changes in the Condobolin (0-15 cm) due to amendment with pea hay organic matter ● (0, 5, 10, 15t/ha), chicken manure organic matter ▼ (0, 5, 10, 15t/ha) and lime ○ (0, 2.5, 5, 10 t/ha). Vertical bars are standard deviation of the mean (n=4).

The application of pea hay organic matter resulted in decreased Colwell P and sulfate concentrations, with no change in mineral N concentration. These results may possibly be due to net immobilisation of the low nutrient content of pea hay relative to the chicken manure. The application of lime increased mineral N, sulfate and Colwell P content which would occur if the increase in soil pH resulted in greater mineralisation of the initial labile organic matter present in the soil.

Individual application of lime to the upper subsoil (15-50 cm) of the Condobolin soil resulted in linear increase in soil pH, Ca:Mg, electrical conductivity and sulfate concentration (Figure 4.16).

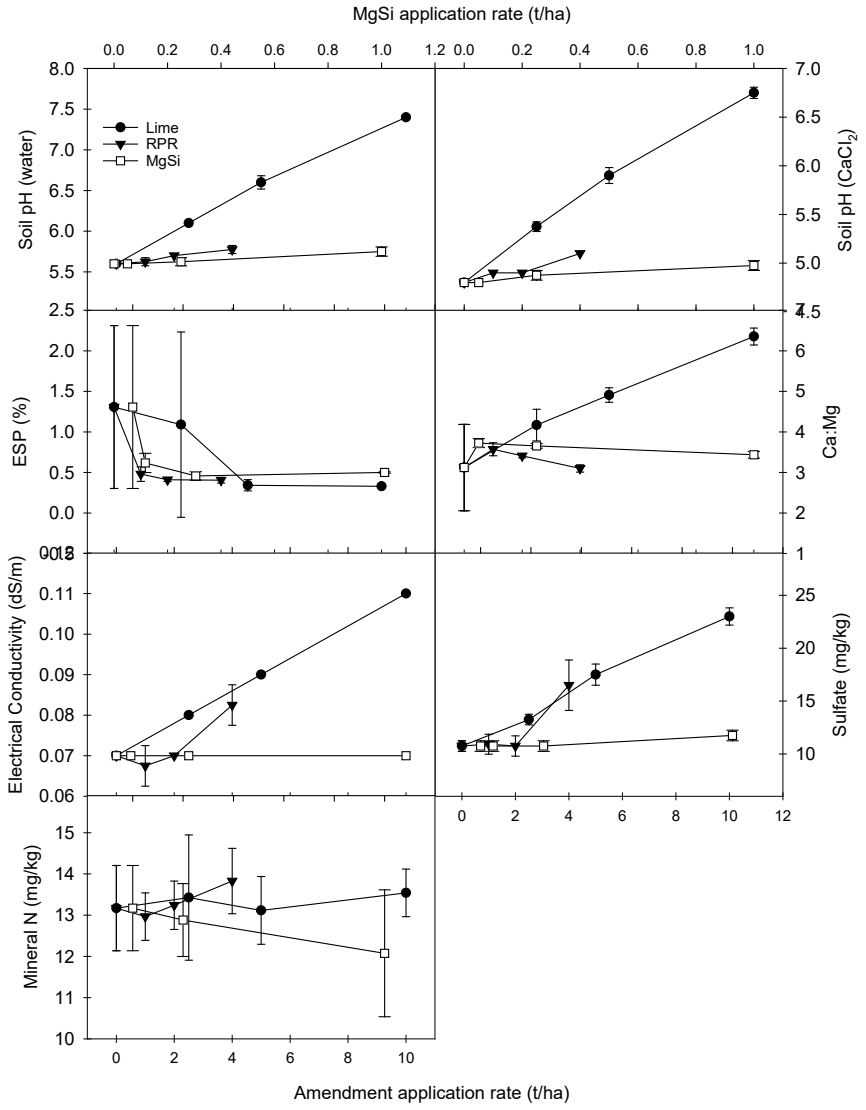


Figure 4.16. Soil chemistry changes in the Condobolin subsoil (15-50 cm) due to amendment with lime , ● (0, 2.5, 5, 10 t/ha, lower x axis), reactive phosphate rock (RPR) ▼ (0, 1, 2, 4 t/ha, lower x axis) or magnesium silicate (MgSi) □ (0, 0.05, 0.25, 1 t/ha, upper x axis). Vertical bars are standard deviation of the mean (n=4).

Reactive phosphate rock resulted in increased soil pH however Colwell P remained below detection limits (data not shown). These would occur if the reactive RPR dissolved to increase pH but the phosphate released was subsequently bound, possibly due to the high iron content of the Condobolin subsoil.

The magnesium silicate was relatively ineffective in altering measured soil parameters other than resulting in relatively small increases in pH with application rate (pH 4.8 to 5.0, control to 1 t/ha MgSi, respectively)

Individual application of lime or magnesium silicate to the deep subsoil (50-100 cm) of the Condobolin soil (Figure 4.17) resulted in the same treatment trends as the upper subsoil.

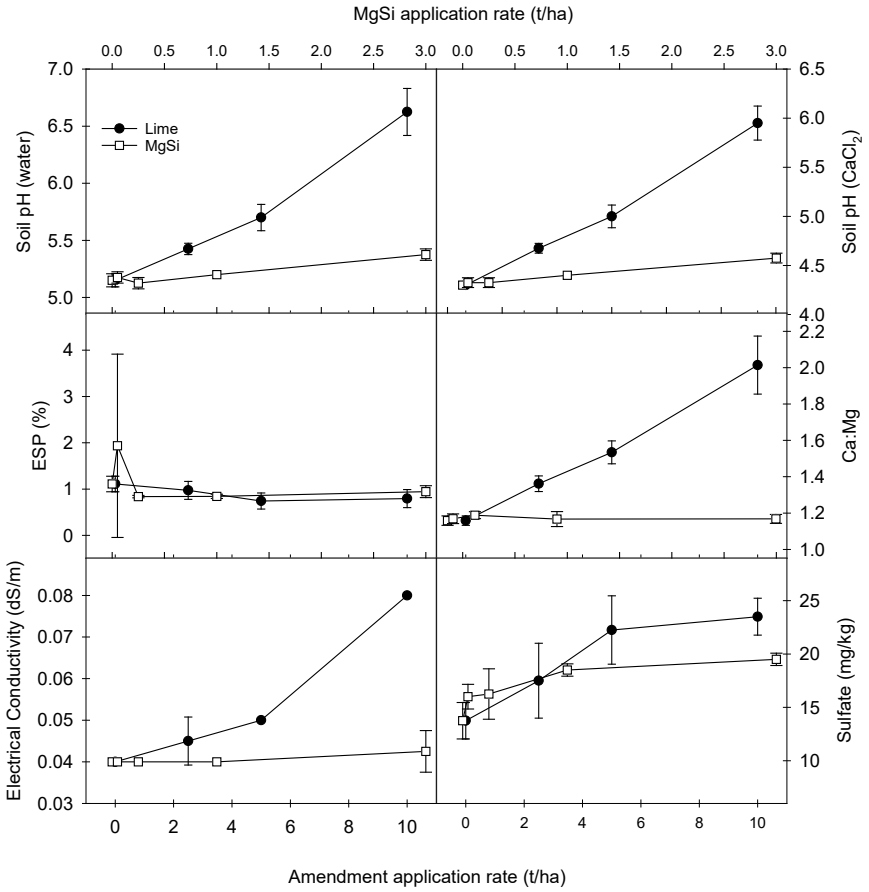


Figure 4.17. Soil chemistry changes in the Condobolin subsoil (50-100 cm) due to amendment with lime , ■ (0, 2.5, 5, 10 t/ha, lower x axis) or magnesium silicate □ (0, 0.05, 0.25, 1, 3 t/ha, upper x axis). Vertical bars are standard deviation of the mean (n=4).

Lime increased soil pH, Ca:Mg ratio and electrical conductivity. The application of magnesium silicate resulted in similar trends but at much smaller magnitude compared with that of lime. In general, the same outcomes could be obtained from approximately 1 to 2 t/ha of lime than 2.6 t/ha of magnesium silicate.

4.4 Discussion

Inorganic treatments

Treatments containing gypsum increased KCl extractable S and Ca^{2+} concentration as rate of application increased. The addition of gypsum decreased both turbidity and exchangeable sodium percentage (ESP), with effectiveness increasing with rate. However, the higher rates of gypsum treatments increased EC to levels likely to constrain plant growth (1.5-3 dS/m). Both gypsum plus elemental sulfur and gypsum plus CMP compounded increases in EC. Gypsum treatments were more effective than either organic matter treatments in reducing turbidity. There is a strong linear relationship between turbidity and dispersion (Zhu et al. 2016) and dispersive soils have degraded physical properties resulting in reduced water and air flow and high soil strength, that inhibit plant growth (Oster 1998). At the higher rates of gypsum application (4 and 8 t/ha), EC increased to levels potentially constraining to plant growth (Maas 1990; Rengasamy 2010); this increase in EC outweighed the positive benefits from decreased turbidity. The lowest rate of gypsum applied (2 t/ha) was still effective in significantly reducing turbidity while keeping EC at moderate levels (<0.6 dS/m).

Treatments containing elemental sulfur, which were applied to the Birchip, Oaklands and Trungley Hall subsoil, increased available S and reduced pH, with this effect increasing with

application rate. This translated to reduced turbidity as seen in previous studies (Chorom et al. 1994) where the effect of soil pH on dispersion was generally linear between pH 4 and 9. Elemental sulfur also increased soil EC and at the higher rates of application and these would have likely constrained plant growth.

The use of reactive phosphate rock (RPR) in the acidic conditions of the Condobolin soil was ineffective in increasing available P in that soil possibly due to the soil pH (pH_{Ca} 5.5) not being acidic enough to dissolve the RPR (Sale et al. 1997). The use of magnesium silicate was largely ineffective in altering measured soil properties of the Condobolin soil.

Organic matter treatments
Both organic matter ameliorants (WSP and CMP) increased soil organic carbon (SOC) with WSP producing greater increases than CMP. CMP increased available N, Colwell P, available potassium and KCl extractable S. WSP plus nutrients only increased P and S and WSP did not increase any nutrients and significantly reduced KCl extractable nitrate. CMP treated soil had higher available nitrate than soil treated with WSP plus nutrients, even though both treatments were balanced for total nitrogen. Interestingly, at the highest rate of CMP application there was often a significant and sharp decrease in soil nitrate which also corresponded with an increase in soil pH. The link in decreasing nitrate concentration and increasing soil pH may be due to either immobilisation or denitrification of nitrate present (Condon et al. 2004). Either process is possible given the addition of labile carbon, but denitrification should have been avoided given the control of soil water content to avoid anaerobic conditions in the soil.

In the absence of added nutrients, applying WSP reduced available nitrate to concentrations below that of the control soils representing net immobilisation favoured by the high (>70) C:N ratio of the WSP. CMP and WSP plus nutrients significantly ($P< 0.05$) increased soil EC but unlike the gypsum treatments these increases were not likely to constrain plant growth (<0.6 dS/m); adding WSP alone had no effect. The effect of organic amendments on turbidity varied between soils and between topsoil and subsoils layers. With the exception of the Birchip subsoil, application of organic matter amendments to subsoils was effective at reducing turbidity. However, gypsum was more significantly effective in flocculation than organic amendments. Organic matter treatments increased the pH of the acidic topsoils of Wonwondah, Trungley Hall and Condobolin soils, either from their own alkaline content, or from subsequent mineralisation (Lauricella et al. 2021). The rate of pH rise increased with rate of application.

OM treatments, even at the highest rates, did not increase EC to levels likely to constrain crop growth. In addition, OM application had positive effects on soil health in terms of increased SOC and nutrition. Increased SOC is related to improved physical properties of soils (Keller and Dexter 2012; Soane 1990) and increased microbial activity (Kay 1998). Immobilisation of soil nitrate was seen in soils treated with OM amendments and this could reflect increased microbial activity - fungal hyphae was observed on the surface of soils treated with OM.

The ability of organic amendments to supply nutrients was greatest for CMP. However, when added with gypsum, the available P released from CMP did not increase with application rate.

4.5 Conclusion

The addition of organic amendments can influence microaggregate flocculation as measured by turbidity however the effectiveness was much less than gypsum. Gypsum and S treatments also have the potential to increase soil EC above the thresholds for healthy plant

growth. Organic amendments have the advantage of simultaneously increasing N, P, S and K nutrition. However, clear differences in nutrient release from different organic matter sources were apparent with CMP providing greater nutrition compared to WSP. The potential of creating excessive salt loads from organic matter addition can be controlled by monitoring rate of application but the inherent salinity of the soil also needs to be considered, especially in subsoils.

Interactions between organic and inorganic amendments can occur and are not always beneficial. For example, CMP and gypsum provided no benefit to turbidity but decreased the available P release that would have otherwise occurred from CMP.

5. Phase 3: – Amendment addition, plant response and mechanism

5.1 Introduction

Based on experiments of Phases 1 and 2 the Wonwondah soil was selected for the Victorian node for detailed mechanistic experiments. This soil was selected as it had poor structure, high clay content, increasing sodicity and alkalinity with depth. These properties were common to soils of the Riverine Plains, HART, and Birchip Cropping Group farming system groups.

The NSW node selected both the Trungley Hall and Condobolin soils due to both having measured positive plant response in experiments of Phase 1 and the understandings of treatments in Phase 2. The root growth was improved in the Trungley Hall soil when organic amendments were applied resulting in increased plant performance. Nutrient rich organic amendment to the Condobolin soil resulted in increased yield without any change in the root growth relative to the untreated control, indicating response to nutrition deficiency rather than soil structural constraint.

5.2 Victorian node glasshouse experiment: The interaction of organic amendment and growth of plant roots

5.2.1 Objectives

Dispersive subsoils have degraded physical properties with low porosity, high density and high strength resulting in slow water infiltration (Rengasamy and Olsson 1991). These soil conditions restrict crop growth due to their impact on root growth and function and propensity to waterlog (Oster 1998). Subsoil amelioration strategies with organic matter amendments have been shown to improve crop productivity on these soils (Gill et al. 2009). Although the exact mechanism(s) whereby this amelioration can improve crop production is unclear, it appears to result from improved nutrition in the shorter term and better soil structure in the longer-term. Recent evidence (Wang et al. 2020) suggested that the success of these strategies change with time and differ in the presence of plants.

A long-term glasshouse experiment was conducted to investigate the effectiveness of a range of soil amendments on a highly dispersive subsoil over time, with or without plants. Soil treatments included application of organic and inorganic amendments and nutrients with the aim of better understanding the mechanism(s) of how organic matter amendments alter subsoil properties and improve plant production. The hypotheses of this experiment were:

1. Plants (roots) enhance the effectiveness of organic matter subsoil amendments to improve soil biophysical properties and plant production;

2. The effectiveness of organic matter subsoil amendments increases over time; and

3. Crop responses to organic matter subsoil amendments result from improved soil structure and not just better nutrition.

5.2.2 Methods

Collection and preparation of soil

Bulk soil was collected with an excavator from a site near Wonwondah in the Wimmera region of Victoria (36°52'47.2"S 142°11'43.9"E). Two depths of soil were collected and kept separate, a non-dispersive topsoil 0-20 cm and a dispersive subsoil 60-100 cm. Each soil layer was air dried, crushed and passed through a 5 mm sieve.

This soil was reconstituted in 20 L plastic pails (400 mm height x 255 mm diameter increasing to 285 mm diameter at the top). Each pail was filled with 29 cm of subsoil which was then covered with 7 cm of topsoil. Pots were arranged in a randomised complete block design of seven treatments (Table 5.1) with four replicates in a factorial design with or without plants (7 x 4 x 2 = 56 units).

The 20 t/ha treatment rate of CMP was based on the rate used by (Gill et al. 2009) where significant soil and crop responses were observed. The 15.4 t/ha rate of WSP was determined by matching the carbon content of WSP with the CMP (Table 5.2). Treatment rates of added nutrients (N, P, K, S) were determined by matching with those contained in the CMP treatment. Gypsum was applied at a rate of 5 t/ha based on an average of rates from previous studies: 3 t/ha (Baldock et al. 1994), 5 t/ha (Bennett et al. 2015) and 7.5 t/ha (Armstrong et al. 2015). Treatments were mixed throughout the subsoil in a tarpaulin by two people alternately lifting opposite ends repetitively. Nutrient treatments were applied in 25 mL aliquots and allowed to dry for a day before being mixed into the soil. Organic matter treatments were crushed and graded between 2-5 mm before being mixed. Initially the subsoil was reconstituted and moistened to 70% plant available water (PAW) using individual drippers for each pot. The dripper system was constructed using adjustable micro sprayer irrigation nozzles screwed into the base of 500 mL polypropylene containers and placed on a section of supporting pipe on the soil surface. A geotextile was placed inside the supporting pipe to disperse and distribute the drops of water. To avoid soil surface dispersion and encourage infiltration the nozzles were adjusted to drip roughly every 6 s achieving an infiltration rate of around 0.5 mm/hr. The topsoil was then reconstituted and basal nutrients, calculated on a surface area basis, were applied to the topsoil of all pots at the following rates: KH_2PO_4 (25 kg P/ha and 31.5 kg K/ha), NH_4NO_3 (50 kg N/ha), $ZnSO_4.7H_2O$ (10 kg Zn/ha), $MgSO_4.5H_2O$ (10 kg S/ha) and $CuSO_4.5H_2O$ (10 kg Cu/ha) (equalling application of 23.1 kg S/ha). These were added to the topsoil in 5 ml aliquots and allowed to dry for a day before being mixed throughout. The topsoil was then gradually moistened to 70 % PAW by surface watering.

Table 5.1. Treatments and rates on a surface area and weight basis

Treatment	Equivalent Field Rate (t/ha)	Rate (g/kg soil)	Plant
Control	-	-	+ / -
Chicken manure pellets (CMP)	20	4.92	+ / -
Wheat straw pellets (WSP)	15.4	3.79	+ / -
WSP + (NPKS)	15.4 + (CMP 20 equiv. – WSP 15.4 equiv.)	3.79*	+ / -
Gypsum	5	1.23	+ / -
Gypsum + (NPKS)	5 + (CMP 20 equiv.)	1.23*	+ / -
NPKS	CMP 20 equivalent	*	+ / -

Table 5.2 Wheat straw and chicken manure pellet elemental analysis

Analysis	[WSP] g/100 g	[CMP] g/100 g	WSP g/pot	CMP g/pot
Carbon	43.44	33.47	37.90	37.90
Nitrogen	0.588	3.320	0.51	3.76
Calcium	1.247	2.400	1.09	2.72
Phosphorus	0.049	2.000	0.04	2.26
Potassium	0.633	2.700	0.55	3.06
Sulfur	0.080	0.680	0.07	0.77

Measurements and analysis

Three plants of strawberry clover, *Trifolium fragiferum* cv. Palestine inoculated with rhizobium (Group B), were grown per pot and pots were watered to weight (70 % PAW) once a week using reverse osmosis water. Evapotranspiration was recorded as the cumulative mass of water added to each pot. The experiment commenced on 13/10/2020 in a glasshouse set at 12^{o}C nights and 24^{o}C days.

Shoot sampling occurred at 62, 100, 161, 181, 269, 360, and 430 days after sowing (DAS). Plants were cut 2 cm above ground level and dried at 70^{o}C for 48 hrs before being weighed then ground for subsequent tissue analysis including total N (Leco FP-428 Dumas combustion-method total-N analyser) and ICP (ELEMENT High Resolution ICP-MS) (Ca, Mg, Na, K, B, Cu, Mn, Fe, Zn, P, S) following acid digestion.

Soil sampling occurred at 100, 181, 269, 360, and 430 DAS. Soil was sampled with a 25 mm diameter soil corer; the voids were maintained with 25 mm PVC pipe. The subsoils were separated from the topsoils for analysis including organic carbon (Heanes 1984), wet sieving water stable aggregate analysis (Blaud et al. 2016), turbidity (Hach Turbidimeter 2100N) method adapted from Zhu et al, 2016 using 1 g soil in 25 mL water, pH (1:5 soil/water), pH (1:5 soil/0.01 M $CaCl_2$), EC (1:5 soil/water), exchangeable cations (Tuckers procedure), Mineral N (2 M KCl extractable) and Colwell P (0.5 M $NaHCO_3$).

Soil was air dried, crushed and then sieved to obtain 1-2 mm aggregates for water stable aggregate analysis. For all other analysis air dried soil was processed through a jaw crusher to obtain aggregates less than 2 mm.

