Rural R&D for Profit Program

Waste to Revenue: Novel Fertilisers and Feeds

Final Report

Australian Pork Limited

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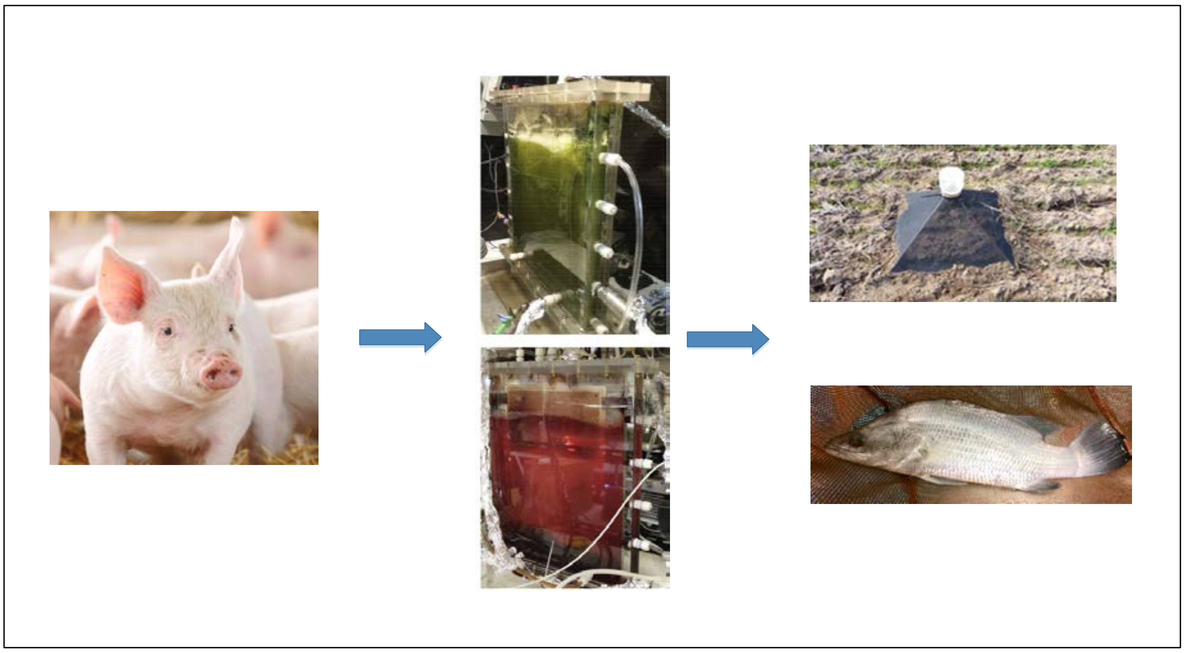

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Note: the department will publish the final report on its website but *<u>will not</u>* *publish the appendices.*

Turning waste into novel fertilisers and feeds

Agricultural industries produce large volumes of waste containing valuable nutrients, carbon and water. Unfortunately, this waste doesn't have the right nutrient balance to be directly used as a crop fertiliser. It is typically diluted with moisture, making transportation and reuse off-farm impractical and unfeasible. Food crops don't use the nitrogen in fertilisers very efficiently and there are significant losses in the process of converting food protein into meat protein. These are widely recognised issues, with the majority of carbon, and almost all nitrogen and phosphorus lost in emissions or waste streams. This project addressed these issues by converting agricultural waste and wastewater into novel feeds and fertilisers demonstrating reduced farming inputs and decreasing costs of primary production.

Benefits to producers

As a result of this project, producers will benefit through:

- New treatment technologies that treat wastewater whilst producing a high-value microbial biomass which offer commercial and revenue opportunities
- Some waste-derived by-products can be used as partial fertilizers, reducing the input costs and increasing fertiliser use efficiency by up to 40%. Other by-products could be used as soil conditioners to ameliorate the soil constraints on yield through; increased pH, C and N retention in soils, resilience to water and heat stress reducing the need for soil amelioration practices (such as liming or adding clay) that can cost approximately $124/ha/year.
- The amended Biosecurity & Agricultural Management Act 2007, now permits WA farmers to apply poultry litter/compost in broad acre agriculture in previously banned Shires. This is a major cost saving as the industry currently loses up to $8/m^3 in removal costs.
- The semi-arid soils of WA were low emitters of nitrous oxide (N_2O) and a major sink of methane when amended with manures and composts. Methane uptake could lead to significant reductions in GHG emissions by negating on-farm GHG emissions. This mitigation methodology could be included as Australian carbon credit units (ACCUs) issued under the Emissions Reduction Fund, currently trading at between $13-15 per tCO2-e of emissions abated.

Objectives

The project aimed to:

- develop new waste treatment technologies using algae and purple phototrophic bacteria (PPB) to recapture nutrients in waste as feed and fertiliser products;
- evaluate the nutritive, economic and agronomic value (e.g. increased soil biology and quality, crop productivity, fertiliser use efficiency) of new waste-derived products against alternatives; and
- overcome some of the key barriers to adoption by involving primary producers and regulators during field trials to assist early adoption.

Methods and Outputs

The project developed technologies for treating and reusing agricultural waste between 2015 and 2018.