Statistical analysis was conducted with Genstat (18th version; VSNI Hertfordshire, UK) software using repeated measure analysis of variance ANOVA, REML random coefficient regression analysis, linear regression and Pearson's correlation. Prior to ANOVA analysis, data was checked for normality using the Shapiro-Wilk test for normality and significance was expressed using least significant differences at the 5 % level.

5.2.3 Results

Plant effects on OM amendments

Soil structural improvement was measured as a decrease in turbidity expressed as Nephelometric Turbidity Units (NTU) and an increase in water stable aggregates (WSA) expressed as Mean Weight Diameters (MWD). MWD and NTU data was analysed using repeated measure ANOVA, which did not show any significant plants effects on MWD ($p=0.095$) or NTU ($p=0.327$). When the MWD and NTU data from the final harvest only was analysed, there was a trend towards a significant plant effect, MWD ($p=0.058$) and NTU ($p=0.099$) (Figure 5.1). When CMP was added the plant was able to increase aggregate stability compared to the CMP where no plants were present. This effect was not evident in the turbidity; an index of clay flocculation.

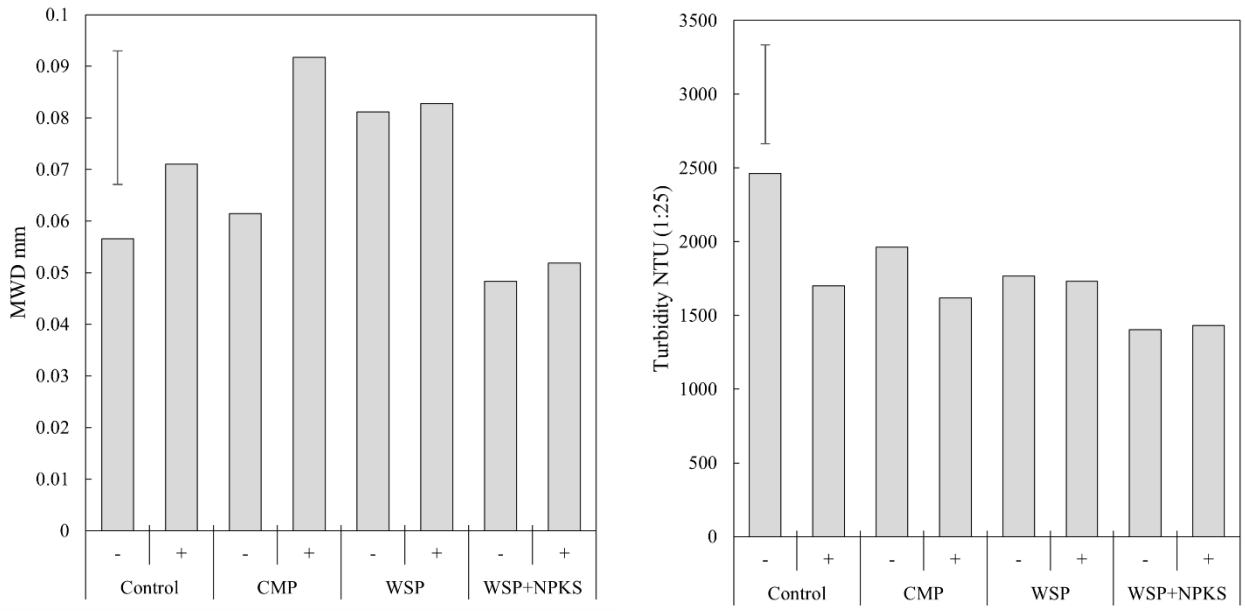


Figure 5.1. Control and OM treated subsoil water stable aggregates (MWD) and turbidities (NTU) at 430 DAS with or without plants. Vertical bars indicate (Treatment x Plant) least significant difference (P= 0.05).

Time effects of OM amendments

The effect of OM amendments on MWD and NTU changed significantly over time ($p<0.01$) when analysed using repeated measure linear mixed modelling. MWD increased over time in soil treated with CMP +/- plants and WSP +/- plants but not *WSP plus nutrients* +/- plants or control +/- plants. Turbidity however also increased over time with all OM amendments and the control soils, regardless of whether plants were present or not. There was a large increase in turbidity measured at the second sampling time (181 days) in all treatments especially *WSP plus nutrients*; levels then gradually decreased over time as the experiment progressed. At the conclusion of the experiment (430 days), turbidity levels remained higher than time zero levels for all treatments +/- plants.

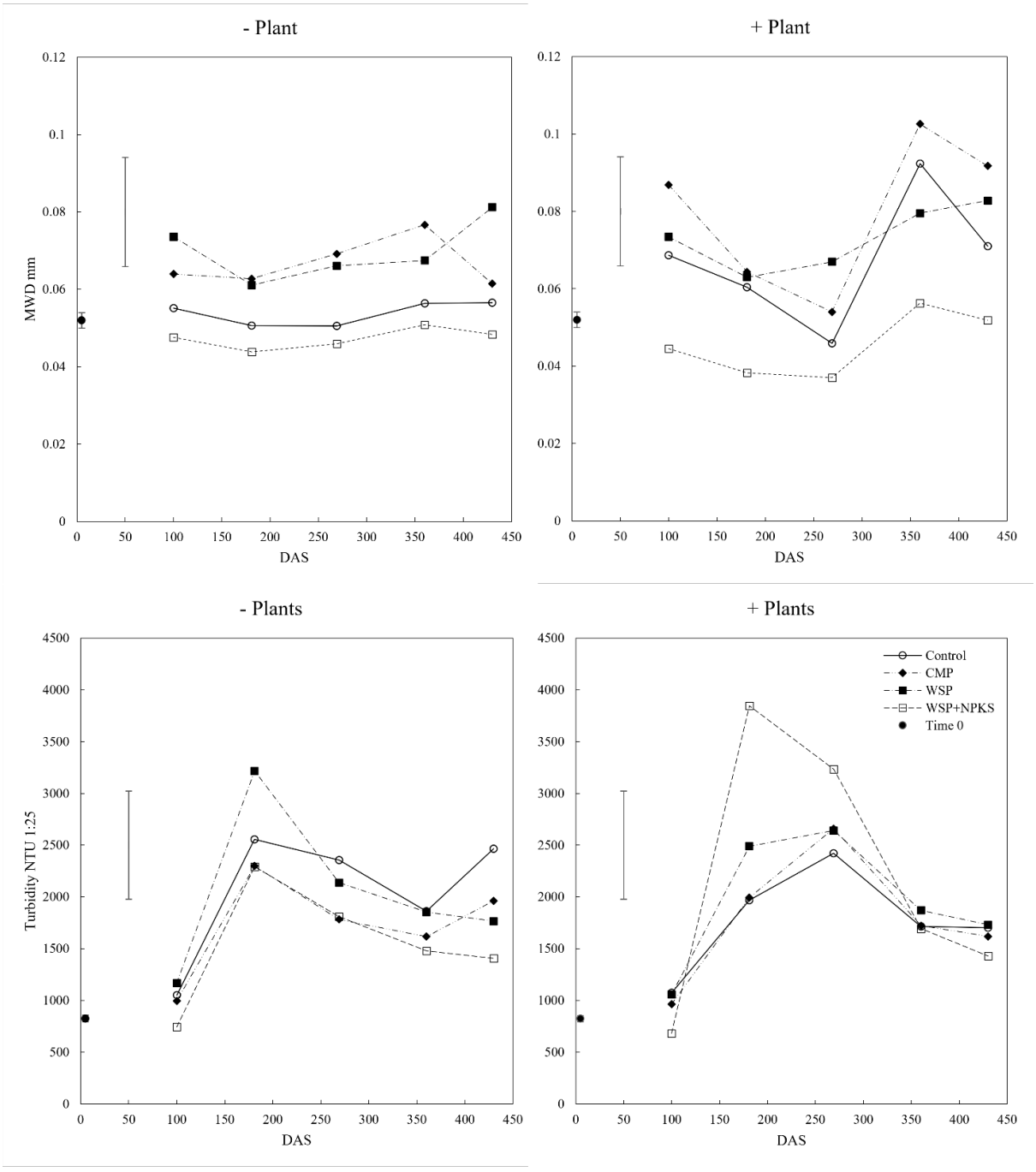


Figure 5.2. Control and OM treated subsoil water stable aggregates (MWD) and turbidities (NTU) over time with or without plants, the vertical bars indicate (DAS x Treatment x Plant) least significant differences (P = 0.05).

Plant responses to OM amendments

Plant biomass was significantly affected ($P < 0.001$) by both amelioration and time. Soil treated with CMP and *WSP plus nutrients* produced more biomass than control and WSP treated soil (Figure 5.3). Relative to control soil the total biomass produced in *WSP plus nutrients,* CMP and WSP treated subsoil was 207, 170, 91% greater, respectively. The biomass of plants growing in control soil decreased over time while it increased when CMP and *WSP plus nutrient* was applied to subsoil. Soil treated with WSP alone produced similar amounts of biomass to control soil, but unlike the control treatment, biomass production was maintained over time.

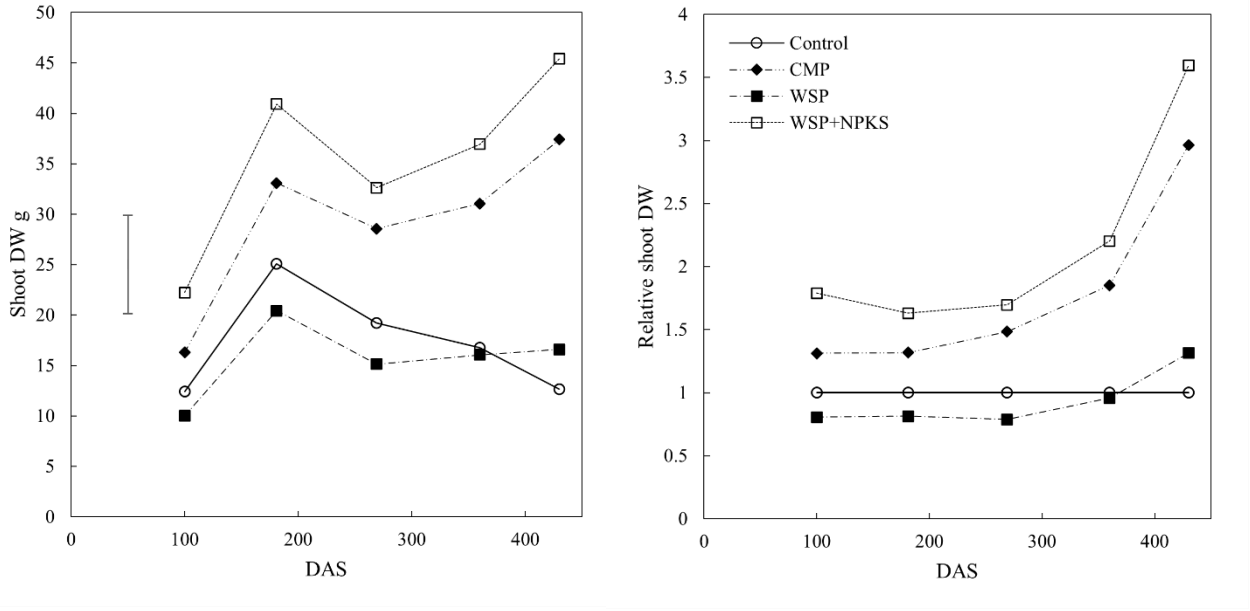


Figure 5.3. OM treated subsoil shoot dry weight mean values and relative shoot dry weights over time, the vertical bar indicates (DAS x Treatment) least significant difference (P=0.05).

Gypsum and nutrient treatments

Gypsum and nutrient treatments were used in this experiment to separate the effects of nutrition and soil structure on plant growth. Gypsum treatments were effective at improving soil structure as measured by decreased NTU and an increase in WSA (Figure 5.4). Nutrient and gypsum plus nutrient treatments significantly ($P < 0.001$) increased plant dry weight (DW) over time (Figure 5.5). The gypsum plus nutrient treated subsoil did not result in more DW produced than nutrients alone. In the absence of additional nutrients, gypsum treatment did not result in additional DW produced relative to control treatments.

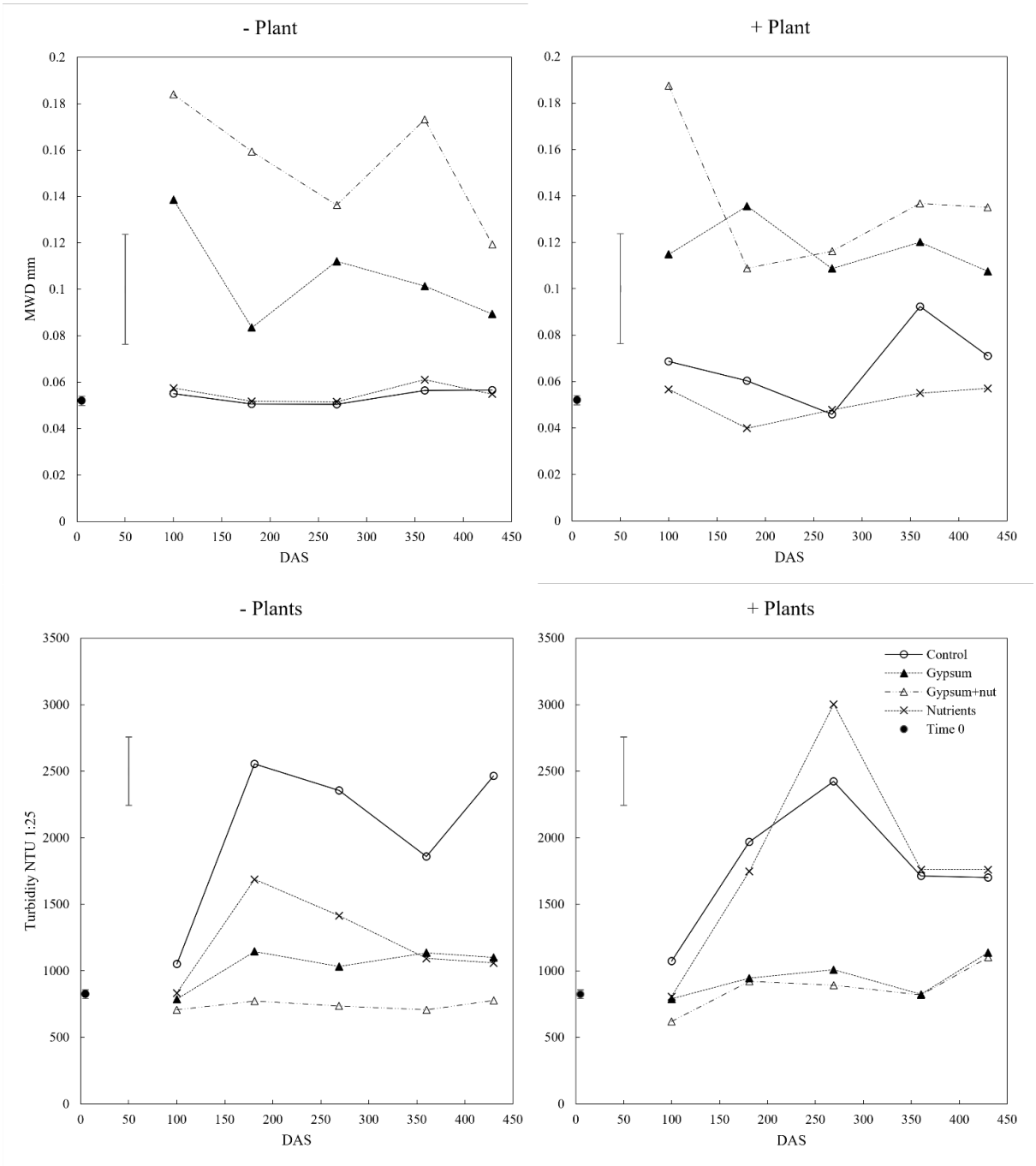


Figure 5.4. Changes in water stable aggregates (MWD) and turbidities (NTU) over time with or without plants for control and inorganic ameliorant treatments. Vertical bars indicate (DAS x Treatment x Plant) least significant differences (P= 0.05).

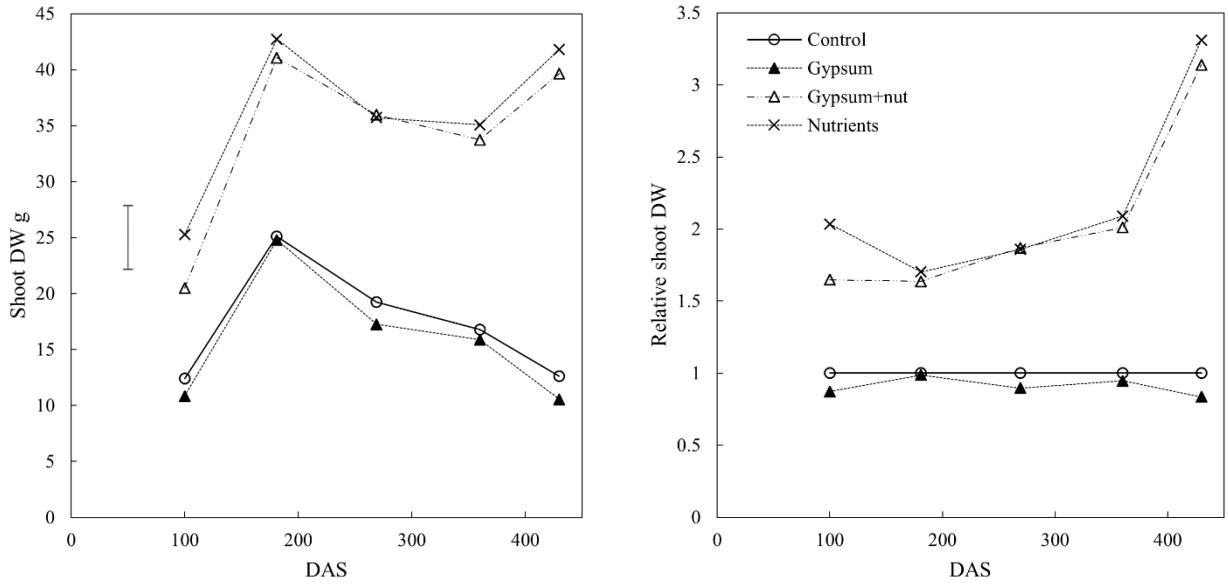


Figure 5.5. Inorganic treated subsoil shoot dry weight mean values and relative shoot dry weights over time. Vertical bar indicates (DAS x Treatment) least significant difference (P= 0.05).

Plant response correlations

Water use and shoot DW were highly correlated (correlation coefficient = 0.94) with a linear relationship (linear regression R^2=0.853) (Table 5.3). All pots were watered weekly to maintain soil at 70% PAW, resulting in larger plants receiving more water. The next highest correlations to shoot dry weight were nutritional viz. Colwell P and available N (NH_4). The Colwell P correlation (correlation coefficient = 0.534) value was around twice that of available N (NH_4) (correlation coefficient = 0.277). Correlations between shoot biomass and measured soil physical properties (MWD and turbidity) were weaker but significant (-0.226 and 0.264, respectively).

*Table 5.3. Shoot dry weight correlations and linear regression adjusted R^2 values for water use and soil properties, *p < 0.05; **p < 0.01; ***p < 0.001.*

Variable	Correlation values	Regression Adj. R^2
Water use mm	0.9401***	0.853***
Colwell P mg/kg	0.5343***	0.279***
Avail. N (NH_4) mg/kg	0.2766**	0.068**
Turbidity NTU	0.2639**	0.028**
WSA MWD mm	-0.2258*	0.033**
EC (1:5 H_2O)	0.2043*	0.005
pH (1:5 H_2O)	-0.2009*	0.025
Na%	0.1375	0.010
Ca%	-0.1336	0.009
K%	0.1182	0.005
Avail. N (NO_3) mg/kg	0.0861	-
pH (1:5 $CaCl_2$)	-0.0791	-
Mg %	0.0292	-

Random coefficient regression

Random coefficient regression analysis was performed to gain an insight into treatment trends over time (Figure 5.6). Plant responses to CMP and treatments containing added

nutrients increased over time. In contrast, plant growth in the control and gypsum treated soil decreased with time but was maintained in the WSP treated soil.