- *Novel waste processing technology work* - Wastewater treatment technologies were developed and tested using photobioreactors to treat wastewater whilst producing high-value microbial biomass.
- *Novel feed* - Microbial biomass produced from synthetic wastewater was tested using bacterial reactors. The biomass was then used as a bulk fishmeal replacement in feed trials with barramundi.
- N*ovel fertilizers work* - The biological, economic and agronomic value of alternative fertilizer products derived from wastes was tested and greenhouse gas (GHG) emissions and stable fly emergence (pest fly species) quantified

Outcomes

- *Novel wastewater treatment technology* - We have shown that it is feasible to treat real wastewaters from agricultural industries with the PPB continuous wastewater treatment system and photobioreactor technology.
- *Novel feeds* - This project tests the bulk replacement of fishmeal with PPB biomass, specifically for barramundi. When we consider savings on wastewater treatment by instead treating with PPB, up to 1400 USD $tonne^{-1}$ PPB product could be saved. This results in a net production cost of 200 USD $tonne^{-1}$ PPB. However, we note that this needs to be confirmed in a demonstration plant.
- *Novel fertilizers* - A number of novel fertiliser products have been developed and tested, with three of them, specifically Black Stable Fly (BSF) frass, microalgae and advanced compost, performing as well as synthetic fertiliser in pot trials. A three-year field trial showed there were increased financial gains in terms of increased productivity and reduced input costs by applying beef compost with synthetic fertiliser at the reduced rate of 60%, without affecting crop yield.
- The research outcomes were used to negotiate and amend the *Biosecurity & Agricultural Management Act 2007*. As a result, poultry litter/compost can now be applied to broad acre agriculture in previously banned Shires in WA. This is a major cost saving to WA producers ($4 million/yr.).
- Applying compost to dairy pastures in WA led to improved shoot growth and P uptake in pasture soils under water stressed conditions and was more effective than clay additions. Currently, the average cost of soil amelioration practices such as clay and lime amendments are about $41/ha and $124/ha/year (annualised value). Thus, applying compost has a benefit: cost ratio of 3:1 and a net benefit of about $83/ha/year.
- Applying chicken compost to soils, raised soil pH, and improved biological function, nutrient retention and nutrient availability in field trials. This could partially overcome current constraints of grain production in WA.
- Overall, semi-arid soils of WA amended with manures and composts are low emitters of nitrous oxide (N2O) and a significant methane (CH4) sink when amended with manures. Methane uptake was greatest in soils amended with composted manures (pig, beef and dairy) with the exception of chicken manure that performed better when untreated. Methane uptake could lead to significant reduction in GHG emissions by negating on-farm N2O and CH4 emissions.

Abbreviations and glossary

C:N ratio	Carbon to nitrogen ratio
CH_4	Methane gas
CO_2	Carbon dioxide gas
COD	Chemical oxygen demand, a measure of chemical energy (also tCOD for total fraction, or sCOD for soluble/filtered fraction)
GHG	Greenhouse gas
Microcosm	An artificial, simplified ecosystem, in this case of soil, used to emulate under controlled conditions, the behaviour in natural soils
Heterotroph	In this case, a microorganism that cannot produce its own food and instead uses carbon in wastewater and oxygen or nitrate as an electron acceptor.
Autotroph	In this case, a microorganism capable of producing its own food from CO2 and inorganic nutrients using light.
PPB	Purple phototrophic bacteria
UWA	The University of Western Australia
UQ	The University of Queensland
WA	Western Australia (A state in Australia) or Western Australian
HRT	Hydraulic retention time
N_2	Nitrogen gas
N_2O	Nitrous oxide
NH_3	Free ammonia
QLD	Queensland, A state in Australia
SRT	Solids retention time
NH_4Cl	Ammonium chloride
TS	Total solids
TSS	Total suspended solids
VFAs	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids

1 Project rationale and objectives

Agricultural industries produce large volumes of waste containing valuable nutrients, carbon and water. Unfortunately, this waste is nutritionally unbalanced for agricultural crop growth, and typically diluted with moisture, making transportation and reuse off-farm impractical and unfeasible. Poor conversion of nitrogen fertilizers into food crops and losses in the conversion of food protein into meat protein, are widely recognised issues, with the majority of carbon, and almost all nitrogen and phosphorus lost in emissions or waste streams. This project addressed these issues by converting agricultural waste and wastewater into novel feeds and fertilisers. By doing this, the carbon and nutrients in wastes and wastewater are not lost, but instead become valuable products for sale or use, generating new revenue streams, reducing farming inputs and decreasing costs of primary production. The project specifically:

- developed new waste treatment technologies that use algae and purple phototrophic bacteria (PPB) to recapture nutrients in waste as feed and fertiliser products;
- evaluated nutritive, economic and agronomic value (e.g. increased soil biology and quality, crop productivity, fertiliser use efficiency) of new waste derived products against other alternatives;
- overcame some of the key barriers to adoption by involving primary producers and regulators during field trials to assist early adoption.

The new wastewater treatment technologies use assimilative microorganisms, namely microalgae and PPB, to convert organic matter, nitrogen and other nutrients into consistent bioavailable microbial biomass. The wastewater treatment thereby removes nutrients and carbon, and in this way simultaneously treats the wastewater. The wastewater provides the nutrients and carbon for producing the microbial biomass, in this way reducing production costs for the microbial biomass. Other key issues include the cost and added complexity of harvesting, separating the microbial biomass from the wastewater matrix, and up-concentrating and drying the biomass for applications.