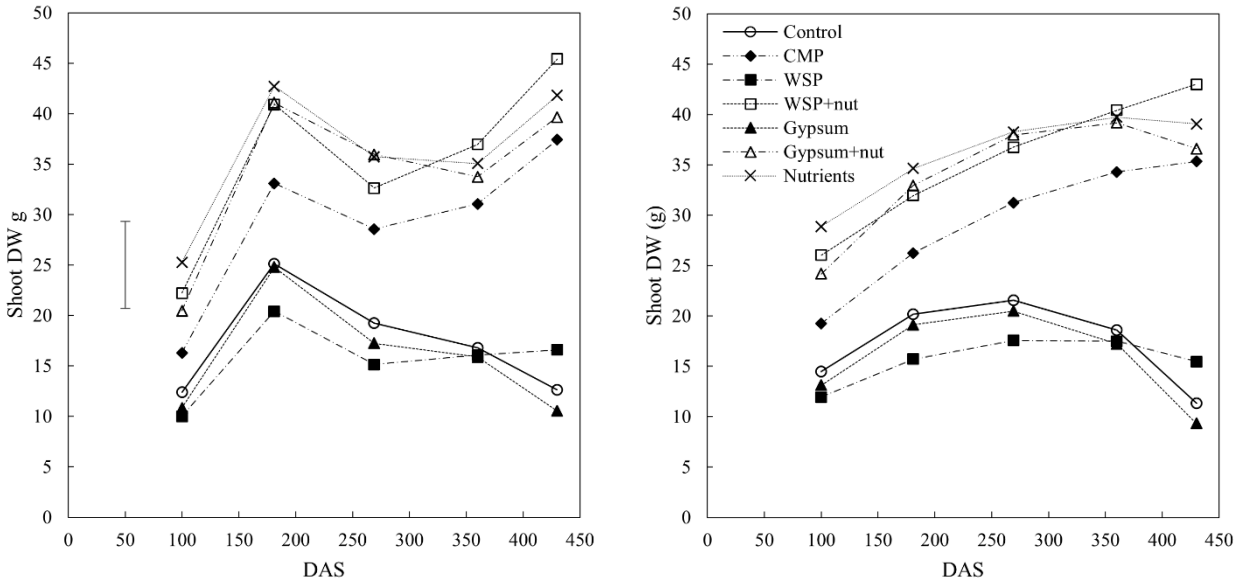


Figure 5.6. Shoot dry weight mean values and random coefficient regression predicted means for each treatment over time. The vertical bar indicates (DAS x Treatment) least significant difference (P= 0.05).

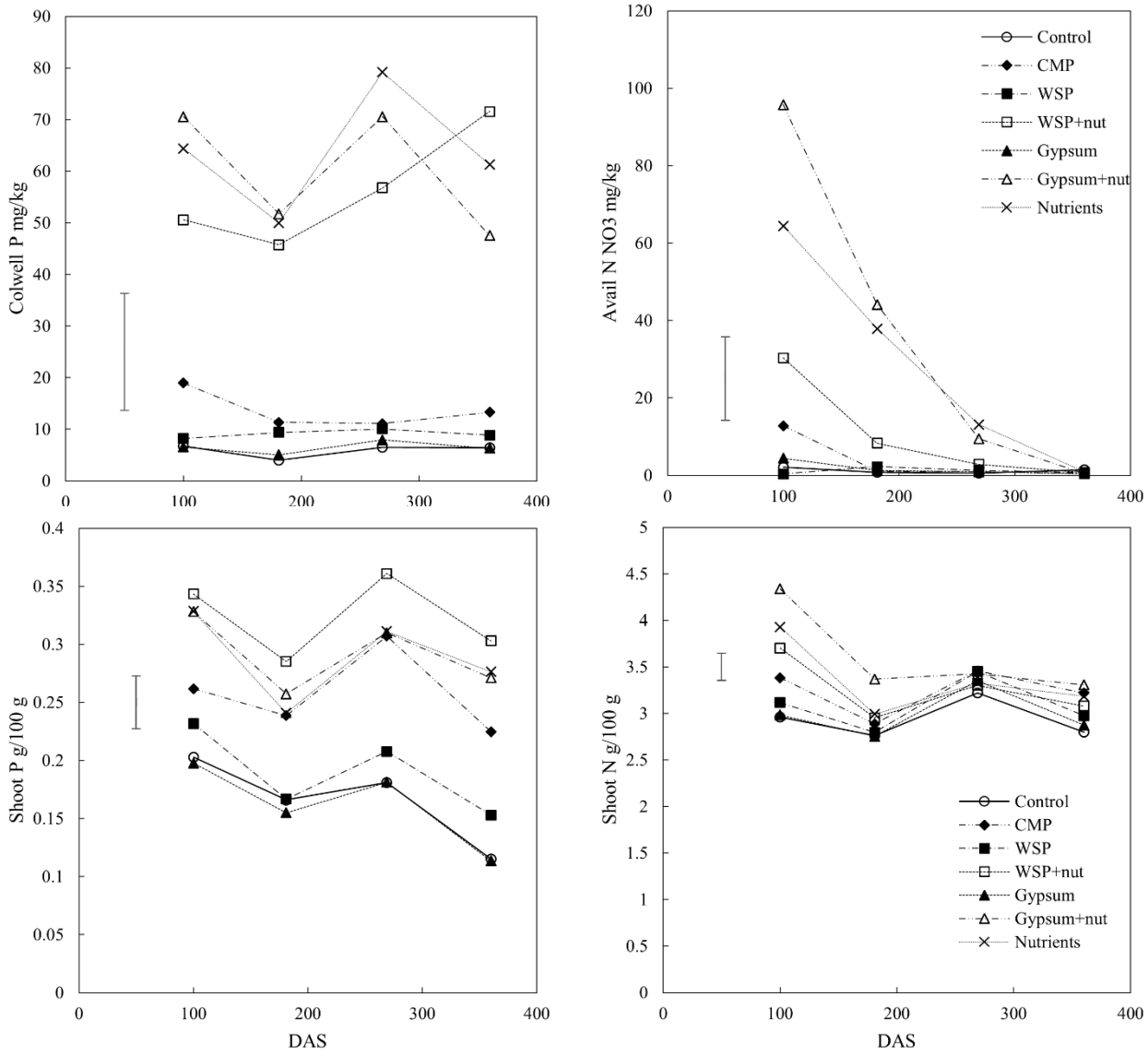


Figure 5.7. Planted subsoil Colwell P, available N (NO_3), shoot P and shoot N concentrations for all treatments over time. Vertical bars indicate (DAS x Treatment) least significant differences (P= 0.05).

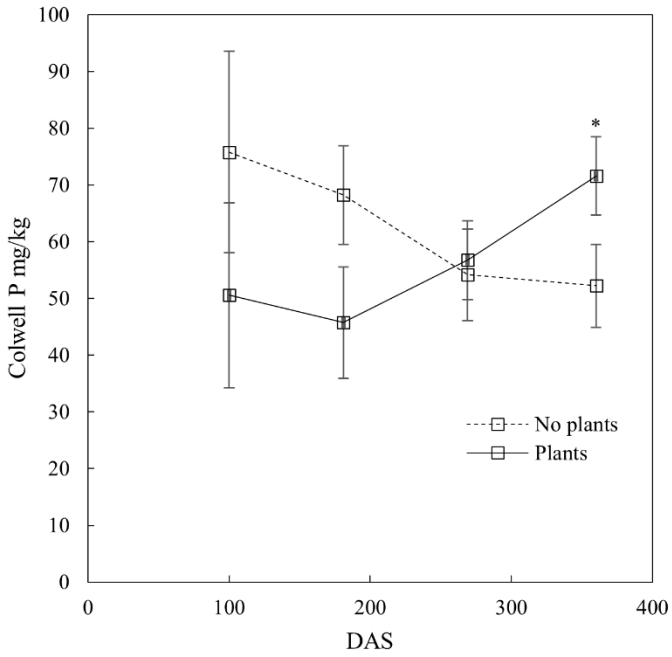


*Figure 5.8. WSP+NPKS treated subsoil Colwell P mean values with and without plants over time, error bars indicate SEM and * indicates ANOVA significant difference (P<0.02).*

5.2.4 Discussion

Plants (roots) and organic matter amendments

Organic matter amendments have been shown to improve the soil structural properties of dispersive clay subsoils, with the presence of plants increasing subsoil aggregation (Wang et al. 2020). The results of this experiment indicated that the presence of plants had no significant effect ($p<0.05$) on two key indicators of poor soil structure: WSA and turbidity, (MWD $p=0.095$, NTU $p=0.331$). The presence of plants had a greater effect on WSA than turbidity and significant at the $p<0.1$ level. Possible explanations for these differing findings compared to Wang et al. (2020) include use of different soils and plant species (wheat vs clover). Although subsoils in both studies had high ESP (21 vs 23%), dispersion is also affected by other soil properties including SOC, EC and pH (Chorom et al. 1994; Rengasamy and Olsson, 1991). By the time of the final subsoil sampling (430 days), the effects of plant roots were beginning to show a trend towards significance (MWD $p=0.058$, NTU $p=0.099$), suggesting time is an important factor. Wang et al. (2020) also found, but over only a three-month period, the presence of plants increased the turbidity of OM treated soil, which in the absence of plants decreased turbidity. In this experiment turbidity was increased in OM treated soil regardless of the presence of plants.

Time and organic matter amendments

The effectiveness of OM amendments on improving subsoil structure changes has been demonstrated to change over time. Gill et al. (2009) showed that OM amendments improved soil structure in the field over an eight-month period. Our controlled environment experiment showed OM amendments required a relative long period of time (12 months) before starting to produce changes in soil structure (MWD $p=0.002$, NTU $p<0.001$). These results were not clear however as both MWD (i.e. improved structure) and NTU (decreased soil structure) increased over time whereas we had anticipated that an increase in MWD would result in a decrease in NTU. Soil turbidity had initially increased at the start of the experiment, before decreasing steadily with time. This was the case with all OM treatments and the control soil. By the end of the experiment turbidity levels remained higher than time zero levels across all treatments.

Crop responses to organic matter amendments

Crop responses to OM amendments in this experiment appear to be the result of different subsoil nutrient availability rather than soil structure. Nutrient rich OM treatments (CMP and WSP+NPKS) resulted in greater shoot DW compared to either the control or OM treatments where the C:N ratio was high (WSP). Both the gypsum and nutrient treatments (NPKS and gypsum+NPKS treated subsoil) resulted in greater shoot DW compared to control and gypsum treatments (Figure 5.5). Gypsum application improved subsoil structure, as indicated by significantly larger aggregates and lower turbidity levels compared to the control but this did not result in improved shoot biomass production throughout the length of the experiment. In contrast to previous experimentation (controlled environment and field) examining subsoil amelioration on poorly structured subsoils, we used a legume in this experiment which supplied its own N supplies via N_2 fixation. Previous studies (Celestina et al. 2019) have argued that improvements in crop production following application of OM ameliorants with relative high nutrient concentrations such as animal manure was due to improved N nutrition. This experiment suggests that improved growth was not due to an N effect but may, in part, be due to other nutrients. Correlation and linear regression analysis indicated a highly significant relationship between shoot growth and subsoil Colwell P levels (Table 5.3). These results suggest phosphorus levels in the subsoil were driving shoot growth which in turn resulted in higher water use. In contrast, the low correlation between KCl extractable soil NO_3-N reflects the contribution of nitrogen fixation. Nodulation was evident when soil sampling and nitrogen fixation was confirmed by natural abundance analysis on all treatments. A perennial legume was used to avoid any confounding effects of applying N which would likely have been required to sustain the cereal growth over the 14 months that this experiment was conducted.

There is evidence to suggest the positive effects of OM amendments on plant growth resulted from more than just improved inorganic nutrition. When plant growth in WSP+NPKS and NPKS treatments is compared (Figure 5.6), there is a trend showing the WSP+NPKS treatment outperforming the NPKS treatment over time. These two treatments were balanced for NPKS levels suggesting the increased plant growth from the WSP+NPKS was not just the result of added inorganic nutrition. Subsoil Colwell-P levels were identified as the most important element in this experiment for increases in shoot DW and when observing WSP+NPKS Colwell P levels (Figure 5.7), these increased significantly over time. A possible explanation for the increased Colwell P levels is the addition of organic matter stimulated microbial activity which mineralised soil phosphorous, which in turn stimulated plant growth. Phosphorous mineralisation by mycorrhizal fungi has previously been demonstrated (Bowen and Theodorou 1973; Rosendhal 1942) and clover species have been observed to be highly responsive to mycorrhizal associations (Facelli et al. 1999; Smith et al. 2004). Mycorrhizal growth and activity stimulated by the addition of WSP+NPKS may provide a possible explanation to the changes in shoot DW over time between WSP+NPKS and NPKS treatments, but this hypothesis needs testing. Trends in differences in Colwell P levels between planted and unplanted WSP+NPKS treatments (Figure 5.8) support the hypothesis of a symbiotic mycorrhizal relationship. Hosting mycorrhizal fungi involves a metabolic cost and it has been shown plants tend to reduce or suppress associations in high P content soil (Braunberger et al. 1991). It has also been shown that mycorrhizal relationships improved plant growth and P uptake in both unfertilised and P fertilised soil (Cozzolino et al. 2013).

5.2.5 Conclusion

This experiment supports the hypothesis that plants (roots) do enhance the effectiveness of subsoil organic matter amendments. Improvements in soil structure resulting from amelioration of subsoils with OM compared to unamended soil only became evident towards the end of the experiment (> 12 months of continuous plant growth). These improvements in soil structure did not translate into increased plant growth however in this experiment. There were large plant responses seen with nutrient rich treatments and plant available P levels in the subsoil appear to play an important role in this greater shoot growth. An interesting result from this experiment was the effect of plants (roots) in WSP+NPKS treated subsoil. There appears to be a trend with this treatment which supports the three hypotheses of this experiment which include plant roots enhance the effectiveness of OM amendments, that this effect develops over time and that plant responses to OM amendments are more than just nutritional driven. Further investigation of this treatment, its interactions with plants (roots) and changes in soil biota over time may reveal greater insights into the mechanisms underpinning additional plant growth seen between nutritionally balanced WSP+NPKS and NPKS treatments.

5.3 NSW node glasshouse experiments: Understanding plant response of soil amendments in responsive soils

5.3.1 Objectives

The initial column study of the NSW component resulted in increased plant yield in response to amendment on the Trungley Hall and Condobolin soils. Based on root growth data, it was hypothesised that plants grown on the Trungley Hall soil increased yield due to improved root growth enabling exploitation of deeper water and nutrients. The mechanism causing root growth, chemical, biological or physical requires investigation. Yield increases due to amendment in the Condobolin soil occurred without significant increases in root growth, compared to an untreated control, suggesting that nutritional benefits of the amendment was the driver of improved plant performance. The mechanisms causing the improved plant performance were then investigated in glasshouse column studies matching nutritional benefits of amendments with inorganic nutritional addition.

5.3.2 Methods

Treatments

The experiment was run as a complete randomised block design with four replicates. Treatments were applied in columns of soil reconstructed to represent soil profiles from the field. Each soil pot/column had a range of treatments applied to them, based on results from the initial pot experiment and the incubation study. These treatments are summarised in Table 5.4 (Trungley Hall) and Table 5.5 (Condobolin) below. The Trungley Hall soil had treatments added to the surface and subsurface layers as treatment application to these layers resulted in increased root growth in previous experiments. Gypsum was not applied as an amendment due to the risk of increasing salinity, a known constraint of the Trungley Hall subsoil. Treatments were only applied to the surface layer of the Condobolin soil based on plant responses in previous experiments.

The manure amendment consisted of chicken manure pellets (CMP) (Table 5.2) ground to pass a 0.5 mm sieve and applied at rates of either 2.5 and 5 t/ha (Condobolin soil) or 5 t/ha for the Trungley Hall soil. Nitrogen (N) was applied as urea and phosphorus (P) applied as Dicalcium Phosphate (DCP) applied either individually, or in combination, at rates equivalent

to the N and P mineralised from manure treatments studied during the incubation experiments. Sulfur was applied as elemental sulfur at a rate equivalent to 300 kg/ha.

Table 5.4: Trungley Hall soil amendment treatment summary

Soil 2. Farmlink FSG – Trungley Hall															
Trt #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0-10 cm	C	C	C	C	C	M	M	M	M	M	M	M	M	M	M
10-40 cm	C	M	S	N	P	C	C+S	M	M+S	N	N+S	P	P+S	N+P	N+P+S
40-70 cm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

C = control, M = manure, S = elemental sulfur, N = urea, P = DCP, (2.5) = equivalent to N or P mineralised from 2.5 t/ha manure), (5) = equivalent to N or P mineralised from 5 t/ha manure

Table 5.5: Condobolin soil amendment treatment summary

Soil 1. Central West Farming Systems FSG – Condobolin									
Trt #	1	2	3	4	5	6	7	8	9
0-15 cm	C	M(2.5)	N(2.5)	P(2.5)	N(2.5)+P(2.5)	M(5)	N(5)	P(5)	N(5)+P(5)
15-50 cm	-	-	-	-	-	-	-	-	-
50-100 cm	-	-	-	-	-	-	-	-	-

Soils

Bulk soil samples were collected from the three upper morphological layers of the Condobolin and Trungley Hall soils, at sites identified by Farmer Support Groups (FSGs) (see Milestone 5 report for site details). It should be noted that these layers differed in depth, depending on the field morphology of the soil type in question and the same profile morphology was replicated in the production of the soil columns, namely; 0-15, 15-50 and 50-100 cm for the Condobolin soil, and 0-10, 10-40 and 40-70 cm for the Trungley Hall soil. The Condobolin pots were 100 cm in length. However, the height of Trungley Hall soil pots were reduced from 100 cm to 70 cm (30 cm shorter for layer 3), because of limited availability of the third layer soil.

Pots were made from lengths of 150 mm diameter PVC stormwater pipe. The columns had two holes drilled through the top part of each to fit a lifting bolt through the top end. The bottom end was fitted with an end cap which was then secured with screws and sealant. A drainage hole was positioned in the bottom cap. The weight of each empty pot was recorded prior to filling. A plunger was used for packing soil to the desired bulk density

The soils were air dried (40°C) and ground using a brush mill (<2 mm). Larger clay aggregates were crushed mechanically and then passed through the brush mill. Any gravel or other concretions were removed as part of the brush mill grinding process. The moisture content at field capacity (FC) was determined for each of the soils using pressure plate apparatus. Where amendments were applied, the required rate of ameliorant for each treatment were added to the test soils and thoroughly mixed using a rotary cement mixer prior to placing in the columns.

In an effort to replicate the bulk densities of the field, each of the soil treatment batches were moistened to around 60% FC, covered and left for the soil to absorb the added moisture (24 hours), before finally adding to the soil columns. For the filling process, soil was gradually poured into the columns in 2 cm layers while compacting with a plunger in order to replicate field bulk densities of around 1.5 g/cm^3 for subsoil and 1.3 g/cm^3 for surface soils. Once the soil columns had been filled, they were housed in a temperature-controlled glasshouse to

equilibrate for three months prior to sowing. The glasshouse temperature was monitored throughout experiment.

Plants, growing conditions, and sample analysis
After the equilibration period, six pre-soaked, spring-wheat seeds were sown per core (Vixen). After germination, the six seedlings were thinned to a maximum of four plants per core. Soil moisture was maintained at 60% FC using deionised water throughout the experiment and until the start of head formation, when watering was ceased to encourage deeper root penetration into the subsoil. All pots received a basal application of fertiliser (equivalent to 11 kg P/ha and 10 kg N/ha) using both urea and DCP, and two subsequent applications of urea (equivalent to 30 kg/ha N), which was applied to all columns at day 30 and 45 after germination.

Sampling and measurements
Plant height and water usage was measured throughout the growing season, while at anthesis, plant height and tiller number was recorded. At grain maturity, the mass of soil columns was recorded. Plants were then cut at ground level, weighed, and dried (60°C). Grain was separated from heads and weighed and shoot (straw) weight recorded.

After plant harvest, the soil columns were cut open and subsamples of soil from each layer were taken for measuring gravimetric water content and for chemical analysis, including pH and EC (1:5 water extract), pH in calcium chloride (pH_{Ca}), Colwell P (Colwell 1963), mineral N, available S (KCl), exchangeable cations and CEC (Gillman and Sumpter 1986), and water soluble cations and anions including chloride (1:5 soil:water extract). Soil dispersion was assessed by measuring the turbidity (Hach Turbidimeter 2100N) via a method adapted from (Zhu et al. 2016) using 1 g soil in 60 mL water. A further subsample (30-50 g) was placed into sterile tubes and immediately frozen (-80°C) for subsequent analysis for microbial DNA.