The microbial biomass has a high protein content suitable for use as intensive animal feed (Ponsano et al., 2004). Microalgae can be a source of protein-rich feed additive for humans as well as animals (cattle, sheep, swine, poultry and fish). Food safety has been assessed in several nutritional, digestive and toxicological studies (Becker, 2004). Thousands of tons of algae are already produced every year. Of particular interest, is the reality that many agro-industrial waste streams, e.g. meat processing facilities (pig, poultry, cattle), are already providing viable sources of commercial protein meal (e.g. meat and bone meal, blood meal, poultry by-product meal, fish meal). The microbial biomass is also rich in nutrients suitable as a renewable novel fertilizer. For example, microalgae biomass produced from animal wastes, specifically from piggery farms, as a nitrogen (N) fertilizer is comparable to commercial fertilizers (Mulbry et al., 2005) and using the microalgae biomass instead of untreated animal wastes helps to sequester CO_2 and reduce greenhouse emissions (Wang et al., 2015).

Although some microalgae species can grow photoheterotrophically, meaning they harness chemical carbon and light for growth, most are photoautotrophs and synthesise organic carbon from inorganic carbon. In high-strength wastewater, up to 50% of the inorganic carbon utilised by algae is provided by heterotrophic bacteria that symbiotically use oxygen produced by the algae. Unfortunately, the performance of microalgal systems is commonly adversely affected by contamination, culture crashes and overall low productivities, with a key example documented in a 10-year, comprehensive study in Singapore (Taiganides, 1992a). When treating wastewater, the culture in open ponds is usually comprised of a mixture of algae, bacteria, zooplankton and detritus, referred to as "ALBAZOD" (Maazouzi et al., 2008) which will reduce the product consistency. However, the consortium simultaneously removes chemical energy, N and phosphorus (P) very efficiently, with up to 90% or more removal extents (de Godos et al., 2009).

In contrast, PPB can directly assimilate chemical carbon in wastewater, with energy for growth coming from infrared light instead of from organic matter. Most of the research to date with PPB has focussed on removal of chemical energy (measured as chemical oxygen demand or COD) from synthetic or dilute wastewaters, and there have been very limited real wastewater studies (Azad et al., 2004; Prachanurak et al., 2014). This is a key technical risk for PPB wastewater treatment because (1) agricultural industrial wastewaters and wastes are complex and high-strength, and (2) agricultural industrial wastewaters at single sites vary widely over time and (3) treatment performance may vary between different wastewaters. Most prior studies have reported encouragingly high microbial biomass yields and high protein contents, but the PPB technology should be tested on real high-strength wastewaters from agricultural industries.

Algal and PPB waste treatment and nutrient recapture technology may be more suitable for medium to large enterprises, whereas the Black soldier fly technology and mobile aerated floor (MAF) composting systems may provide more flexibility for smaller enterprises. MAF converts waste organic matter into high quality soil improvers that are easier to transport and handle. The system requires minimal space and operational management making it potentially attractive to producers. The aeration system combined with minimal disturbance can reduce greenhouse gas (GHG) emissions compared to traditional composting methods (Biala et al., 2016). Black Soldier Fly (BSF) (*Hermetia illucens*) converts waste by-products into a high protein and lipid source that can be utilization in both fish and animal feed formulations (Schiavone et al., 2017; Wang & Shelomi, 2017). The BSF reproduces rapidly, has high feed conversion efficiency, and can be reared on a range of waste streams, especially animal wastes (Moula et al., 2018). The BSF castings (frass) were of particular interest in this project, because of a high N content making it suitable for fertiliser production.

Evaluating the agronomic and economic value of alternative fertilisers, such as the above-mentioned waste-derived products, is key to increasing adoption. It is essential to provide products tailored to crop nutrient requirements, machinery and operations. Adding organic matter to soil has been shown to increase soil quality and crop performance (Jenkins et al., 2009), and to improve the soil's resilience to heat stress and drought (Mickan et al., 2018). Furthermore, Australia's organic produce is a growing market, with the domestic consumer market predicted to be worth $2 billion by the end of 2018 according to the Australian Horticulture Annual Review February 2018 - Rural Bank. Waste-derived fertilisers can play a key role in providing nutrients to this market.

Agricultural semi-arid soils of Western Australia (WA) are characterised by poor soil structure coupled with low soil fertility, organic matter and carbon content (Hoyle et al., 2013). This makes WA soils susceptible to soil water repellence, soil acidity, soil compaction and herbicide resistance (M. Roper et al., 2015; Scanlan et al., 2017). In particular, subsoil acidity has been identified as the most significant economic impact. It is a major yield-limiting constraint, affecting 8.5 million hectares of cropping soils in the south-west with an estimated cost of at least $1.6 million/year in lost production potential. Consequently, the adoption of soil amelioration practices such as adding compost as a soil improver could address these soil constraints and improve crop productivity across the Western cropping region. In the Eastern States of Australia, yield gains of up to 80 per cent are attainable when organic amendments are applied to address soil constraints (especially acidity) (Celestina et al., 2018).

Figure 1 Compost prepared via mobile aerated floor system

Unfortunately, high costs associated with transporting and applying organic amendments mean that there has been limited uptake by growers in both the East and West. This is a key barrier to adoption. The project addresses this barrier by waste treatment that converts farm by-products into concentrated fertiliser products (frass, algae, PPB) and balanced soil conditioners (including composts, pellets, granulates). These products can be more readily transported and are likely to see adoption if agronomic and economic value in broadacre agriculture can also be demonstrated by the project.

The Western Australian Broiler growing industry produces large quantities of spent broiler litter every year, long recognized to be an important soil amendment. Unfortunately, poor management practices in the past have led to stringent application restrictions through Health (Poultry Manure) Regulations 2001 and more recently through the Biosecurity and Agriculture Management Act 2007 (BAM Act). These have required manure disposal at significant cost (> 4 million). This was considered a major barrier to adoption of waste-derived soil amendments, and the project sought to overcome this barrier by quantifying true impacts of broiler litter applications, specifically the emergence of Stable Flies.

2 Method and project locations

The project investigated new waste treatment technologies to improve sustainability, productivity and profitability of primary industries, and explored the conversion of low-value agricultural waste products into innovative fertilisers and feeds to create new markets for primary industries and to decrease primary production costs. The project also sought to overcome key barriers to adoption of novel fertilizers by engaging with regulatory bodies via project activities.

Novel waste processing technology work developed and tested novel wastewater treatment technologies, treating wastewater whilst producing high-value microbial biomass. This was done at (a) small laboratory scale, (b) larger scale laboratory continuous mode, and (c) large pilot scale at a partner site, all using real wastewaters from agricultural industries.

The *novel feed work* tested microbial biomass produced from synthetic wastewater, as a bulk fishmeal replacement in feed trials with barramundi. The fish growth performance and carcass composition were measured, and commercial feasibility was explored.

The *novel fertilizers work* tested in laboratory screening trials, glasshouse trials, and a 3-year field trial, the economic and agronomic value of alternative fertilizer products derived from wastes. Greenhouse gas (GHG) emissions were also quantified.

2.1 Novel Waste Processing

The activities in this section tests novel processing technologies that turn nutrients and carbon in agricultural industrial wastewaters into high-protein high-value products, whilst simultaneously treating the wastewaters. This was done at small laboratory batch scale, then at larger laboratory pilot scale and finally at pilot-scale on-site at an agricultural industrial site.

2.1.1 Small-scale Batch Proof-of-Concept Testing (UQ, St Lucia, Queensland)

Wastewaters

Real wastewaters for the batch tests were provided by five different agricultural industrial sectors in Australia, namely pork (Western Australia), poultry processing (Queensland), dairy processing (Tasmania), sugar milling (Queensland) and red meat processing (New South Wales). The sample locations and sample handling details are given elsewhere by Hülsen et al. (2018a). The samples were analysed at the UQ St Lucia laboratories in Brisbane, Queensland. The results obtained from these batch tests are expected to be translatable to treatment of wastewaters from other similar sites to those sampled in this study.

Inoculum

Purple phototrophic bacteria (PPB) inoculum was enriched on domestic wastewater as described elsewhere (Hülsen et al., 2014) and following enrichment, the PPB cultures were incubated in the absence of oxygen (anaerobic) and using light energy (phototrophic) to build microbial biomass, as described by Hülsen et al. (2018a). For inoculation of the batch tests, the PPB inoculum culture was pre-concentrated by centrifuging, which enhances settling and separation of solids under an enhanced gravity field. The concentrated PPB inoculum was analysed for physicochemical and microbial composition as described in Section 2.1.4.

Microalgae inoculum was prepared by first isolating a strain from effluent evaporation ponds at a piggery in South Australia. It was then enriched and grown as described by Hülsen et al. (2018a). This culture comprised of a native *Chlorella vulgaris* and a *Scenedesmus* species, frequently used for wastewater treatment. Prior to inoculation of the batch tests, the microalgae were pre-concentrated as for the PPB inoculum and analysed for physicochemical and microbial composition as described in Section 2.1.4.

Batch growth tests

The batch tests examined the treatment of the various agricultural industrial wastewater by growing microalgae or PPB that remove and sequester carbon and nutrients present in the wastewater. The tests were performed in batch in small serum flasks (160 mL), inoculated either with PPB inoculum or microalgae inoculum and filled with a respective wastewater, as described by Hülsen et al. (2018a). The PPB test flasks were made anaerobic by flushing the headspace once with N_2 and then sealing the flask with a gastight rubber septum. The microalgae tests were open to the atmosphere. The flasks were illuminated with fluorescent lamps and the PPB flasks were covered with UV-VIS absorbing foil. All the flasks were continuously shaken in an orbital shaker and heated to and kept at 30°C. Wastewater composition was measured for samples collected from each flask over time as described in Section 2.1.4. For each wastewater test, illuminated and non-illuminated controls were also performed in parallel with no added inoculum, to show net effects of added inoculum and the net effects of illumination. The method and results for these control tests have been described by Hülsen et al. (2018a). Samples of the original inoculum and each test flask contents were collected for analysis of the initial and grown microbial composition. DNA was extracted from these samples after which 16S Amplicon sequencing was performed using an Illumina Miseq Platform as described in Section 2.1.4. Data processing of the sequencing results were as described by Hülsen et al. (2018a).