At the same time, the visual presence of roots in each soil increment was recorded. Subsequent to subsampling for chemical analysis, columns were sealed up prior to root separation and stored in a temperature-controlled glasshouse (see above). For root separation, columns were sectioned into 10 cm increments and soaked in tap water overnight. Roots were then removed from each 10 cm soil increment by repeated washing with water and passing the solution through a 0.5 mm sieve. The root plus organic matter samples were dried (60°C) and roots separated from the visible soil organic matter by hand. The mass of roots in each soil increment was measured.

Soil biology analysis
Soil samples (n=120) were collected for microbial analysis from the 0-10 cm layer (n=60) and 10-40 cm layer (n = 60) from the 60 soil columns with 15 treatments and four replicates of the Trungley Hall experiment. Soils (>50 g) were frozen at -80°C and shipped interstate on ice. Upon arrival at laboratory samples were stored at -20°C.

DNA was extracted from 0.25 g of soil using a DNeasy PowerSoil Pro Kit (Qiagen, Clayton, Australia) as per manufacturer's instructions using a Qiagen QIACube. The quantity and quality of the DNA was analysed using a spectrophotometer (NanoDrop Technology, Wilmington, USA). DNA extracts were diluted to 5 ng μL^{-1} with ultrapure molecular grade water and stored at −20°C before use.

A total of 72 genes including 71 functional genes related to C, N, P and S cycling and one bacterial 16S rRNA gene (Table S1) were quantified using QMEC: a high-throughput quantitative-PCR-based chip to assess the microbial functional potential (Zheng, Zhu et al. 2018). Amplification was conducted in a 100 nL reaction system on the Wafergen SmartChip Real-time PCR system (Wafergen, Fremont, CA). The reaction consisted of forward primer

(0.5 µM), reverse primer (0.5 µM), SYBR Green (1X), Bovine serum albumin (BSA) (1 mg/mL) and DNA (1 ng/uL). QPCR reactions were dispensed using a SmartChip Multisample Nanodispenser (Takara Biomedical Technology) into microwells on the high-throughput qPCR chip (Takara Biomedical Technology).

All qPCR reactions were conducted in triplicate for each primer set. A negative control with no DNA template was included in each HT-qPCR run to eliminate false-positive detections. The initial enzyme activation was performed at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s. Melting process was automatically generated and analysed using the SmartChip qPCR Software. The criteria used for positive detections of functional genes were: (1) a threshold cycle (CT) value of 31 was considered as the detection limit and (2) amplicons with multiple melt curves or amplification efficiencies beyond the range (0.9–1.1) were removed from the analysis.

Relative gene abundance was calculated for each gene detected based on a CT <31 using the method of Looft et al. (2012). Relative gene abundance was defined as the proportion of the abundance of a functional gene (equation 1) to the abundance of the 16S rRNA gene (equation 2), where CNi is the copy number of one of the 71 functional genes and CN16S is the copy number of the 16S rRNA gene for the same sample.

$$\text{Gene copy number} = 10^{(31-Ct)/(10/3)} \quad (1).$$

$$\text{Relative gene copy number} = CN_i / CN_{16S} \quad (2).$$

Statistical analyses

Significance tests (at the 0.05 significance level) were performed by one-way ANOVA (LSD) using Genstat (Vers. 19.1.0.21390, VSN International Ltd). Several analyses were carried out with the R statistical package (v 4.2.0) (Team 2014) with R-studio 2022.02.2 (Team 2014). The similarity of microbial communities was compared between treatments(depth in the column, treatment within a column layer and column treatments) using a permutational multivariate analysis of variance (PERMANOVA) using the adonis2 function in the package “vegan” (Oksanen, Simpson et al. 2022). The “vegan” package was also used to represent the relationship between each of the samples in a Nonmetric Multidimensional Scaling (NMDS) ordination. Samples are more similar in their functional diversity as the points representing each soil sample become spatially closer to each other.

The abundance of functional genes varied between genes and HT-qPCR chips, thus it was normalised by conversion to a Z score using the ‘scale’ function in R for the downstream analyses.

Statistical analysis of plant growth and soil data were carried out using analysis of variance ANOVA (Genstat,18th version; VSNI Hertfordshire, UK). Prior to ANOVA analysis, data was checked for normality using the Shapiro-Wilk test for normality. Statistical significance was expressed for each of the treatment effects using least significant differences and Tukey’s confidence interval test at the 5 % level.

5.3.3 Results and discussion

Trungley Hall Experiment

Plant response

Root growth, expressed as total root mass per plant, was not significantly changed by amendment to the subsurface (10-40 cm) layer when no amendment (control) was made to the surface layer (0-10 cm) with the exception of the addition of N (Table 5.6). When manure was added to the surface soil, root mass was increased by application of manure, N or P into

the 10-40 cm layer. Investigation of root mass with depth through the profile indicates that the root mass of the Manure,P treated soil was only significantly greater than the control in the 0-10 cm layer (Figure 5.9). Manure,N and Manure,Manure significantly increased root growth in the 10-30 cm layers relative to other treatments. The Control,N treatment also had significantly more root mass than the untreated control in the 20-30 cm layer. These results demonstrate that the addition of nitrogen in the 10-30 cm, either as urea or manure can increase root growth. However, there was a lack of response from the Manure,N+P and Manure N+P+S treatments.

Table 5.6. Wheat plant response to treatment on Trungley Hall soil. Root mass, above ground Dry Matter Yield (DMY), tiller and head number, grain yield expressed on a per plant basis. Values within columns designated with different letters are significantly different. lsd = least significant difference at p<0.05. Treatment labels represent "topsoil,subsurface" treatment amendments.

Treatment	Root mass (g/plant)	DMY (g/plant)	Tillers/plant	heads/plant	Grain yield (g/plant)
Control,Control	0.22ab	2.09a	1.88abcd	1.81abcd	1.26ab
Control,Manure	0.15a	1.97a	1.33a	1.33ab	1.1a
Control,S	0.23ab	1.97a	1.56ab	1.56abc	1a
Control,N	0.34cd	3.09bcd	2.06bcd	2.06cd	1.52bcd
Control,P	0.19ab	2.91bc	1.69abc	1.69abcd	1.5bc
Manure,Control	0.25abc	2.44ab	1.75abcd	1.75abcd	1.25ab
Manure,S	0.29bc	3.24cd	2.06bcd	2.06cd	1.59bcd
Manure,Manure	0.46e	3bc	1.63ab	1.63abc	1.49bc
Manure,Manure +S	0.23ab	1.91a	1.33a	1.27a	1.01a
Manure,N	0.41de	3.13bcd	2.31de	2.25de	1.5bcd
Manure,N+S	0.29bc	3.08bcd	2.25cde	2.25de	1.49bc
Manure,P	0.47e	3.54cd	1.88abcd	1.88bcd	1.76cd
Manure,P+S	0.28bc	3.23cd	2.06bcd	2.06cd	1.65cd
Manure,N+P	0.24abc	3.8d	2.75e	2.75e	1.84d
Manure,N+P+S	0.23ab	3.04bcd	2bcd	2cd	1.46bc
P value	<0.001	<0.001	<0.001	<0.001	<0.001
lsd (p=0.05)	0.10847	0.7656	0.5919	0.5973	0.3476

The treatments that resulted in increased root growth also caused significantly greater above ground biomass (DMY) (Table 5.6). However, unlike root growth, DMY increased with addition of P to the 10-40 cm layer when no amendment to the surface soil was made. Of the treatments amended with Manure in the surface soil, only the Manure,control and Manure,Manure+S did not result in significantly more DMY than the untreated control. The DMY of all other subsurface amended soils were similar ranging from 3 to 3.8 g/plant. The plant response to amendment in DMY was highly correlated to grain yield (r =0.96). However grain yield was not significantly increased from the untreated control for the treatments Control,N and Control,P. As for the DMY, grain yield was significantly increased relative to both the control and the Manure,control in columns amended with Manure,P, Manure,P+S, and Manure,N+P.

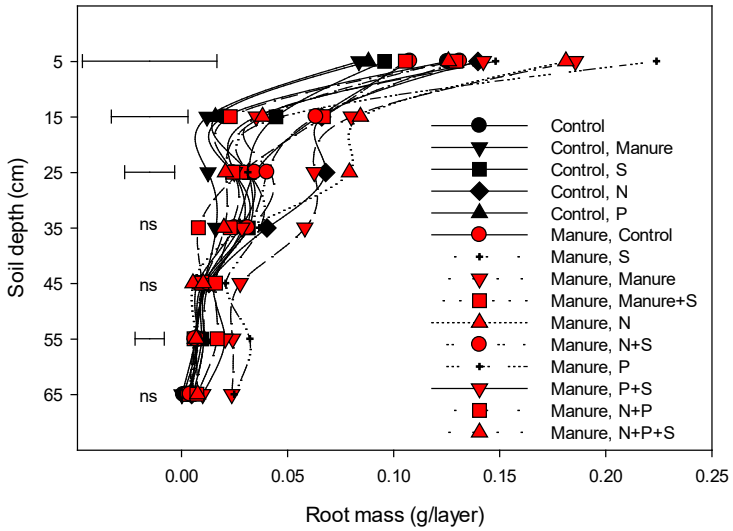


Figure 5.9. Wheat root mass per soil layer for soil amendment treatments on Trungley Hall soil. Horizontal bars represent lsd ($p<0.05$), ns = not significant. Treatment labels represent "topsoil,subsurface" treatment amendments.

The N content of above ground plant material was significantly greater when N was applied as a fertiliser in the subsoil (Table 5.7). The presence of manure in the topsoil had no effect on N content in straw, grain or total N. Where P was applied to the subsoil, N content of the grain was significantly increased relative to the control and was not different to that of the plus N treatments. However, the plus P treatments had lower nitrogen content in the straw and total plant compared to the plus N treatments. Nitrogen content of straw, grain and plant was maximised when N and P were added together to the subsoil and exceeded that of when manure was added (Table 5.7).

Table 5.7. Nitrogen content (mg N per plant) in straw, grain and the above ground plant (sum of straw and grain) in response to treatment on Trungley Hall soil. Values within columns designated with different letters are significantly different. lsd = least significant difference at p<0.05. Treatment labels represent "topsoil,subsurface" treatment amendments.

	Nitrogen content (mg/plant)		
Treatment	Straw	Grain	Plant
Control,Control	11.2ab	32.2ab	43.3ab
Control,Manure	9.6a	26.9a	36.5a
Control,S	11.3ab	26.8a	38.1a
Control,N	20.0c	40.9c	60.9ef
Control,P	11.9ab	37.5bc	49.4bc
Manure,Control	12.7ab	32.7a	45.4abc
Manure,S	13.2ab	37.9bc	51.1bc
Manure,Manure	12.3ab	39.3c	51.6bc
Manure,Manure +S	10.3ab	27.2a	37.5a
Manure,N	21.5c	40.7c	62.2efg
Manure,N+S	22.2cd	41.9c	64.1fg
Manure,P	12.5ab	41.4c	53.9cde
Manure,P+S	13.9b	40.7c	54.6cdef
Manure,N+P	30.5d	50.7d	81.2h
Manure,N+P+S	25.5d	41.0c	66.5g
P value	<0.001	<0.001	<0.001
lsd (p=0.05)	4.00	7.66	9.79

Soil response

The addition of manure to the surface soil (0-10 cm) significantly increased soil pH_{Ca} in that layer by approximately 0.4 pH units relative to the untreated control (Figure 5.10). The pH amelioration effect of manure has been reported (Lauricella et al. 2021) and whilst increases in pH on an acid soil can have benefits to nutrient availability, the pH of the control (pH 5.5) soil was not acidic enough to limit plant performance.

There were no significant differences in soil pH of the 10-40 cm layer in response to amendment application. The soil pH of the deepest layer (40-70 cm) decreased significantly, relative to the control, in treatments receiving nitrogen or elemental S when manure was also applied in the surface. Whilst it is understood that application of elemental S will decrease pH due to microbial oxidation of sulfur and nitrification of ammonium sourced from N applied will also decrease pH in the layer of application, it is not clear why these treatments caused acidification of the deepest layer. Elemental S is not soluble and ammonium should have been retained on the exchange sites within the layer were amendments were made. Additionally, experiments of phase 2 of the project demonstrated that oxidation of elemental S was limited in deeper soil layers presumably due to lower numbers of *Thiobacillus spp.* bacteria. As the soil pH of the deepest layer was greater than pH 8, acidification may have been beneficial to plant performance. However, there was no statistical relationship between pH at 40-70 cm and plant DMY or grain yield indicating that soil pH was not a responsive mechanism causing observed plant performance when amendments were applied.

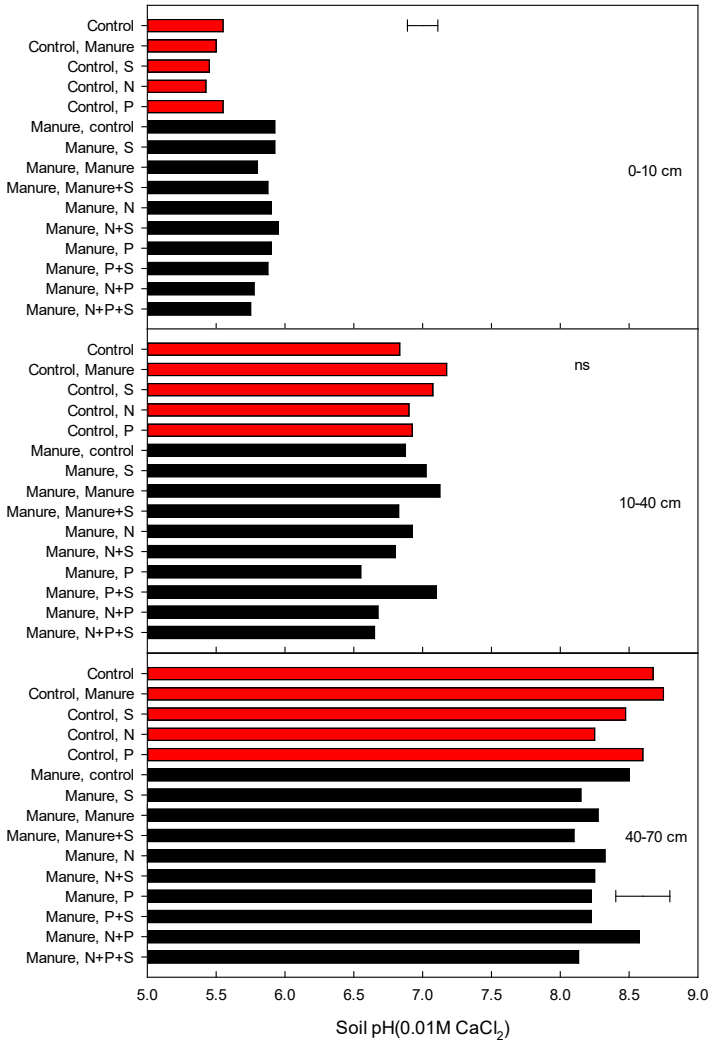


Figure 5.10. Soil pH (0.01 M $CaCl_2$) of soil from each sampled layer at the time of plant harvest for soil amendment treatments on Trungley Hall soil. Horizontal bars represent lsd (p<0.05), ns = not significant. Treatment labels represent "topsoil,subsurface" treatment amendments.

Colwell P

There were no significant differences in the concentration of Colwell P in the surface soil (0-10 cm) at harvest for treatments that had no amendment addition to that layer (Figure 5.11). Where manure was applied to the soil surface, significantly greater Colwell P concentrations were present in the Manure,Manure and Manure,N+P treatments relative to the control. There were no significant differences in Colwell P concentration between treatments receiving Manure in the 0-10 cm layer. It should be noted that the Colwell P concentrations present are much greater than the critical value required to obtain maximal yield (approximately 40 mg/kg) and therefore the plants should not be responsive to P addition in the 0-10 cm layer. Within the subsurface layer (10-40 cm), Colwell P concentrations were significantly greater than the control in any treatment that had P applied as inorganic fertiliser with the exception of Manure,P (Figure 5.11). The Manure,P treatment had relatively large root growth possibly resulting in greater exploitation of soil Colwell P. This may also be the reason when the Manure,Manure treatment did not result in increases in Colwell P.

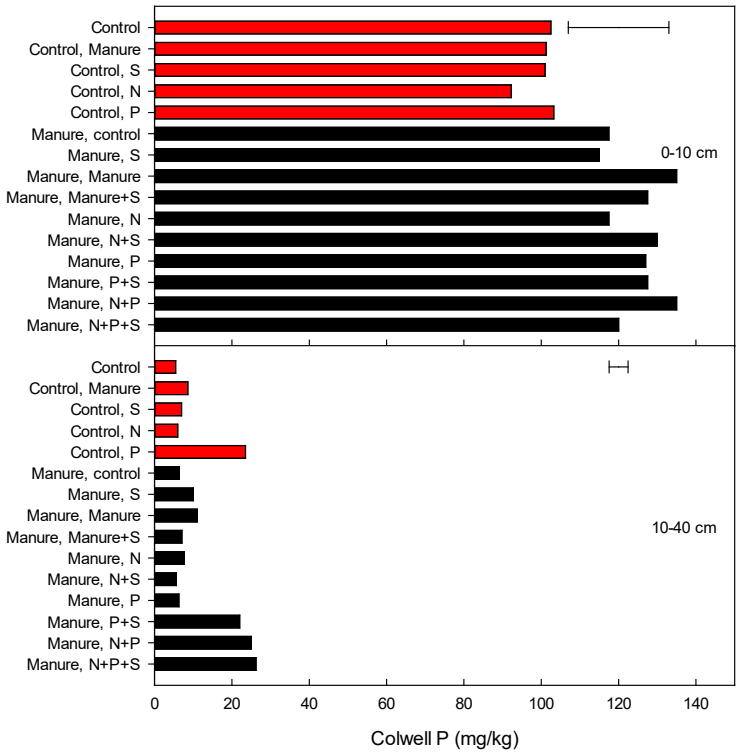


Figure 5.11. Soil Colwell P (mg/kg) of soil from each sampled layer at the time of plant harvest for soil amendment treatments on Trungley Hall soil. Horizontal bars represent lsd (p<0.05), ns = not significant. Treatment labels represent "topsoil,subsurface" treatment amendments.

Soil Mineral N

The majority of mineral N present at the time of sampling was in the nitrate form. This mobile form of nitrogen is free to move due to either diffusion or in mass flow of water as a result of watering (downwards) or evaporative flux (upwards). As a result, the treatments that resulted in the surface 0-10 cm layer having significantly greater mineral N concentrations, Control,Manure, Control,P, Manure,Manure and Manure,P+S treatments were not necessarily the treatments of greatest N addition. In the 10-40 cm layer, the Control,N, Manure,N+S and Manure,P had significantly greater soil mineral N concentrations than other treatments (Figure 5.12).