2.1.2 Larger continuous system testing (UQ, St Lucia, Queensland)

Wastewater

Batches of around 60 L wastewater were collected monthly as grab samples from a poultry-processing facility in Brisbane, Australia. The wastewater was a combination of process water from feather removal and degutting, as well as general cleaning water (e.g. from cleaning of floors). The sample locations and sample handling details are given elsewhere by Hülsen et al. (2018b). After collection, the wastewater was transported in jerry cans at room temperature (<30 min transport time) and when received at the UQ, St Lucia laboratories. The wastewater was immediately stored in a cold room at 4°C. From this location, wastewater was pumped on a weekly basis to a stirred influent tank in the laboratory after discarding residues from a previous week of operation. A 1 mm sieve was used at the suction side of the influent pipe to prevent clogging of a pump and piping with solids. This was likely due to discharge of different wastewater streams such as process water from feather removal and degutting at different operational times, as well as differences in cleaning water amounts. Such changes in wastewater composition and strength are typical of poultry-processing facilities. These variations in wastewater strength were useful to test the robustness of the waste treatment technology.

Apparatus

Based on the results of the batch testing in Section 2.1.1, a continuous wastewater treatment system was designed, built and operated. For this system, a membrane configuration was selected, because it permits tight control of biomass product concentration and produces a high quality treated wastewater. This is done by drawing treated wastewater through a membrane filter (in this case an A4 sized Kubota flat sheet membrane), which retains any particulate matter in the system whilst allowing treated wastewater to pass through the membrane as a filtrate free of particulate matter.

Two systems were operated in parallel as photobioreactors (Figure 2), with one being illuminated with IR light using LED Illuminator to grow PPB (termed photo anaerobic membrane bioreactor or PAnMBR) and the other the other was illuminated with a full spectra fluorescence lamp to grow microalgae (termed photo aerobic membrane bioreactor or PAMBR). Illumination intensity was measured on the outside of the reactor wall using a spectroradiometer and further quantified as described by Hülsen et al. (2018b).

The PAnMBR (PPB based) was continuously mixed by recirculating headspace gas with a vacuum pump via a condensate trap and an air stone at the bottom of the bioreactor. The PAnMBR was maintained at a pressure of 1.1 kPa. This gas recycling also functioned to scour the membrane and inner reactor wall clean, to minimize biofilm attachment and clogging. The PAMBR (microalgae based) was instead open to atmosphere and air was supplied by an air compressor to mix the bioreactor and to supply CO_2 and O_2 for microalgae and heterotrophic growth.

Both bioreactors were continuously fed with poultry processing wastewater and the draw-off via the membrane was controlled by a pressure sensor operating as a level switch to turn the effluent pump on or off to draw treated wastewater through the membrane. Both bioreactors were operated at room temperature averaging 22 °C for the PAMBR and 24 °C for the PAnMBR. The pH and temperature of the reactors were continuously measured and recorded.

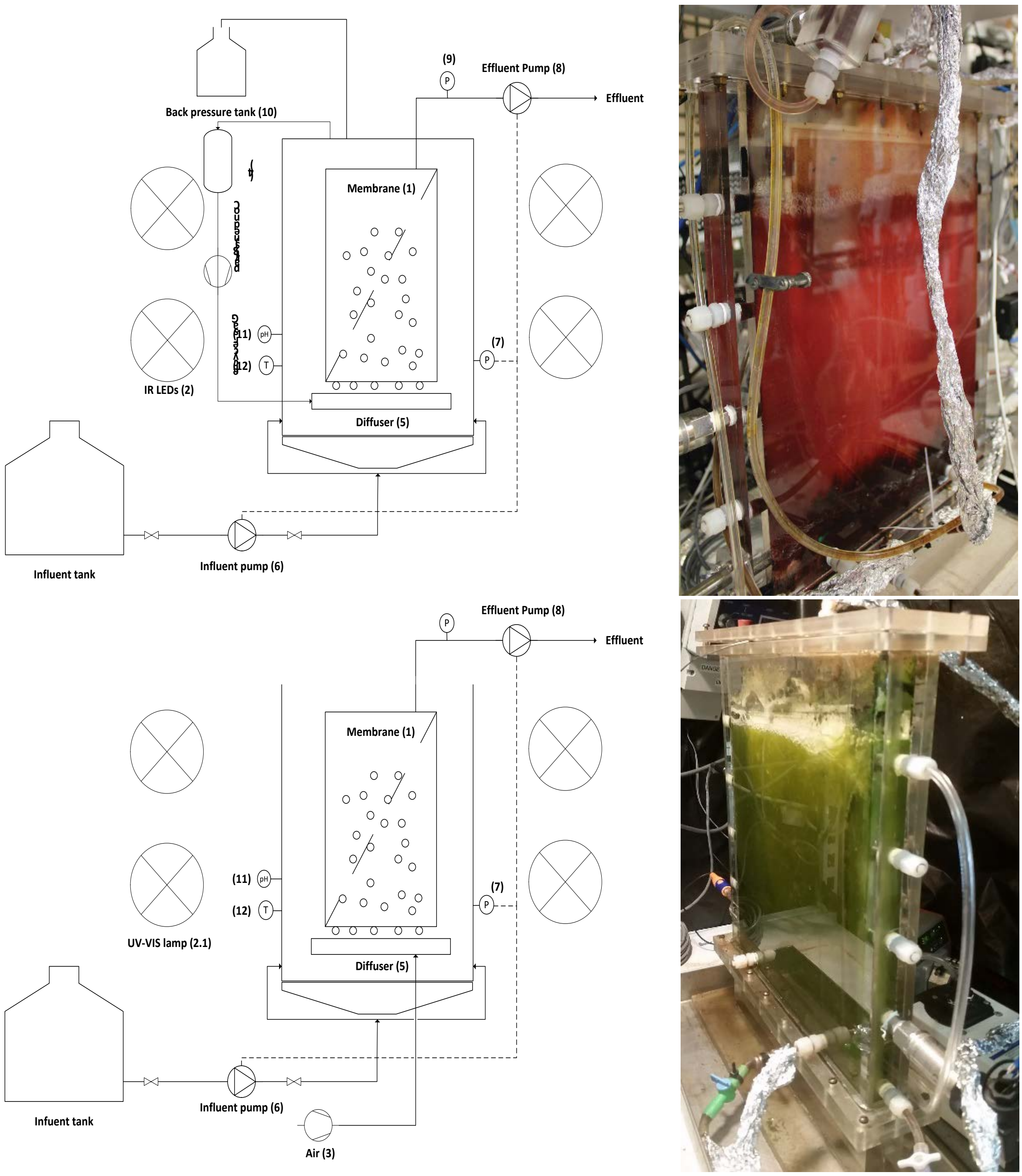

Figure 2. Schematics and photos of the laboratory-scale continuous wastewater treatment systems; (Top) PAnMBR growing PPB; (Bottom) PAMBR with microalgae and heterotrophs.

Inoculum and Operation

The PAnBR was inoculated with PPB from a domestic wastewater enrichment culture as described elsewhere (Hulsen et al., 2016; Hülsen et al., 2018b). The PABR was inoculated with microalgae isolated from an effluent evaporation pond at a piggery in South Australia and enriched and adapted as described by Hulsen et al. (Hulsen et al., 2016; Hülsen et al., 2018b) (Figure 3).

Figure 3. Enrichment cultures of purple phototrophic bacteria (red colour, on left) and microalgae (green colour on right).

The bioreactors were operated for 243 days, split into five periods of operation based on the operating hydraulic retention time (HRT = bioreactor volume/influent flow rate) and general differences in wastewater composition. Contents were regularly withdrawn from each bioreactor to control the solids concentration at a reasonable level to allow on-going PPB and microalgae growth, because if solids concentration becomes too high, light attenuation becomes excessive and impedes phototrophic growth. The volumetric loading rates of both reactors were from 2.0 to 3.0 g tCOD $L^{-1}.d^{-1}$ during Periods I-III and peaked during Period IV at around 9.3 g tCOD $L^{-1}.d^{-1}$. Further details of operating conditions are described elsewhere (Hülsen et al., 2018b).

Approximately once per week, the biofilm from the membrane and walls of each bioreactor was removed and re-suspended in the bioreactor contents. The bioreactors were routinely sampled from the middle of each bioreactor, and these samples were assumed to be representative of the well-mixed contents in each respective bioreactor. Membrane filtrate was also regularly sampled. These samples were analysed for tCOD, TKN, TP, NH_4-N,NO_x-N, NO_2-N, PO_4-P, Sulfur species, metals, VFAs, microbial composition, protein content and amino acid profile, using the methods in Section 2.1.4. For total characteristics, the samples were analysed as is. For soluble characteristics the samples were filtered at 0.45μm (Milipore, Darmstadt, Germany) and the filtrate was analysed for sCOD, NH_4-N, PO_4-P, VFAs, and filtered elemental analysis via ICP, pH, TSS/VSS) (See Section 2.1.4). To determine losses and recovery of carbon and nutrients in the biomass product, and to quantify the effectiveness of the wastewater treatment, cumulative mass balances were performed on tCOD, TN, TP, K and magnesium (Mg), as described elsewhere (Hülsen et al., 2018b).

2.1.3 Piggery Pilot Testing and Development (UQ Gatton Piggery, QLD)

The activities described in this section aimed to use attached growth to separate the biomass product from the wastewater particulates and produce a high quality concentrated biomass. In addition, this was to be done at a relevant scale and at an agricultural industrial site to demonstrate the concept for applications. The design of the pilot system was based on earlier testing of a 1000L field pilot attached growth system in domestic wastewater, which was funded by the Water Sensitive Cities CRC and the Smart Water Fund. The artificial energy input for illumination of the domestic wastewater pilot was cost-intensive. Consequently, in the present work filtered sunlight was used instead to improve the energy efficiency and reduce operational costs. No membrane was used in this pilot system. Instead, the wastewater contents in the system was drained batch-wise, followed by harvesting of the biomass separate to the wastewater.

The batch tests (Section 2.1.1) and continuous bioreactor testing (Section 2.1.2) provided an important proof-of–concept of wastewater treatment by growing of phototrophic biomass. However, in a full-scale system it would not be trivial to separate the desired biomass product from the undesired particulates present in the wastewater. In fact, the bioreactor testing used suspended growth (Section 2.1.2) with continuous mixing of the biomass product with the undesired particulates in the wastewater, making the biomass product diluted and potentially compromising product quality. The attached growth mode bypasses these problems and allows for consistent biomass quality almost independent from the wastewater source,

Apparatus

The pilot PPB growth system was constructed and operated in an open field (27°33'20.5"S, 152°20'28.1"E) at UQ's Gatton piggery, which is a conventional farrow-to-wean piggery with slatted floors (depth of underfloor drain pits 1-1.5m). The system consisted of six custom made acrylic flat-plate, flow-through cartridges (dimensions: two of 1×1×0.1 m, two of 1×1×0.08 m, and two of 1×1×0.06 m, with 0.015 m wall thickness) with detachable lids (fastened via six clasps). The top 0.1 m of each cartridge was freeboard (Figure 4). The cartridge was connected via multiple drain ports at the base of each cartridge to a single manual drain valve for each cartridge located below the cartridge frame. The upstream connection tapped off the side of the cartridge near the top and connected to 25 mm PVC manual ball valves.