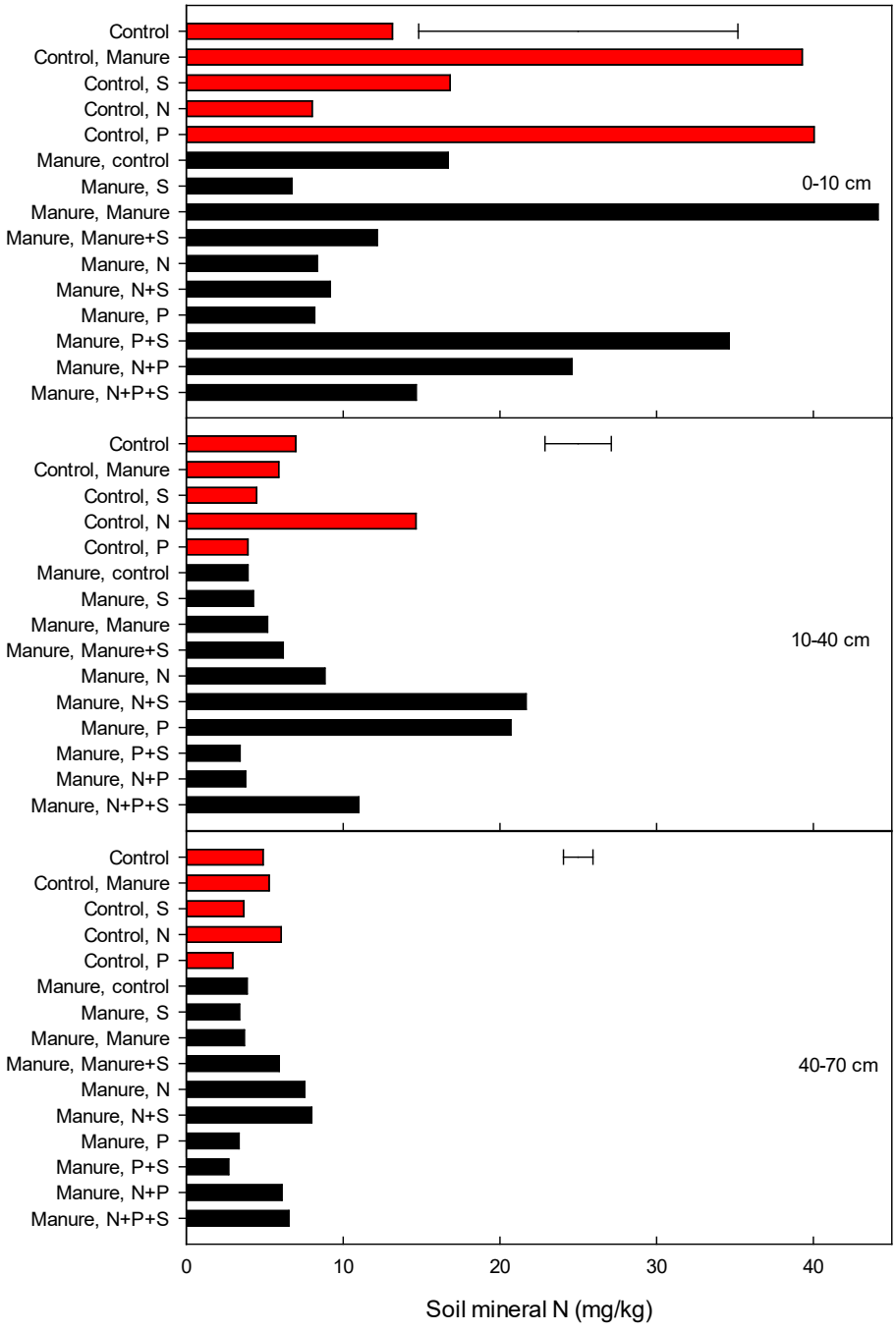


Figure 5.12. Soil mineral N, sum of ammonium and nitrate (mg/kg) of soil from each sampled layer at the time of plant harvest for soil amendment treatments on Trungley Hall soil. Horizontal bars represent lsd (p<0.05). Treatment labels represent "topsoil,subsurface" treatment amendments.

Soil mineral N concentrations in the deepest layer (40-70 cm) were greater in the treatments receiving N as fertiliser in the subsurface layer. The majority of mineral N recovered in the 40-70 cm layer was in the nitrate form. The rapid release of N from urea (Condon et al. 2004) would have caused mineral N to be present quantities in excess of plant requirements during early growth resulting in downward movement of nitrate to the deepest layer of the soil pots. The mineralisation of mineral N from manure would have occurred at a slower rate possibly favouring uptake higher in the profile.

Given the mobile nature of nitrate and its ability to move between layers of the soil columns, the mineral N was summed between layers to provide a total mineral N mass per column. Only Manure,S and Manure,P had significantly less mineral N (both 14 mg/kg) than all other treatments (34 ± 8 mg/kg).

Available S

Sulfate-S is also highly mobile in the soil solution, so the concentrations present in individual layers were summed to study total profile treatment differences (Figure 5.13). Treatments that resulted in the application of S had significantly greater sulfate-S concentrations compared to other treatments. This result indicates that S applied was in excess of plant requirements and therefore it is not expected that positive plant responses observed were related to S nutrition. The sulfate-S concentrations were above the critical value (7 mg/kg, 95% relative yield) (BFDC 2022) which is evidence that sulfur was present in concentration in excess of plant requirements.

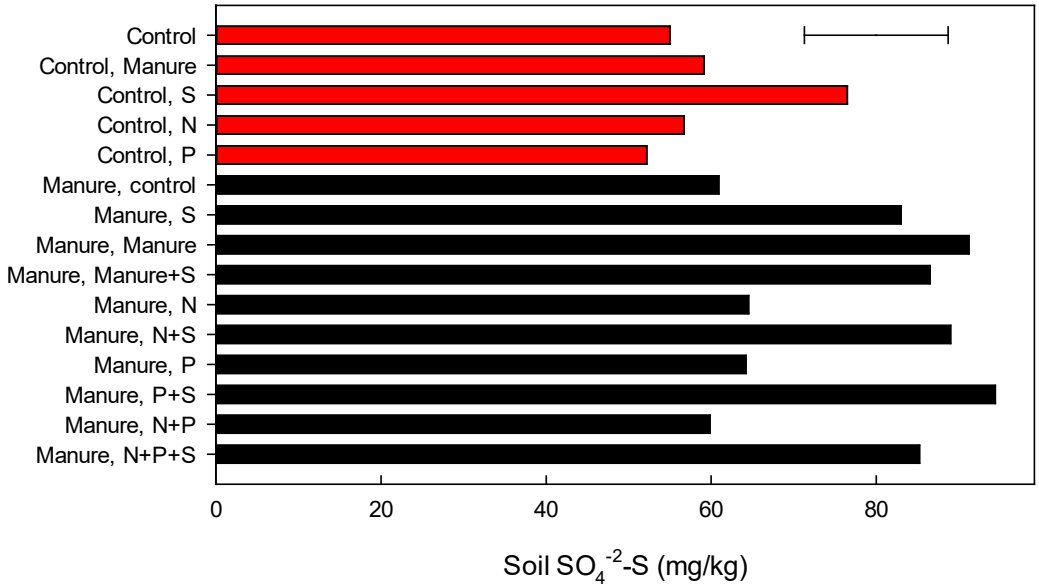


Figure 5.13. Available Sulfate-S (mg/kg), summed from each soil layer, at the time of plant harvest for amendment treatments on Trungley Hall soil. Horizontal bars represent lsd (p<0.05). Treatment labels represent "topsoil,subsurface" treatment amendments.

Exchangeable cations

There were no significant differences in Ca or K as a percentage of exchangeable cations, Ca:Mg or exchangeable sodium percentage (ESP) as a result of treatment amendments.

Soil biology

The QMEC approach used in this study can detect a wide range of microbial functional genes representing the C, N, P and S cycles in a high-throughput manner, by measuring all genes simultaneously within a sample. Of the 72 genes represented on the HT-qPCR chip the bacterial 16S rRNA gene was detected and 11 functional genes among samples.

Of the C cycle, the genes *acsA* (acetyl-coenzyme A synthetase) and *rbcL* (ribulose-bisphosphate carboxylase large chain) were highly prevalent and encode carbon fixation proteins. Carbon degradation gene *gmGDH* (glucose dehydrogenase) and methane metabolism gene *emGDH* (methanol dehydrogenase) were present in a limited number of soil samples. The N cycle genes *nxrA* (nitrite oxidoreductase α subunit), *nirK* (nitrite reductase), and *ureC* (urease) were detected, representing the processes of nitrification, denitrification and ammonification respectively. P cycle genes detected were *phnK*

(phosphonate transport system ATP-binding protein) and *phoD* (alkaline phosphomonoesterase) which regulate organic phosphorus mobilisation, and *pqqC* (pyrroloquinoline-quinone synthase) which regulates inorganic P solubilisation. The gene y*edZ* (sulfite oxidase) is responsible for sulfur oxidation in the S cycle.

Functional community structure for all samples is shown in an NMDS ordination (Figure 5.14), with ten of the genes detected as significant vectors (P<0.05) (Figure 5.14). Samples from the upper layer of the column are scattered predominantly across the negative side of the NMDS1 axis, while samples from the lower layer of the column are mostly in the middle of the ordination and positive side of NMDS1 axis. This separation on depth was confirmed by PERMANOVA analysis with adonis 2 (pseudo-F = 9.7619, P < 0.001) and treatment layer (pseudo-F= 1.4415, P < 0.05). NMDS ordinations for the upper and lower depth and shown in Figure S1 and Figure S2 respectively. PERMANOVA analysis of samples within a depth was not significant for treatments applied to the layer (upper depth: pseudo F = 2.9212, P =0.056; lower depth pseudo F 1.4896, P = 0.074).

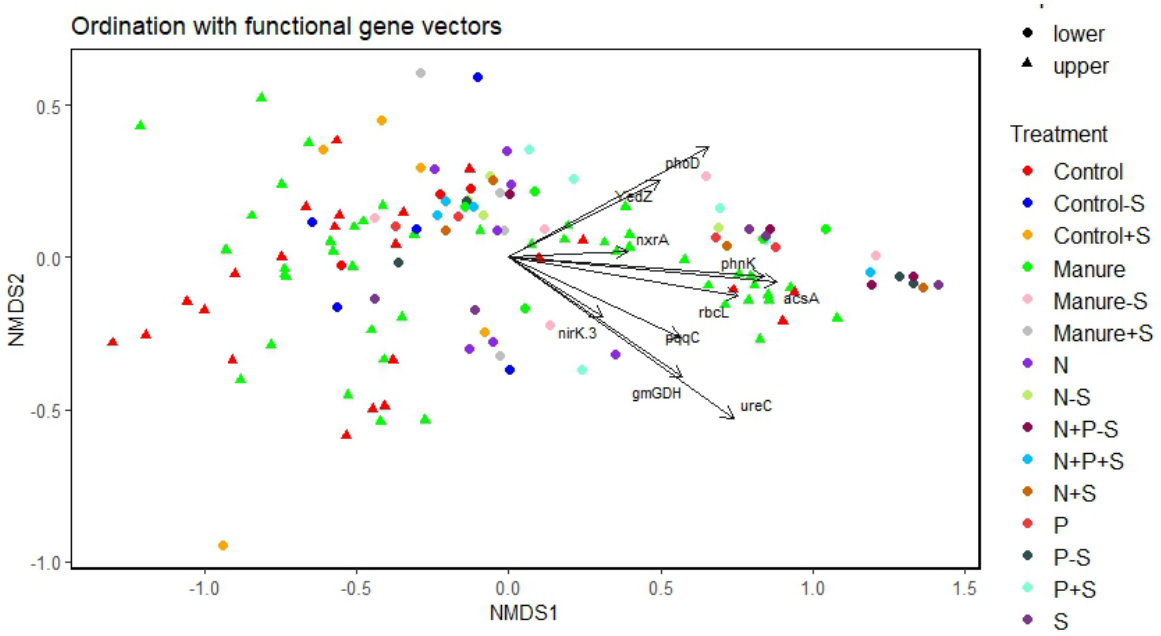


Figure 5.14: NMDS ordination of all soil samples (n =120) from two depths in the soil column (0-10 cm "upper" and 10-40 cm "lower") and the treatment applied within the sampled layer of the column. The closer together samples are spatially, the more similar they are in their relative functional gene profile. Ten of the genes detected are overlaid as significant vectors (P<0.05) on the ordination. Stress = 0.066.

Z-value (unitless) was used to compare the abundance of functional genes between treatments within a depth to assess trends for each gene detected. In the upper depth, two genes had great abundance in soils treated with manure, *nxrA* (control -0.1995, manure 0.2069; P < 0.05) and *emGDH* (control -0.1072, manure 0.2593; P < 0.05). In the lower depth, *YedZ* showed greater abundance in some of the treatments with sulfur added (control -0.5178, S 1.7969, P 1.6508, N+S 1.0367, P+S 1.1200 and N+P-S 1.1138; P< 0.05). Patterns of gene abundance are shown across all the different treatment columns for genes in C cycling (Figure 5.15), N cycling (Figure 5.16), P cycling (Figure 5.17), S cycling (Figure 5.18A) and methane metabolism (Figure 5.18B).

.

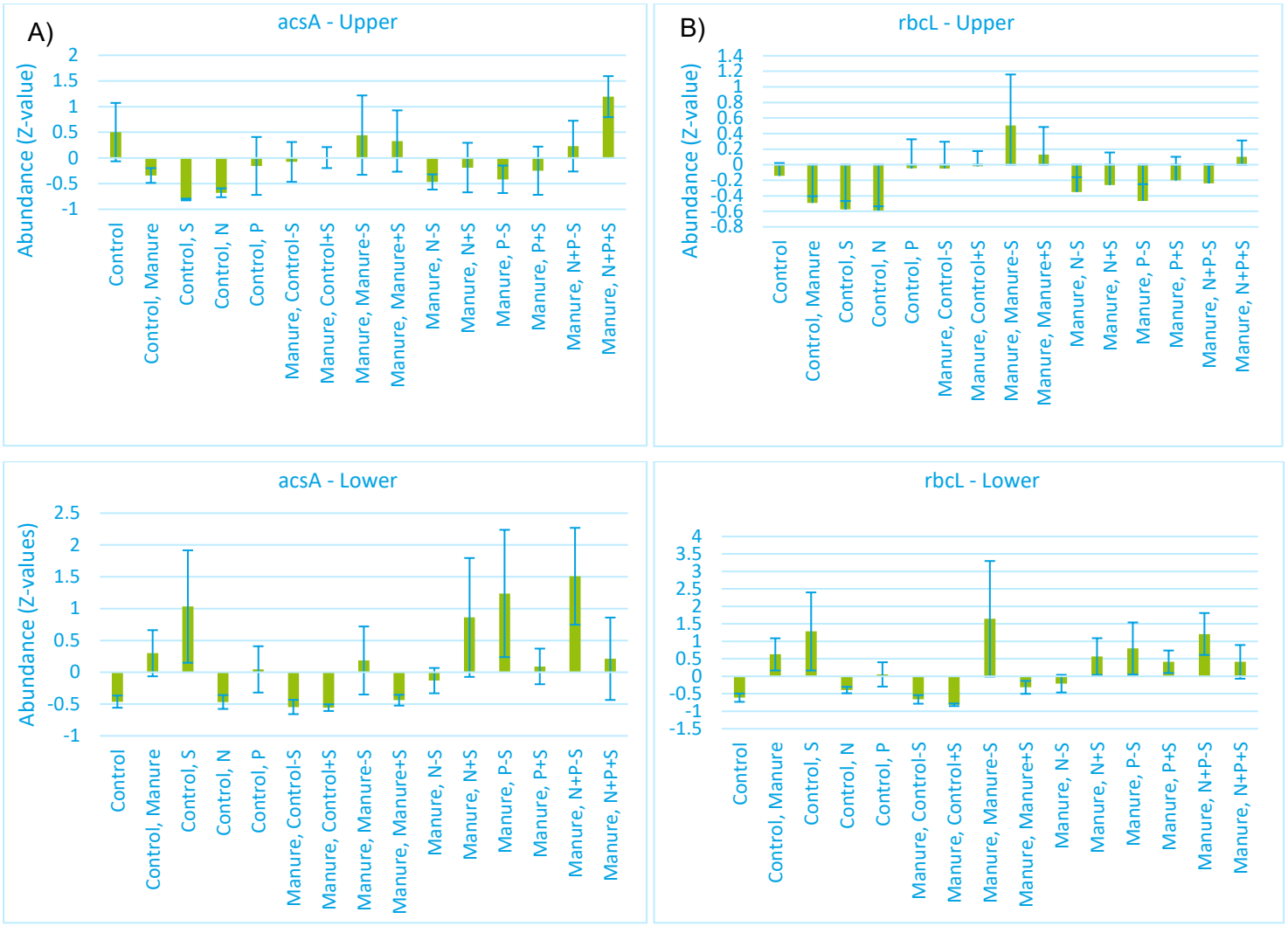


Figure 5.15. Carbon cycling gene abundance in upper and lower column depths A) acsA, B) rbcL. of the Trungley Hall amendment treated soil

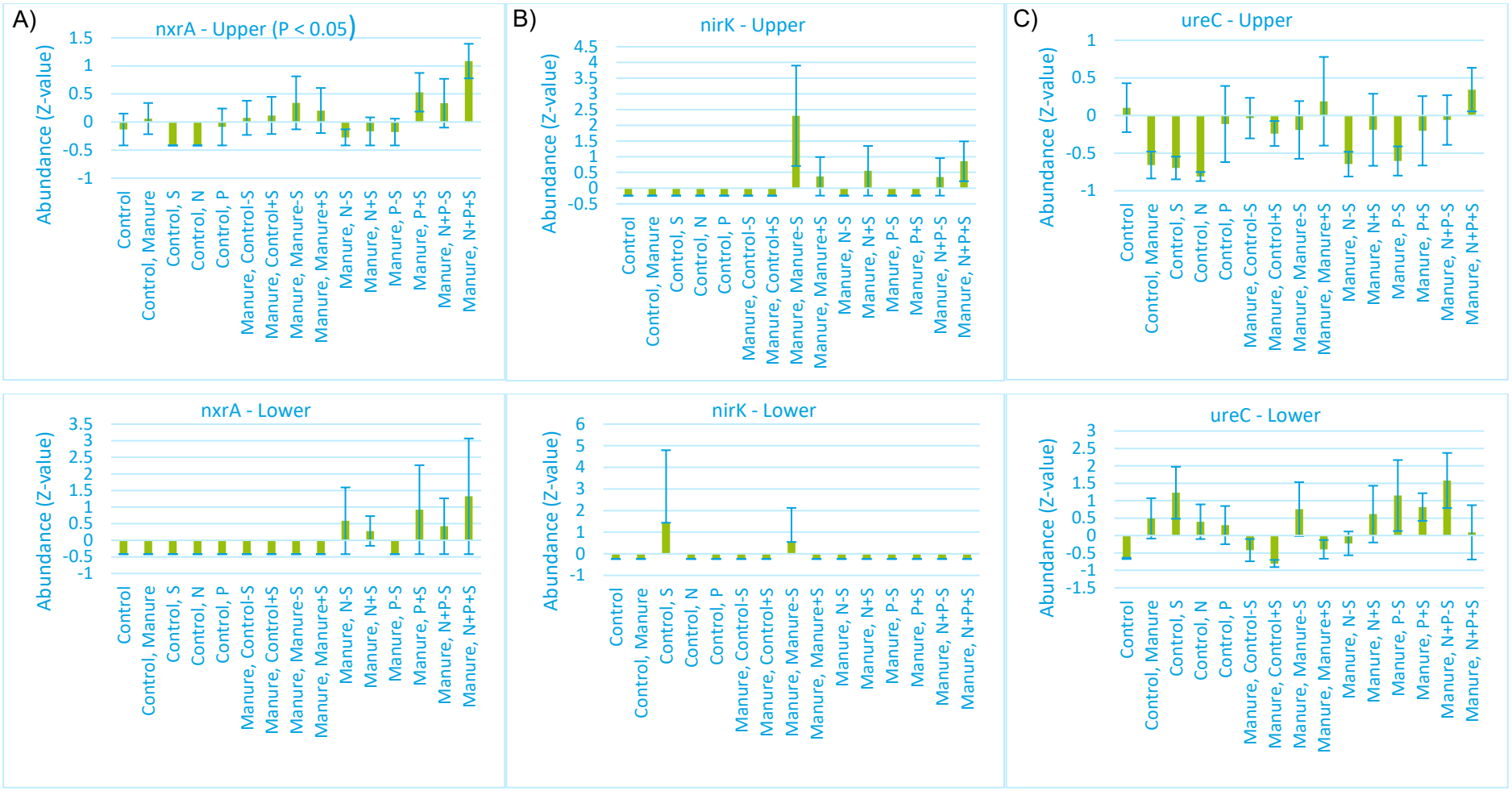


Figure 5.16. Nitrogen cycling gene abundance in upper and lower column depths A) nxrA, B) nirK, C) ureC of the Trungley Hall amendment treated soil