The upstream and downstream connections used 25 mm ID food grade, braid reinforced PVC tubing. The tanks were illuminated only with sunlight filtered for IR-radiation using black UV-VIS foil. Temperature (℃) and dissolved oxygen (mg/L) of the liquid contents in each cartridge were continuously measured with a pre-calibrated sensor.

Operation, sampling and measurements

Wastewater was periodically pumped from the underfloor pits in a grower shed of the piggery into a 1 m^3 high density polyethylene intermediate bulk container (IBC) located near the cartridges. The wastewater was then left to settle in the IBC for about 30 minutes before pumping the supernatant into the cartridges at 25 $L.min^{-1}$. The wastewater was typical of a commercial piggery operation, containing faeces, urine, wash water, undigested feed, and other gritty particulates (e.g. pig hair, sand). PPB readily grow on dissolved carbon, and particulate matter caused some interim problems with pump and blockages. For these reasons, the wastewater was usually pumped from near the top of the standing liquid level in the underfloor pits, to not draw in the large quantity of settled solids mass that was present at the base of these pits. The narrow edge of each cartridge faced North-South, so that the sun shone on the large outer cartridge faces early in the morning and late in the afternoon.

Once PPB growth was observed on the walls of the cartridges, multiple batch operations proceeded, which involved;

1. draining the cartridges by gravity into an existing effluent well onsite, whilst collecting approximately 30% of each cartridge liquid volume in a separate bucket. pH was measured during this step using a pre-calibrated HI 83141 portable pH meter (Hanna Instruments);
2. harvesting the PPB manually by scraping the cartridge walls with a rubber Squeegee wiping upwards;
3. collecting the PPB biomass, which was then weighed with HT-3000 Compact Scale (Labtek) and placed on ice for transport;
4. adding the liquid collected from the previous batch into the respective cartridge; and
5. refilling the cartridge with fresh wastewater.

Each cartridge was mixed daily with a pulse/pause setting between 05:00 and 18:00 by recirculating contents at 25 $L.min^{-1}$ from a side port via a mono pump and back into the cartridge via four drain ports at the base. Outside of these hours, the cartridges were stagnant.

Figure 4. Schematic of flat-plate flow-through PPB reactor tank, (middle) a photo of the set-up prior to commissioning at the UQ Gatton piggery, and (bottom) a photo of the PPB biomass grown and harvested.

Wastewater samples were collected before each cartridge was drained (treated wastewater), after each cartridge was refilled (untreated wastewater), and periodically from the IBC. These samples and the collected biomass were placed on ice for transport to the UQ St Lucia laboratory where they were stored at -2°C until analysis. The wastewater samples were analysed as is for total characteristics (tCOD, unfiltered TKN, unfiltered TP and unfiltered elemental analysis using ICP). For soluble characteristics the samples were filtered at 0.45µm (Milipore, Darmstadt, Germany) and the filtrate was analysed for sCOD, NH_4-N, PO_4-P, VFAs, and filtered elemental analysis via ICP, pH, TSS/VSS) (See Section 2.1.4). Wet harvested biomass was analysed as is for TS, VS, tCOD, TKN, TP, and elemental analysis by ICP (See Section 2.1.4).

2.1.4 Analytical methods

COD was determined according to Standard Methods 5220D with potassium dichromate in sulfuric acid (colorimetric) using Merck Spectroquant® COD cell tests (114560, 114540, 114541 and 114555 AD). Volatile fatty acids (VFA) were analysed after pre-filtering sample with a 0.45 µm membrane filter followed by gas chromatography equipped with a flame ionisation detector and a polar capillary column. NH_4-N, NO_x-N, NO_2-N and PO_4-P were determined by a Lachat QuickChem800 Flow Injection Analyser. NH_4-N and PO_4-P were also analysed with test kits (Merck, 114752 and 114848). Soluble and total Kjeldahl nitrogen (TKN) and TP were determined using sulfuric acid, potassium sulfate and copper sulfate catalyst in a block digester followed by analysis as for NH_4-N above. Dissolved sulfide, sulfate, thiosulfate and sulfite concentrations were measured by ion chromatography (IC). Elemental analysis was performed by inductively coupled plasma optical emission spectrometry (ICP) after 10% nitric acid digestion. Protein content was estimated by calculation based on NH_4-N and TKN contents (Eding et al., 2006) and determined separately using the bicinchoninic acid assay (Walker, 1994). Amino acid profiles were externally analysed by the Australian Proteome Analysis Facility (Sydney, Australia) using a Waters Acquity UPLC. Carbohydrates were determined by the sulphuric phenol method (Nielsen, 2010) using D-glucose as standard. Total solids (TS) and volatile solids (VS) were determined by Standard Methods (APHA, 2005). Total suspended solids (TSS) and Volatile suspended solids (VSS) were determined as for VS and TS, after glass fibre filtration, also in accordance with standard methods (APHA, 2005). Further details on analytical methods have been provided by Hülsen et al. (2018a; 2018b).