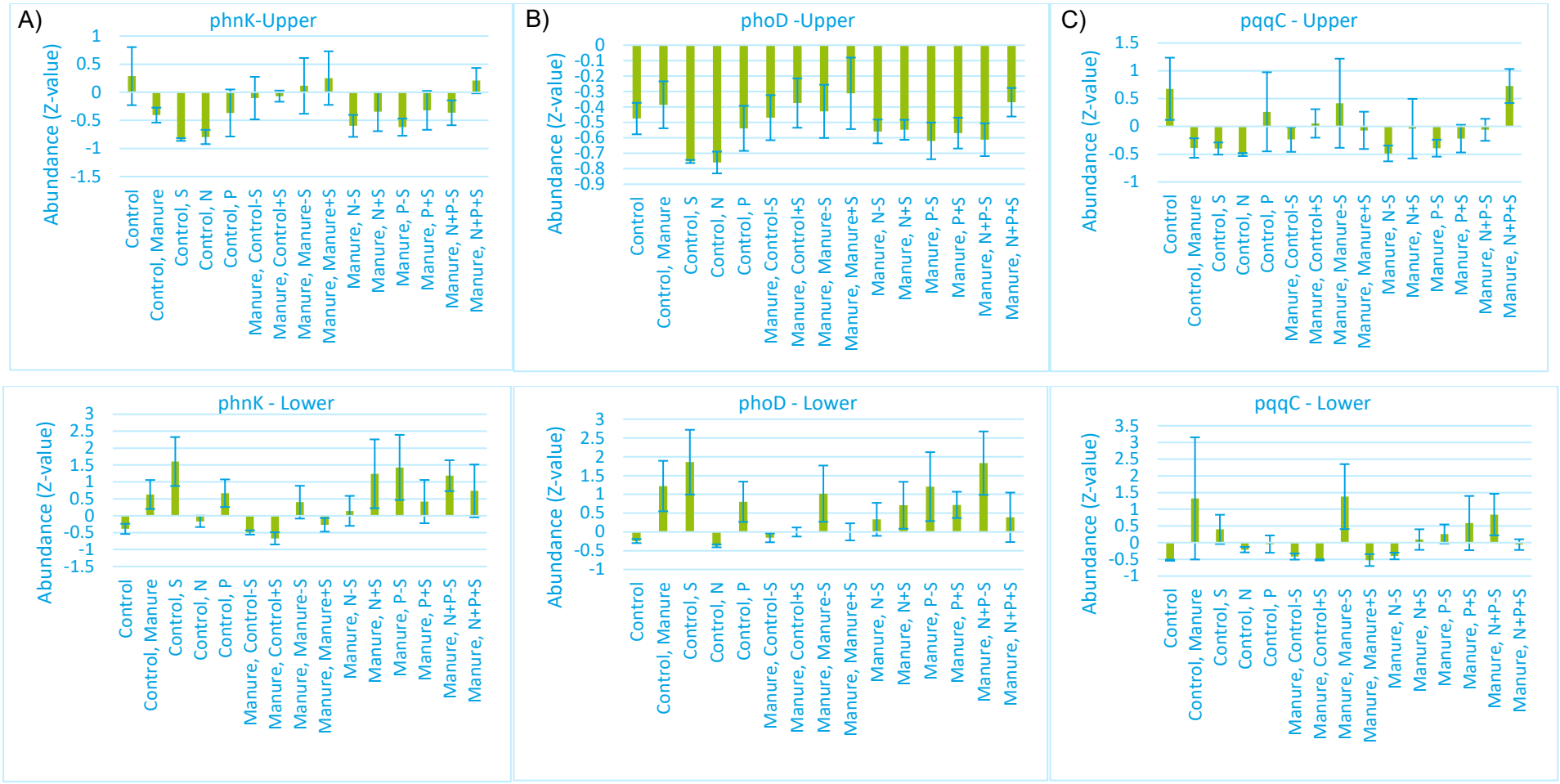


Figure 5.17 Phosphorus cycling gene abundance in upper and lower column depths A) phnK, B) phoD, C) pqqC of the Trungley Hall amendment treated soil

Figure 5.18. Sulfur cycling gene abundance A) yedZ, and methane metabolism gene B) emGDH in upper and lower column depths of the Trungley Hall amendment treated soil

Interpretation of biological data and QMEC approach

The QMEC high-throughput qPCR method is increasingly being used in agricultural soils to examine soil microbial responses to fertiliser and organic matter additions (Chen, Ding et al. 2020, Bi, Jin et al. 2021, Li, Cui et al. 2022, Xiao, Dong et al. 2022). The method is attractive because of the large number of genes that can be detected simultaneously compared to single gene analysis in conventional quantitative PCR. This experiment is the first time the QMEC method has been used on Australian soils.

The number of functional genes detected in the Trungley soil (n=11) was lower than expected compared to Zheng et al. (2018), which describes the development and primer validation of 71 functional genes on the QMEC chip. In fairness, the Trungley soil is the first soil type to be used on the QMEC Wafergen system in the laboratory, so primers and amplification conditions were not optimised specifically for this soil. A soil sample from another site, Dookie, was also used in preliminary testing of the functional gene primers Wafergen system, amplifying the same genes as most of the Trungley soil samples. The absence of other functional genes in the Trungley soil may be explained by: a) absence of the gene in the soil; b) the gene being below the QMEC chip detection limit; c) the primers not detecting the functional gene variant present in Australian soils, as new primers designed by Zheng et al. (2018) may have been optimised for Chinese soils; and d) reduced efficiency in gene amplification because unlike conventional qPCR where annealing temperatures and extension times can be optimised for each assay, QMEC relies on a single amplification profile for all 72 genes. Additional experiments utilising conventional qPCR on the same soil samples would help elucidate which of the above is the most likely explanation. The Bacterial 16S rRNA gene on the QMEC chip is necessary to normalise all functional genes for a soil sample. Measuring the 16S rRNA gene using conventional qPCR for each soil sample, with a standard curve of known gene copy numbers, would also mean QMEC relative abundance data could be converted to absolute gene abundance, and gene abundance per gram of dry soil for additional data analyses.

For the upper layer of columns, the main treatment comparison was control and amendment with manure. Interestingly, two genes responded with increased abundance in the manure treatment, *nxrA* and *emGDH*. Nitrification is the process of oxidation of ammonia through to nitrate, with the enzyme nitrite oxidoreductase (encoded by the *nxrAB* genes) facilitating the conversion of NO_2^- to NO_3^-.The application of manure to soils can provide additional nutrients that facilitate growth of plants including nitrate (Edmeades 2003). It is interesting that the greatest abundance of *nxrA* occurred particularly in soils where combinations of N, P and/or S were applied at the lower depth as well as manure at both depths (Figure 5.16). In general, these treatments also had greater mineral N concentrations in the surface 40 cm; Manure,N+S and Manure,P had significantly more mineral N than other treatments in the 10-40 cm layer and Manure,P+S and Manure,N+P had relatively large mineral N concentrations in the 0-10 cm layer (Figure 5.12). The effect of increased plant growth on stimulating manure decomposition/mineralisation by the microbial community should be investigated further for these treatments.

The abundance of acSA genes (Figure 5.15A) matched those of ureC genes (Figure 5.16C) in the subsurface layer and seem to be enhanced by N or P addition in the absence of S. The presence of S decreased abundance of these genes when manure or N and/or P nutrients were present.

The abundance of the phosphorus cycling genes phnK and phoD were generally greater in the subsurface layer when P was added (Figure 5.17) and Colwell P concentrations were also increased in that layer (Figure 5.11). The addition of manure to the 10-40 cm layer caused increased abundance of pqqC gene in that layer (Figure 5.17). The treatments receiving the addition of S decreased the abundance of phoD compared to paired treatments not receiving S (e.g. Manure,P+S compared to Manure,P). The negative influence of S on gene abundance may need further research.

The methane metabolism gene *emGDH* was more abundant where manure was applied in the upper layer in combination with lower depth applications of control or manure +S or -S. Manure is populated by methanogenic bacteria, which are anaerobic organisms that convert CO_2 to methane (Li, Zhao et al. 2021), so that may explain why microbes that utilise methanol are present. Bacteria capable of utilising methanol and dimethylsulfide, which plays a globally significant role in carbon and sulfur cycling have been described (Koch and Dahl 2018). Further study is needed to understand the links between the C and S cycles and manure amendments in the soil.

In the lower soil, the abundance of the sulfite oxidation gene, *YedZ*, was also linked with soils manured in the upper layer with different combinations of N and or P, + or -S in the lower layer. The mineralisation of organic manure in the upper layer might have released S. Given *YedZ* oxidases inorganic sulfite, and this gene which was hardly detectable in the manure treatments of the upper layer, it is more likely to be associated with enhanced S supply from soil after fertilisation. The treatments that resulted in significantly greater concentrations of available S in the 10-40 cm (Figure 5.13) had lower yedZ gene abundance.

Trungley Hall Summary

The unamended Trungley Hall soil is constrained by poor root growth into the subsurface soil (10-40 cm). In an initial glasshouse experiment, amendment with chicken manure pellets enabled improved root growth resulting in increased grain yield. The experiment reported here demonstrated that placement of N into the subsoil was able to counteract the soil constraint, increasing root growth and grain yield and N content of the plant. Placing manure into the subsurface layer without amendment to the surface soil provided no benefit to plants, a reflection of surface soil constraint. However, when the surface was amended with manure, the addition of P and N into the subsurface soil increased yield as much or more than manure alone.

Surface applied manure and fertilising with N and/or P in the subsurface layer provided biological benefit to that layer as evident in increased abundance of genes associated with C, P and N cycling. As these treatments also resulted in improved plant performance, with N also increasing root growth into the subsurface layers, the link between plant and biology may provide benefits to soil health and function beyond the initial crop. The longevity of these effects needs to be studied.

Condobolin Experiment

Plant response

When manure was applied at either 2.5 or 5 t/ha the root mass per plant was not significantly different to the untreated control (Table 5.8). Application of synthetic fertiliser as N, P or both N+P resulted in significantly less root mass compared to either the control or the equivalent manure application. This effect was evident within the surface 20 cm of the pots (Figure 5.19). There were no differences in root growth due to treatment below 20 cm. The lack of treatment response in roots below the surface 20 cm was also observed in previous experiments on the Condobolin soil.

The application of manure at 2.5 and 5 t/ha caused a significant increase in DMY relative to the untreated control (4.9 and 5.15 g/plant respectively, compared to 4.2 g/plant for the control). Fertiliser was able to match the plant response of 2.5 t/ha with the addition of N but not P. By contrast, fertiliser P was able to match the DMY gain due to manure at 5/ha but N was not.

Table 5.8. Wheat plant response to treatment on Condobolin soil. Root mass, above ground Dry Matter Yield (DMY), tiller and head number, grain yield expressed on a per plant basis. Manure is ground chicken manure pellets at 2.5 or 5 t/ha, N= nitrogen, P=phosphorous applied at rates equivalent to that applied in manure treatments. Values within columns designated with different letters are significantly different. lsd = least significant difference at p<0.05.

Treatment	Root mass (g/plant)	DMY (g/plant)	Tillers/plant	heads/plant	Grain yield (g/plant)
Control	1.71ef	4.18a	3.13	2.88	1.46a
Manure 2.5	1.86f	4.9bc	3.06	3.06	1.96cd
N2.5	1.26abc	4.54ab	3.13	3.13	1.66ab
P2.5	1.09a	4.17a	2.81	2.81	1.82bcd
N+P2.5	1.32bc	4.59abc	3.25	3.25	1.79bcd
Manure 5	1.56de	5.15c	3.77	3.77	1.97cd
N5	1.12ab	4.5ab	3.31	3.31	1.72abc
P5	1.4cd	5.1c	3.31	3.31	2.03d
N+P5	1.2abc	4.69abc	3.44	3.44	1.72abc
P value	p=0.005	0.01	ns	ns	p<0.001
lsd (p=0.05)	0.207	0.564			0.269

The grain yield data indicates that the manure 2.5 or 5 t/ha treatments significantly increased grain yield compared to the control (Table 5.8) and there was no difference in the yield of manure treatments. The grain yield of the 2.5 t/ha manure treatment could be achieved via application of P either by itself or in combination with N as N+P, however N by itself was not different to the control; this differed compared to the response of DMY. The 5 t/ha manure treatment could be achieved by either N, P or N+P, although the P alone (2.03 g/plant) was the best match of the manure treatment (1.97 g/plant). These results indicate that for the Condobolin soil, available P is the limitation to grain yield.

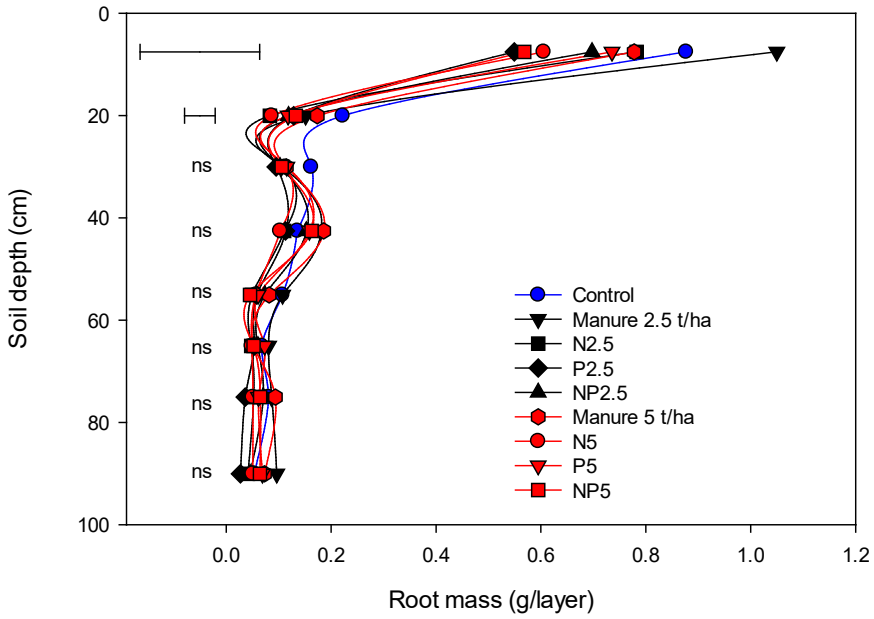


Figure 5.19. Wheat root as per sampled layer in response to amendment treatment on Condobolin soil. Manure is ground chicken manure pellets at 2.5 or 5 t/ha, N= nitrogen, P=phosphorous applied at rates equivalent to that applied in manure treatments. Horizontal bars represent least significant difference (lsd) at p<0.05. ns= no significant difference.

The nitrogen content of the straw and above ground plant was the same for all treatment with the exception of the P at a rate equivalent to 2.5 t/ha of chicken manure pellets (Table 5.9). The P only treatments tended to have less N present in the straw relative to all other treatments. Adding nutrients as manure or synthetic sources increase grain N content relative to the control with the exception of the P at a rate equivalent to 2.5 t/ha of chicken manure pellets (Table 5.9).

Table 5.9. Nitrogen content (mg N per plant) in straw, grain and the above ground plant (sum of straw and grain) in response to treatment on the Condobolin soil. Manure is ground chicken manure pellets at 2.5 or 5 t/ha, N= nitrogen, P=phosphorous applied at rates equivalent to that applied in manure treatments. Values within columns designated with different letters are significantly different. lsd = least significant difference at p<0.05.

	Nitrogen content (mg/plant)		
Treatment	Straw	Grain	Plant
Control	47.3b	49.1a	96.4b
Manure 2.5	40.4b	60.6c	101.0b
N2.5	44.1b	57.6bc	101.8b
P2.5	27.8a	53.7ab	81.5a
N+P2.5	44.2b	59.0bc	103.2b
Manure 5	43.4b	63.7c	107.1b
N5	46.4b	56.5bc	102.9b
P5	36.6ab	63.5c	100.1b
N+P5	46.8b	60.5c	107.3b
P value	0.02	0.007	0.02
lsd (p=0.05)	10.80	7.32	15.54

Soil response

Treatments receiving manure at 5 t/ha and fertiliser P at rates equivalent to that applied in manure had significantly greater Colwell P concentrations compared to the control (Figure 5.20). No significant differences in Colwell P existed in the treatments relating to 2.5 t/ha manure or fertilised equivalents. No differences in Colwell P in the soil at the time of harvest may be due to plant utilisation of Colwell P. The concentrations are lower than the critical value (35-40 mg/kg) representing a slight deficiency of Colwell P, even where P had been applied. The greater Colwell P concentrations of the treatments relating to 5 t/ha manure represent underutilisation of added P from the higher application rates. There were no differences in Colwell P in the subsurface layer as P is relative immobile in the soil profile.

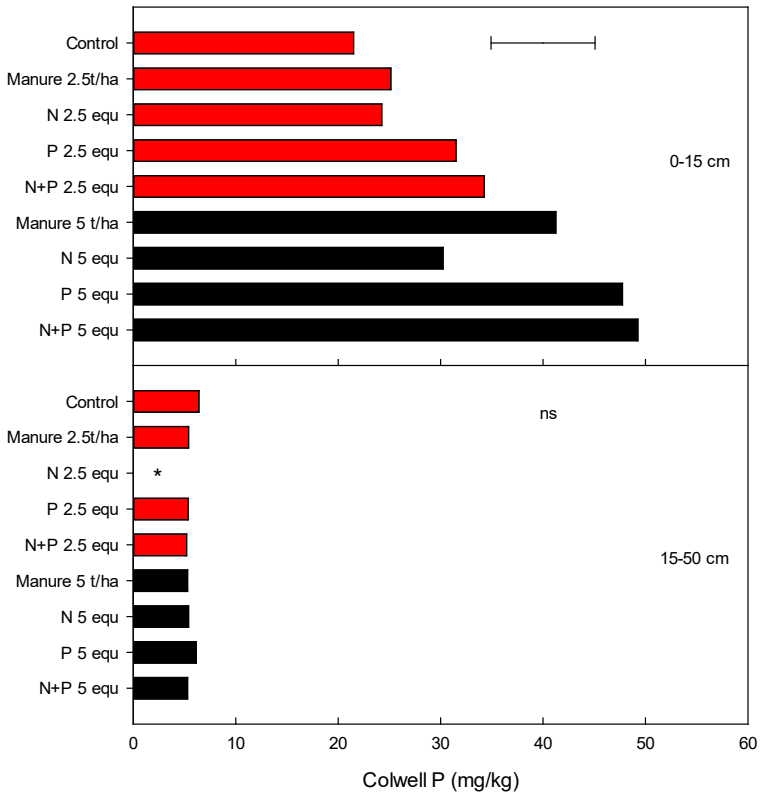


Figure 5.20. Colwell P concentration (mg/kg) of sampled layers of amendment treatment on Condobolin soil. Manure is ground chicken manure pellets at 2.5 or 5 t/ha, N= nitrogen, P=phosphorous applied at rates equivalent to that applied in manure treatments. Horizontal bars represent least significant difference (lsd) at p<0.05. ns= no significant difference.

There was no significant difference in mineral N concentrations between treatments in the treated surface layer (Figure 5.21). Variability in N concentration due to plant uptake and the mobility of nitrate N may explain the lack of statistical difference. In the subsurface (15-50 cm) layer, for the treatments relating to 2.5 t/ha, the P at an equivalent rate to 2.5 t/ha manure had significantly smaller mineral N concentrations than all other treatments relating to 2.5 t/ha applications. In the 5 t/ha suite of treatments, all treatments had significantly less mineral N present compared to the N only treatment. Given the concentration of the control and treatments relating to 2.5 t/ha, the results of the 5 t/ha suite of treatments indicate that the addition of P, from manure or fertiliser, has led to an increase in mineral N utilisation increasing N content of grain (Table 5.9). Combined application of P with N has been shown to be an effective method of enhancing soil nitrogen utilisation (Weng et al. 2021). In the absence of P, N is underutilised in the 15-50 cm layer. This is supported by the DMY data (Table 5.8) in which N only resulted in less DMY than manure or P at 5 t/ha equivalents. It is noteworthy that there appears to be no relationship between N utilisation and root mass in the 15-50 cm layer. This may be due to treatments impacting root architecture in a way that is not measured by examining root mass alone.

There were no significant differences in the concentration of sulfate-S of any treatment, in any layer, nor for the sum of sulfate-S in the pots. However, the concentration of sulfate-S did increase with depth; 6, 13, 17 mg/kg for the 0-15, 15-50, 50-100 cm layers respectively.

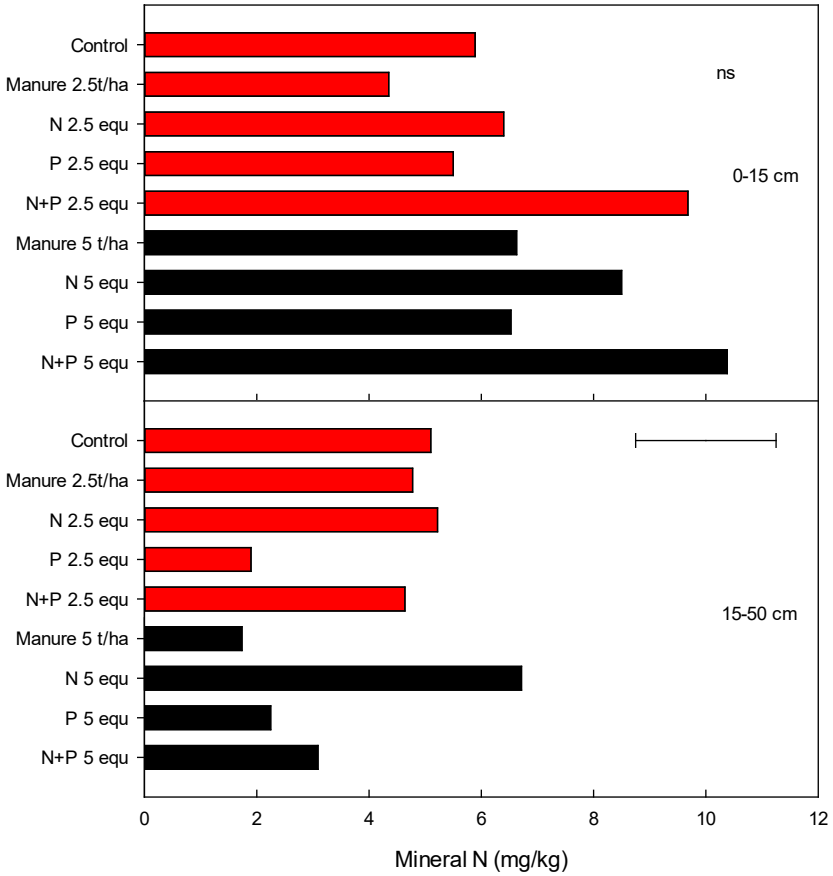


Figure 5.21. Soil mineral N, summed ammonium and nitrate concentration (mg/kg) of sampled layers of amendment treatment on Condobolin soil. Manure is ground chicken manure pellets at 2.5 or 5 t/ha, N= nitrogen, P=phosphorous applied at rates equivalent to that applied in manure treatments. Horizontal bars represent least significant difference (lsd) at p<0.05. ns= no significant difference.

Soil pH decreased significantly due to the application of N alone at the 2.5 t/ha equivalent rate (Figure 5.22) compared to the untreated control presumably due to downward movement of nitrate formed form the nitrification of hydrolysed ammonium from urea applied (Condon et al. 2004). The application of manure at 2.5 t/ha did not alter pH compared to the control whereas the 5 t/ha manure application caused a significant increase in soil pH. The addition of manure should result in short term increases in soil pH due to both the alkali present in manure and the oxidation of organic components within manure which will increase pH (Lauricella et al. 2021). However, the increase in pH due to oxidation of organic sources can be short lived (3-6 weeks) if the organic source has a substantial nitrogen component (chicken manure pellets used had >3% N, Table 5.2) which can undergo nitrification (Nguyen et al. 2018) and re-acidify the soil to near its initial soil pH value. Regardless, the resulting soil pH of the 0-15 cm layer is not sufficiently acidic to inhibit plant performance, being pH>5. The pH of the 15-50 cm layer was quite acidic and exchangeable aluminium percentage (exAl%) exceeded 5 which may harm sensitive roots, however no relationship between root mass and soil pH or exAl% existed for treatments of the 15-50 cm layer.

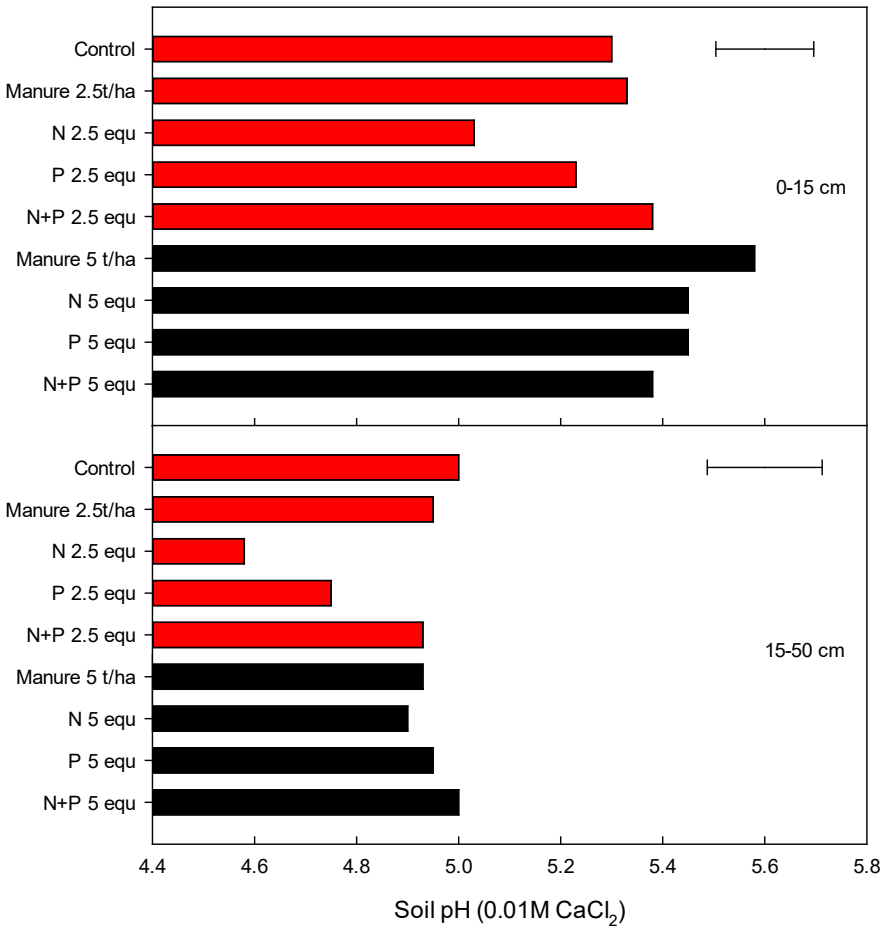


Figure 5.22. Soil pH (0.01 M $CaCl_2$) of sampled layers of amendment treatment on Condobolin soil. Manure is ground chicken manure pellets at 2.5 or 5 t/ha, N= nitrogen, P=phosphorous applied at rates equivalent to that applied in manure treatments. Horizontal bars represent least significant difference (lsd) at p<0.05. ns= no significant difference.

Condobolin Soil Summary

The glasshouse pot trial confirms the results of the initial column study conducted on the Condobolin soil in Phase 1 of the project. Plant response to organic amendment is due to nutritional benefit of the chicken manure pellets. The soil is responsive to P addition, either as fertiliser or applied in manure. The provision of adequate P nutrition improved utilisation of nitrogen and resulted in improved plant performance, relative to an untreated control.

6.Conclusion

The experiments reported here aimed to understand the mechanism(s) responsible for improved plant growth following amendment aimed to overcome known soil constraints to plant growth. Detailed glasshouse experiments were conducted on soils from Wonwondah (Vic), Trungley Hall and Condobolin (NSW) selected based on previous experiments.

The Victorian experiment demonstrated that plants (roots) enhance the effectiveness of subsoil organic matter amendments with benefits increasing with time (>12 months). However structural benefits from organic matter alone did not translate into increased plant growth. Nutrition, especially P, from organic material or synthetic sources applied to the subsoil increased plant shoot growth over the duration of the experiment. Evidence of plant/microbe interaction in phosphorus cycling existed. At the completion of the experiment available P in the soil was greater in the presence of plants compared to where no plants were grown.

The constraints to plant growth found in the Trungley Hall soil could also be overcome by an amendment addressing nutrition. Placement of N into the subsoil was able to counteract the soil constraint, increasing root growth and grain yield. Placing manure into the subsurface layer without amendment to the surface soil provided no benefit to plants, a reflection of surface soil constraint. However, when the surface was amended with manure, the addition of P and N into the subsurface soil increased yield as much or more than manure alone when applied in that layer.

Surface applied manure and fertilising with N and/or P in the subsurface layer provided biological benefit by increasing abundance of genes associated with C, P and N cycling. As these treatments also resulted in improved plant performance, with N also increasing root growth into the subsurface layers, the link between plant and soil biology may provide benefits to soil health and function beyond the initial crop.

The glasshouse pot trial conducted on the Condobolin soil confirmed that the plant response to organic amendment is due to nutritional benefit of the chicken manure pellets. The soil is responsive to P addition, either as fertiliser or applied in manure. The provision of adequate P nutrition improved utilisation of nitrogen and resulted in improved plant performance, relative to an untreated control.

Though the application of organic amendments can benefit soil structure, all three experiments conducted demonstrated that nutrition, either from organic matter or synthetic sources, was able to improve plant performance. The combination of manure application and improved nutrition in the subsurface layers provides biological benefit to the soil system that should carry over to subsequent crops.

7. Recommendations

Field investigation of the longer-term benefit of adequate subsurface/subsoil nutrition to plant performance and plant/soil biology interaction is warranted.

Field evaluation, over different seasons, of the influence of N and/or P fertiliser in subsurface layers is warranted. This should be compared with organic amendments providing the same quantity of nutrient applied.

8. Communication and dissemination

Farming Systems Groups

One field day was conducted at the field location selected by FarmLink at Trungley Hall, NSW. Farmers and advisors attended a soil pit presentation by Jason Condon.

The project progress and findings have been reported in farming system group newsletters and trial booklets.

Presentations

The project progress has been reported to the CRC via input into Program 4 reports and Jason Condon presented a zoom presentation to the CRC research committee in 2021.

The project was showcased in a Soil CRC Webinar presented by Jason Condon on the 9th November 2022. There were 117 registrations, and the YouTube recording of the webinar has been viewed 124 times to date.

Jason Condon presented a brief outline of the project to a delegation of the Soil CRC Board visiting CSU on 23/5/2022.

Jason Condon was interviewed by CRC communications officer in May 2022 for production of a CRC video on CRC projects.

Publications

Weiss, M., Condon, J., Tavakkoli, E., Whatmuff, M. and Armstrong, R. (2022) Mechanistic understanding of mode of action of soil re-engineering methods for complex chemical and physical constraints. System Solutions for Complex Problems. Eds. Bell, L. & Bhagirath, C. Proceedings of the 20th Australian Agronomy Conference, 18-22 September 2022, Toowoomba, Qld, Australia. (http://www.agronomyaustraliaproceedings.org/).

9. Acknowledgments

This research is funded by the CRC for High Performance Soils and supported by the Cooperative Research Centres program, an Australian Government initiative.

The project acknowledges the support of the Soil CRC in all aspects of funding, communication opportunities, project administration and understanding related to the many delays incurred due to COVID-enforced restrictions to travel and facilities access.

The project would not have been possible without the support of collaborators from the farming systems groups. Helen McMillan (CWFS), Eva Moffitt (FarmLink), Cameron Taylor (BCG), Sarah Noack (HART), Dr Cassie Schefe (Riverine Plains) who all helped with the soil prioritisation process and site selection. Sarah Clarry (FarmLink), Michelle Pardy (Riverine plains) helped ensure farmers were kept up to date with findings of the project experiments.

The experiments were conducted with the technical support of Mr Michael Weiss (Ag Victoria) and Mr Chick Chilby (NSW DPI) and Mr Richard Lowrie (NSW DPI).

10. References

Armstrong, et al. (2015). Improving grain yields on a sodic clay soil in a temperate, medium-rainfall cropping environment. *Crop & Pasture Science* **66**, 492–505.

Baldock, J.A., Aoyama, M., Oades, J.M., Susanto, Grant, C.D. (1994). Structural amelioration of a South Australian red-brown earth using calcium and organic amendments. *Aust. J. Soil Res*. **32**, 571-94.

Bennett, J., McL. Cattle, S.R., Singh, B., Quilty, J.R, (2015). Influence of gypsum enhanced chicken manure and wheat-straw compost on amelioration of an irrigated sodic brown vertisol – laboratory experiment. *Arid Land Research and Management* **29**:4, 415-431.

Better Fertilisers Decisions for Cropping (2022), Downloaded from https://bfdc.com.au/ 1/7/2022.

Qing-Fang Bi, Bing-Jie Jin, Dong Zhu, Yu-Gen Jiang, Bang-Xiao Zheng, Patrick O'Connor, Xiao-Ru Yang, Andreas Richter, Xian-Yong Lin, Yong-Guan Zhu (2021). How can fertilization regimes and durations shape earthworm gut microbiota in a long-term field experiment? *Ecotoxicology and Environmental Safety* **224**: 112643.

Blaud, A., Memon, M., van der Zaan, B., Lair, G.J., Banwart, S. (2016). Effects of dry and wet sieving of soil on identification and interpretation of microbial community composition. *Advances in Agronomy* **142**, 119-142.

Bowen, G.D. (1973). Mineral nutrition of Ectomycorrhizae. In Ectomycorrhizae: Their Ecology and Physiology. Eds. G.C. Marks and T.T. Kozlowski. pp 151-205. Academic Press, New York.

Braunberger, P.G., Miller, M.H., Peterson, R.L. (1991). Effect of phosphorus nutrition on morphological characteristics of vesicular–arbuscular mycorrhizal colonization of maize. *New Phytologist* **119**, 107–114.

Qing-Lin Chen, Jing Ding, Chao-Yu Li, Zhen-Zhen Yan, Ji-Zheng He, Hang-Wei Hu (2020). Microbial functional attributes, rather than taxonomic attributes, drive top soil respiration, nitrification and denitrification processes. *Science of The Total Environment* **734**: 139479.

Chorom, M., Rengasamy, P., Murray, R. (1994). Clay dispersion as influenced by pH and net particle charge of sodic soils. *Soil Research* **32**, 1243–1252.

Clark, G.J., Sale, P.W.G., Tang, C. (2009). Organic amendments initiate the formation and stabilisation of macroaggregates in a high clay sodic soil. *Australian Journal of Soil Research* **47**, 770–780.

Condon, J.R., Black, A.S., Conyers, M.K. (2004). The role of N transformations in the formation of acidic subsurface layers in stock urine patches. *Australian Journal of Soil Research* **42**. 221-230.

Cozzolino, V., Di Meo, V., Piccolo, A. (2013). Impact of arbuscular mycorrhizal fungi applications on maize production and soil phosphorus availability. *Journal of Geochemical Exploration* **129**, 40-44.

Edmeades, D.C. (2003). The long-term effects of manures and fertilisers on soil productivity and quality: A review. *Nutrient Cycling in Agroecosystems* **66**(2): 165-180.

Facelli, E., Facelli, J.M., Smith, S.E., McLaughlin, M.J. (1999). Interactive effects of arbuscular mycorrhizal symbiosis, intraspecific competition and resource availability on Trifolium subterraneum cv. Mt. Barker. *New Phytol*. **141**, 535–547.

Gill, J.S., Sale, P., Peries, R., Tang, Caixian (2009). Changes in soil physical properties and crop root growth in dense sodic subsoil following incorporation of organic amendments. *Field Crops Research* **114**, 137–146.

Heanes, D.L. (1984) Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. *Communications in Soil Science and Plant Analysis* **15**, 1191–1213.

Koch, T., Dahl, C. (2018). A novel bacterial sulfur oxidation pathway provides a new link between the cycles of organic and inorganic sulfur compounds. *The ISME Journal* **12**(10): 2479-2491.

Lauricella, D., Butterly, C.R., Weng, Z., Clark, G.J., Sale, P.W.G., Li G., Tang C. (2021). Impact of novel materials on alkalinity movement down acid soil profiles when combined with lime. *J. Soils Sediments* **21**, 52–62.

Looft, T., Johnson, T.A., Allen, H.K., Stanton, T.B. (2012). In-feed antibiotic effects on the swine intestinal microbiome. *Proc. Natl. Acad. Sci. U S A* **109**(5): 1691-1696.

Oksanen, J.A.R.I., Guillaume Blanchet, F., Friendly, M.Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. Wagner, H. (2022). Vegan: Community Ecology Package, R package.

Oster, J.D., Jayawardane, N.S. (1998). Agricultural Management of Sodic Soils. In Sodic Soils: Distribution, Properties, Management, and Environmental Consequences; Sumner, M.E., Naidu, R., Eds.; Oxford University Press: New York, NY, USA, pp. 125–147.

Nguyen, H.H., Moroni, S., Condon, J., Zander, A., Li, G. (2018) Surface incorporation of lucerne pellets and lime alleviates subsurface soil acidity under field conditions. *National Soils Conference. Canberra 2018* Proceedings. pp. 387-388.

Rengasamy, P., Olsson, K.A. (1991). Sodicity and soil structure. *Australian Journal of Soil Research* **29**, 935–952.

Rosendahl, R.O. (1942). The effect of mycorrhizal and nonmycorrhizal fungi on the availability of difficultly soluble potassium and phosphorus. *Soil Sci. Soc. Am. Proc*. **7**, 477-479.

Sale, P.W.G., Gilkes, R.J., Bolland, M.D.A., Simpson, P.G., Lewis, D.C., Ratkowsky, D.A., Gilbert, M.A., Garden, D.L., Cayley, J.W.D., Johnson, D. (1997). The agronomic effectiveness of reactive phosphate rocks 1. Effect of the pasture environment. *Australian Journal of Experimental Agriculture* **37**, 921-936.

Shuailin Li, S. Li, Yongxing Cui, Y. Cui, Zhuqing Xia, Z. Xia, Xinhui Zhang, X. Zhang, Mengmeng Zhu, M. Zhu, Yun Gao, Y. Gao, Siyu An, S. An, Wantai Yu, W. Yu, Qiang Ma, Q. Ma. (2022). The mechanism of the dose effect of straw on soil respiration: Evidence from enzymatic stoichiometry and functional genes. *Soil biology and biochemistry* **168**, 108636.

Smith, S.E., Smith, F.A., Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol* **133**, 16–20.

Team, R.C. (2014). R: a language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.

Wang, W.J., Sale, P.W.G., Hayden, H., Tang, C., Clark G., Armstrong, R. (2020). Plant roots and deep-banded nutrient-rich amendments influence aggregation and dispersion in a dispersive clay subsoil. *Soil Biology and Biochemistry* **141**, 107664.

Weng, Z., Butterly, C.R., Sale, P., Li G., Caixian Tang (2021). Combined nitrate and phosphorus application promotes rhizosphere alkalization and nitrogen uptake by wheat but not canola in acid subsoils. *J. Soils Sediments* **21**, 2995–3006.

Jiannan Xiao, Shikui Dong, Hao Shen, Shuai Li, Kelly Wessell, Shiliang Liu, Wei Li, Yangliu Zhi, Zhiyuan Mu, Hongbo Li (2022). N addition overwhelmed the effects of P addition on the soil C, N, and P cycling genes in alpine meadow of the Qinghai-Tibetan Plateau. *Frontiers in Plant Science* **13**.

Yu Li, Jing Zhao, Janneke Krooneman, Gert Jan Willem Euverink (2021). Strategies to boost anaerobic digestion performance of cow manure: Laboratory achievements and their full-scale application potential. *Science of The Total Environment* **755**: 142940.

Bangxiao Zheng, Yongguan Zhu, Jordi Sardans, Josep Peñuelas, Jianqiang Su (2018). QMEC: a tool for high-throughput quantitative assessment of microbial functional potential in C, N, P, and S biogeochemical cycling. *Science China Life Sciences* **61**(12): 1451-1462.

Zhu, Y., Marchuk, A., Bennett, J. (2016). Rapid method for assessment of soil structural stability by turbidimeter. *Soil Science Society of America Journal*. **80**. 10.2136/sssaj2016.07.0222.

Appendix 1. Collation of Initial Soil Chemical Properties of Field Sampled Soil Used in Project Experiments

Site Name	Depth From (cm)	pH (1:5 Water)	pH (1:5 $CaCl_2$)	EC (1:5) (dS/m)	Chloride (mg/kg)	Nitrate Nitrogen (mg/kg)	Ammonium Nitrogen (mg/kg)	Phosphorus - Colwell (mg/kg)	Phosphorus Buffer Index - Colwell (mg/kg)	Copper (DTPA) (mg/kg)	Iron (DTPA) (mg/kg)	Manganese (DTPA) (mg/kg)	Zinc (DTPA) (mg/kg)	Boron (mg/kg)	Sulfur (KCl40) (mg/kg)	Organic Carbon (%)	Organic Matter (%)	Aluminium ($BaCl/NH_4Cl$) (cmol/kg)	Calcium ($BaCl/NH_4Cl$) (cmol/kg)	Potassium ($BaCl/NH_4Cl$) (cmol/kg)	Magnesium ($BaCl/NH_4Cl$) (cmol/kg)	Sodium ($BaCl/NH_4Cl$) (cmol/kg)	CEC ($BaCl/NH_4Cl$) (cmol/kg)	Aluminium % of Cations ($BaCl/NH_4Cl$) (%)	ESP% (%)	Ca:Mg
Condobolin	0-15	5.9	5.2	0.13	28	55	4	39	58	0.89	14	72	0.69	0.87	5.6	1.11	1.9	<0.10	8.5	2.3	1.7	0.038	13	0.77	0.29	5.0
	15-50	5.8	4.9	0.05	<10	4.7	0.72	5.3	110	0.32	5.4	6.4	0.03	0.74	12	0.46	0.79	0.11	6	0.9	1.4	0.052	8.5	1.3	0.61	4.3
	50-80	5.4	4.4	0.03	<10	1.9	0.94	<5.0	140	0.22	4.5	3.4	0	0.9	16	0.22	0.38	0.51	3.7	0.36	3.2	0.074	7.8	6.5	0.95	1.2
Oaklands	0-10	5.9	5.3	0.3	86	81	15	66	96	2.6	100	78	0.97	1.4	36	1.44	2.5	<0.10	8.5	1.7	6.2	1.4	18	0.56	7.8	1.4
	10-50	8.2	6.7	0.1	22.0	2.9	1.1	5.2	120.0	2.3	30.0	22.0	0.1	2.9	11.7	0.4	0.8	<0.10	9.2	0.7	11.7	4.3	26.0	0.4	16.3	0.8
	50-80	9.2	8.1	0.215	16.5	2.45	0.725	<5.0	88.5	1.55	13.5	3.9	0.1	5.3	14	0.24	0.335	<0.10	10	0.755	13.5	5	29.5	0.34	17	0.7
Trungley	0-10	6.5	5.6	0.14	27	20	5	50	47	0.48	77	15	1	0.53	34	0.76	1.3	<0.10	6	0.74	2.2	1	10	1	10	2.7
	10-40	8.6	7.2	0.12	30	8.25	1.55	9.25	65.5	0.63	25.5	4.55	0.63	1.22	9.7	0.275	0.47	<0.10	6	0.46	7.1	3.15	16.5	0.615	19	0.8
	40-100	9.55	8.6	0.43	205	2.1	0.92	7.3	60	0.84	10.7	2.15	0.13	1.85	51	<0.15	0.26	<0.10	5.75	0.71	10.05	7	23.5	0.43	29.5	0.6
Marrabel	0-20	6.7	6.1	0.19	30	9.3	3.8	61	50	0.72	100	14	3.1	0.92	110	1.14	2	<0.10	5.5	0.24	0.3	0.26	6.4	1.6	4.1	18.3
	20-60	6.7	5.7	0.12	20	10	1.1	8.3	80	0.86	30	5.4	0.5	1.6	38	0.27	0.46	<0.10	3.8	0.28	2.4	0.87	7.5	1.3	12	1.6
	60-100	9.5	8.5	0.34	25	5.8	0.9	<5.0	94	0.7	7.3	0.52	0.34	8.8	44	<0.15	0.26	<0.10	7.5	0.84	11	5.7	25	0.4	23	0.7
Birchip	0-20	8.1	7	0.1	14	11	2.2	20	67	0.73	7	10	1.1	2.8	7.7	0.89	1.5	<0.10	15	1.6	4.4	1.5	23	0.43	6.5	3.4
	20-60	9.7	8.7	0.44	150	4.9	0.98	5.6	120	0.82	5.8	2.2	0.37	21	43	0.32	0.55	<0.10	10	1.1	11	9.1	31	0.32	29	0.9
	60-100	9.3	8.7	1.35	770	4.7	0.81	5.8	110	0.84	6.6	2.8	0.51	23	430	0.19	0.33	<0.10	9.5	1.2	9.9	14	35	0.29	40	1.0
Wonwondah	0-20	5.9	4.7	0.07	27	5.1	2.4	46	39	0.13	200	4.9	1.2	0.62	4.9	1.05	1.8	0.23	3.1	0.25	2.1	0.51	6.15	3.8	8.3	1.5
	20-60	8.2	6.8	0.15	58	1.1	1.5	9.6	35	0.039	26	1.2	0.45	2.5	16	0.22	0.38	<0.10	4.1	0.27	6.5	2.4	13.3	<1	18	0.63
	60-100	9.6	8.7	0.56	320	1.1	0.96	6.5	45	0.092	8.9	0.34	0.26	5.1	72	<0.15	0.26	<0.10	9.5	0.34	8.6	5.4	23.8	<1	23	1.1

Note: exchangeable cations for Wonwondah soil were analysed using ammonium acetate extraction.

Supplementary Table S1 Primers used on Wafergen high throughput quantitative PCR chip (Zheng et al. 2018)

Primer pair No.	Gene name	Encoded protein	Cycle	Function	Predicted length (bp)	Forward primer	Reverse primer	Reference
1	*abfA*	α-L-arabinofuranosidase	C degradation	Hemicellulose hydrolysis	349	AbfA-F	AbfA-R	Zheng *et al.*, 2018
2	*accA*	acetyl-CoA carboxylase carboxyltransferase α subunit	C fixation	C fixation	284	AccA-F	AccA-R	Zheng *et al.*, 2018
3	*aclB*	ATP-citrate lyase β subunit	C fixation	C fixation	333	892F	1204R	Campbell *et al.*, 2003
4	*acsA*	acetyl-coenzyme A synthetase	C fixation	C fixation	331	AcsA-F	AcsA-R	Zheng *et al.*, 2018
5	*acsB*	acetyl-CoA synthase complex β subunit	C fixation	C fixation	420	ACSF1	ACSR1	Gagen *et al.*, 2010
6	*acsE*	5-methyltetrahydrofolate corrinoid methyltransferase	C fixation	C fixation	331	acsE-F	acsE-R	Zheng *et al.*, 2018
7	*amoA1*	ammonia monooxygenase α subunit (Archaea)	N Cycling	Nitrification	635	Arch-amoAF	Arch-amoAR	Francis *et al.*, 2005
8	*amoA2*	ammonia monooxygenase α subunit (Bacteria)	N Cycling	Nitrification	490	amoA-1F	amoA-2R	Rotthauwe *et al.*, 1997
9	*amoB*	ammonia monooxygenase β subunit	N Cycling	Nitrification	501	amoBMf	amoBMr	Calvo & Garcia-Gil, 2004
10	*amyA*	α-amylase	C degradation	Starch hydrolysis	316	AmyA-F	AmyA-R	Zheng *et al.*, 2018
11	*amyX*	pullulanase	C degradation	Starch hydrolysis	365	AmyX-F	AmyX-R	Zheng *et al.*, 2018
12	*apsA*	adenosine-5'-phosphosulfate reductase α subunit	S Cycling	S reduction	279	APS7-F	RH2-aps-R	Ben-Dov *et al.*, 2007

Primer pair No.	Gene name	Encoded protein	Cycle	Function	Predicted length (bp)	Forward primer	Reverse primer	Reference
13	*apu*	amylopullulanase	C degradation	Starch hydrolysis	287	Apu-F	Apu-R	Zheng *et al.*, 2018
14	*bpp*	β-propeller phytase	P Cycling	Organic P mineralisation	160-200	BPP-F	BPP-R	Huang *et al.*, 2009
15	*cdaR*	carbohydrate diacid regulon transcriptional regulator	C fixation	C fixation	433	CdaR-F	CdaR-R	Zheng *et al.*, 2018
16	*cdh*	cellobiose dehydrogenase	C degradation	Cellulose hydrolysis	130	Cdh-F	Cdh-R	Zheng *et al.*, 2018
17	*cex*	exoglucanase	C degradation	Cellulose hydrolysis	380	Cex-F	Cex-R	Zheng *et al.*, 2018
18	*chiA*	endochitinase	C degradation	Chitin hydrolysis	300	ChiA-F	ChiA-R	Zheng *et al.*, 2018
19	*cphy*	cysteine phytase	P Cycling	Organic P mineralisation	380-400	Cphy-F	Cphy-R	Huang *et al.*, 2011
20	*dsrA*	sulfite reductase α subunit	S Cycling	S reduction	222	DSR1F	RH3-dsr-R	Ben-Dov *et al.*, 2007
21	*dsrB*	sulfite reductase β subunit	S Cycling	S reduction	390	DSRp2060F	DSR4R	Geets *et al.*, 2006
22	*exo-chi*	exochitinase	C degradation	Chitin hydrolysis	400-430	ExoChi-F	ExoChi-R	Zheng *et al.*, 2018
23	*frdA*	fumarate reductase flavoprotein subunit	C fixation	C fixation	302	FrdA-F	FrdA-R	Zheng *et al.*, 2018
24	*gcd*	quinoprotein glucose dehydrogenase	P Cycling	Inorganic P solubilisation	300	Gcd-F	Gcd-R	Zheng *et al.*, 2018
25	*gdhA*	glutamate dehydrogenase	N Cycling	Organic N mineralisation	240	GdhA-F	GdhA-R	Zheng *et al.*, 2018

Primer pair No.	Gene name	Encoded protein	Cycle	Function	Predicted length (bp)	Forward primer	Reverse primer	Reference
26	*glx*	glyoxal oxidase	C degradation	Lignin hydrolysis	312	Glx-F	Glx-R	Zheng *et al.*, 2018
27	*hao*	hydroxylamine oxidoreductase	N Cycling	Nitrification	218	hao/hzo_ cl2aF1	hao/hzo_ cl2aR1	Nunoura *et al.*, 2013
28	*hzo*	hydrazine oxidase	N Cycling	Anaerobic ammonium oxidation	224	HzoQPCR1F	HzoQPCR1R	Long *et al.*, 2013
29	*hzsA*	hydrazine synthase α subunit	N Cycling	Anaerobic ammonium oxidation	260	hzsA_1597F	hzsA_1857R	Shen *et al.*, 2013
30	*hzsB*	hydrazine synthase β subunit			381	HSBeta296F	HSBeta742R	Wang *et al.*, 2012
31	*iso-plu*	Isopullulanase	C degradation	Starch hydrolysis	540	Ipu-F	Ipu-R	Zheng *et al.*, 2018
32	*korA*	2-oxoglutarate ferredoxin oxidoreductase α subunit	C fixation	C fixation	252	KorA-F	KorA-R	Zheng *et al.*, 2018
33	*lig*	lignin peroxidase	C degradation	Lignin hydrolysis	243	Lig-F	Lig-R	Zheng *et al.*, 2018
34	*manB*	β- mannanase	C degradation	Hemicellulose hydrolysis	323	ManB-F	ManB-R	Zheng *et al.*, 2018
35	*mct*	mesaconyl-CoA C1-C4 CoA transferase	C fixation	C fixation	320	Mct-F	Mct-R	Zheng *et al.*, 2018
36	*mcrA*	methyl-coenzyme M reductase α subunit	C fixation	C fixation	450	McrA-F	McrA-R	Steinberg & Regan, 2008
37	*mmoX*	methane monooxygenase component A alpha chain	Methane metabolism	Methane oxidation	350	MmoX-F	MmoX-R	Zheng *et al.*, 2018

Primer pair No.	Gene name	Encoded protein	Cycle	Function	Predicted length (bp)	Forward primer	Reverse primer	Reference
38	*mnp*	manganese peroxidase	C degradation	Lignin hydrolysis	294	MnP-F	MnP-R	Zheng *et al.*, 2018
39	*mxaF*	methanol dehydrogenase (cytochrome c) subunit 1	Methane metabolism	Methane production	560	f1003	r1561	McDonald & Murrell, 1997
40	*naglu*	α-N-acetylglucosaminidase	C degradation	Cellulose hydrolysis	217	Naglu-F	Naglu-R	Zheng *et al.*, 2018
41	*napA*	periplasmic nitrate reductase	N Cycling	Dissimilatory N reduction	490	napAf1	napAr1	Feng *et al.*, 2011)
42	*narG*	nitrate reductase α chain	N Cycling	Denitrification	110	1960m2F	2050m2R	Lopez-Gutierrez *et al.*, 2004
43	*nasA*	assimilatory nitrate reductase catalytic subunit	N Cycling	Assimilatory N reduction	750-800	nas964	nasA1735	Allen *et al.*, 2001
44	*nifH*	nitrogenase iron protein	N Cycling	N fixation	400	nifHF	nifHRb	Rosch & Bothe, 2005
45	*nirK1*	nitrite reductase (NO-forming)	N Cycling	Denitrification	514	nirK1F	nirK5R	Braker *et al.*, 1998
46	*nirK2*	nitrite reductase (NO-forming)	N Cycling	Denitrification	450	nirKC1F	nirKC1R	Wei *et al.*, 2015
47	*nirK3*	nitrite reductase (NO-forming)	N Cycling	Denitrification	400	nirKC2F	nirKC2R	Wei *et al.*, 2015
48	*nirS1*	nitrite reductase (NO-forming)	N Cycling	Denitrification	425	nirScd3AF	nirSR3cd	Jung *et al.*, 2011
49	*nirS2*	nitrite reductase (NO-forming)	N Cycling	Denitrification	400	nirSC1F	nirSC1R	Wei *et al.*, 2015
50	*nirS3*	nitrite reductase (NO-forming)	N Cycling	Denitrification	400	nirSC2F	nirSC2R	Wei *et al.*, 2015
51	*nosZ1*	nitrous-oxide reductase	N Cycling	Denitrification	267	nosZ2F	nosZ2R	Henry *et al.*, 2006

Primer pair No.	Gene name	Encoded protein	Cycle	Function	Predicted length (bp)	Forward primer	Reverse primer	Reference
52	*nosZ2*	nitrous-oxide reductase	N Cycling	Denitrification	454	nosZ-F	nosZ1622R	Throback *et al.*, 2004
53	*nxrA*	nitrite oxidoreductase α subunit	N Cycling	Nitrification	322	F1370-F1	F2843-R2	Wertz *et al.*, 2008
54	*pccA*	acetyl/propionyl-CoA carboxylase alpha	C fixation	C fixation	413	PccA-F	PccA-R	Zheng *et al.*, 2018
55	*pgu*	pectinase/polygalacturonase			380	Pgu-F	Pgu-R	Zheng *et al.*, 2018
56	*phnK*	phosphonate transport system ATP-binding protein	P Cycling	Organic P mineralisation	366	PhnK-F	PhnK-R	Zheng *et al.*, 2018
57	*phoD*	alkaline phosphatase D	P Cycling	Organic P mineralisation	370	ALPS-F730	ALPS-R1101	Sakurai *et al.*, 2008
58	*phoX*	alkaline phosphatase/Pho regulon	P Cycling	Organic P mineralisation	600	phoX2-F	phoX2-R	Sebastian & Ammerman 2009
59	*pqq-mdh*	methanol/ethanol family PQQ-dependent dehydrogenase	Methane metabolism	Methane production	293	Mdh-F	Mdh-R	Zheng *et al.*, 2018
60	*pmoA*	methane/ammonia monooxygenase subunit A	Methane metabolism	Methane oxidation	531	A189	A682	Holmes *et al.*, 1995
61	*pox*	phenol oxidase	C degradation	Lignin hydrolysis	155	Pox-F	Pox-R	Zheng *et al.*, 2018
62	*ppk*	polyphosphate kinase	P Cycling	Inorganic P biosynthesis	296	Ppk-F	Ppk-R	Zheng *et al.*, 2018
63	*ppx*	exopolyphosphatase	P Cycling	Inorganic P hydrolysis	310	Ppx-F	Ppx-R	This study

Primer pair No.	Gene name	Encoded protein	Cycle	Function	Predicted length (bp)	Forward primer	Reverse primer	Reference
64	*pqqC*	pyrroloquinoline-quinone synthase	P Cycling	Inorganic P solubilisation	300	PqqC-F	PqqC-R	Zheng *et al.*, 2017
65	*rbcL*	ribulose-bisphosphate carboxylase large chain	C fixation	C fixation	272	rbbLR1F	rbbLR1intR	Selesi *et al.*, 2007
66	*sga*	glucoamylase	C degradation	Starch hydrolysis	375	Sga-F	Sga-R	Zheng *et al.*, 2018
67	*smtA*	succinyl-CoA:(S)- malate CoA transferase	C fixation	C fixation	340	SmtA-F	SmtA-R	Zheng *et al.*, 2018
68	*soxY*	sulfur-oxidising protein	S Cycling	S oxidation	329	SoxY-F	SoxY-R	Zheng *et al.*, 2018
69	*ureC*	urease	N Cycling	Ammonification	340	ureC-F	ureC-R	Koper *et al.*, 2004
70	*xylA*	xylose isomerase	C degradation	Hemicellulose hydrolysis	464	XylA-F	XylA-R	Zheng *et al.*, 2018
71	*yedZ*	sulfite oxidase	S Cycling	S oxidation	291	YedZ-F	YedZ-R	Zheng *et al.*, 2018
72	*16S*	Bacterial ribosomal RNA gene sequence (reference gene)			393	F515	R907	Zhou *et al.*, 2011

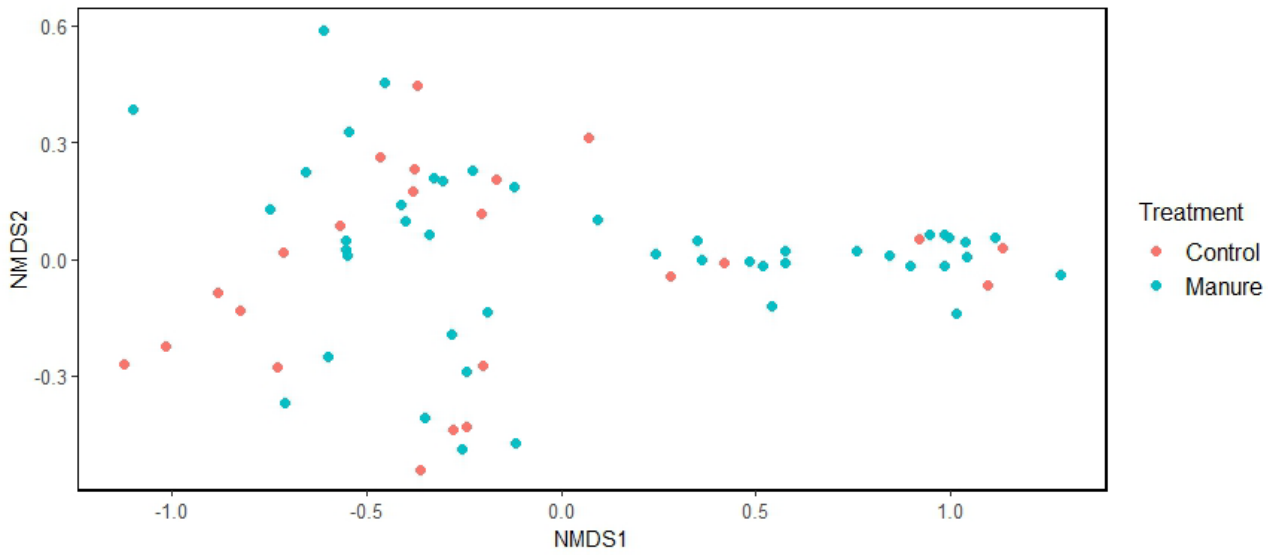


Figure S1 NMDS ordination of soil samples from the upper depth of the column (n=60), shown by the treatment applied in this layer. Stress = 0.046.

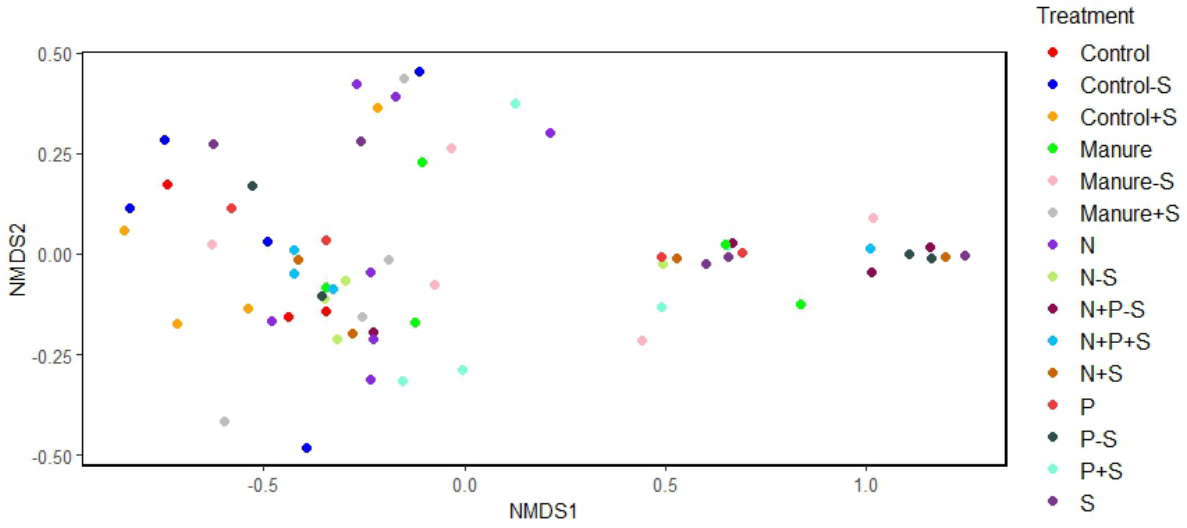


Figure S2 NMDS ordination of soil samples from the lower depth of the column (n=60), shown by the treatment applied in this layer. Stress = 0.062.