For microbial community analysis, genomic DNA was extracted from the samples by FastSpin for Soil Kit (MP-Biomedicals) according to the manufacturer's protocol. DNA of each sample was provided to Australian Centre for Ecogenomics (ACE) for 16S Amplicon sequencing by Illumina Miseq Platform, using 926F (5'-AAACTYAAAKGAATTGACGG-3') and 1392wR (5'-ACGGGCGGTGWGTRC-3') primer sets (Engelbrektson et al., 2010). The results from this analysis were processed as described by Hülsen et al. (2018a; 2018b), providing microbial composition data at the genus level.

2.2 Novel Feeds

The activities described in this section tested as an aquaculture feed, the PPB biomass produced by technologies in Section 2.1. The description given in this section is an excerpt taken from a full journal paper prepared for this study (Delamare-Deboutteville et al., 2018). The co-authors of this journal paper (Delamare-Deboutteville et al., 2018) are acknowledged and thanked for their willingness to include some details of the study below.

2.2.1 Fish feed trials (UQ St Lucia Campus)

This work was carried out under approval granted by the University of Queensland Native and Exotic Wildlife and Marine Animals (NEWMA) Ethics Committee (permit SBS/130/17).

PPB feed

A 1000L (800L working volume) photo anaerobic bioreactor was inoculated with PPB enriched on domestic wastewater (Hülsen et al., 2014) and was then used to grow enough PPB for the fish feeding trial. This bioreactor was a rectangular clear acrylic tank with 100 sealed, hollow acrylic tubes connected to the lid of the tank and submerged into the reactor liquid. Each tube contained a rigid light bar with 126 infrared LEDs to irradiate the bioreactor from the inside-out. The bioreactor was mixed via a conical bottom with two vacuum pumps recycling headspace gas back to the base of the tank. To fulfil the requirements of the ethics approval, the PPB used for the fish trial had to be grown on synthetic wastewater, which consisted of Ormerod medium (Ormerod et al., 1961) with the following modifications: biotin, $(NH_4)_2SO_4$ and malic acid substituted with: yeast extract (0.015 $mg.L^{-1}$), NH_4Cl (50 $mg.L^{-1}$) and acetic acid (500 mg $COD.L^{-1}$). The pH of the medium was set to 6.5. The PPB biomass was harvested batch-wise by removing each illumination tube and manually scraping the biofilm into a bucket (Figure 5). The harvested biomass was weighed, TS and VS determined as described in Section 2.1.4, and then stored in at -20°C before air drying in a commercial food dryer at 45°C (about two days). The dried biomass was further processed in a benchtop mixer-blender to produce a fine homogenous powder. A total of 2.2 kg of dried biomass was grown and prepared in this way on synthetic wastewater.

The PPB was analysed for proteins, fats, carbohydrates, ash content, amino acids, total carotenoids, total chlorophyll and poly-β-hydroxybutyrate (PHB) using the methods described below, and for metals, TKN and TP using the methods described in Section 2.1.4. Pigment analysis (bacteriochlorophyll and total carotenoids) was performed by freezing a sub-sample overnight at -80^{o}C, and thawing to 4^{o}C and weighing a small sub-sample (<7 mg) before pigments were extracted with acetone and methanol at a 7:2 ratio while sonicating on ice for 10 minutes (modified from Bóna-Lovász et al. (2013); Van der Rest and Gingras (1974)). This extraction was repeated until a colourless pellet was obtained. The extracted pigments were then measured by absorbance at 475 nm for total carotenoids, at 771 nm for bacteriochlorophyll in the PPB sample extracts and 750 nm for bacteriochlorophyll in mixed diet sample extracts, using the method described by (Van der Rest & Gingras, 1974).

Figure 5. Manual harvesting and processing of purple phototrophic bacteria (PPB) from illumination tubes, and the resulting biomass at different stages of processing, in preparation for the fish feeding trial.

Gross energy was determined by bomb calorimetry or calculated based on (Garling Jr & Wilson, 1976) crude protein content estimated from nitrogen content as described in Section 2.1.4. Total lipids were determined by acid hydrolysis followed by extraction in a soxhlet apparatus. Ash content was determined gravimetrically at the same time as TS and VS, as described in Section 2.1.4. These analyses were performed by Symbio laboratories in Eight Mile Plains, QLD, 4113). Amino acid analysis from PPB grown on the synthetic wastewater was also performed by the same laboratories. Other amino acid profiles were performed by Ridley Aquafeed (Narangba, Australia) using ultra-high-performance liquid chromatography. Microbial community composition was determined by amplicon sequencing as described in Section 2.1.4.

Diet formulation and preparation

Proximate analysis (protein, fat, ash, gross energy and amino acid profile) enabled formulation of nutritionally complete diets, balanced on an equal protein and energy basis and using standard raw materials of commercial barramundi feeds (Ridley Aquafeeds, Australia). Experimental diets were prepared with 30% fish meal (control), or with substitution of 33%, 67% or 100% of the fish meal with PPB. Adjustments were made in other components to maintain crude protein, phosphorous, and lipid levels. The mixture was prepared, pressed into mince strands, placed on stainless steel mesh trays and steamed at 100% relative humidity and 105°C for 2 minutes. Steamed strands were then dried overnight at 65°C and broken by hand and pulse-blended five times for 1 second each. To produce 1.5-2 mm pellets, the resulting mixed pellets were sieved through a 2 mm sieve shaker and the composition of each diet was determined afterwards to confirm formulation levels.

Fish husbandry and analysis

Juvenile barramundi (n = 540) of approximately 25 mm were sourced from Mainstream Aquaculture hatchery in Melbourne, Victoria, airfreighted to UQ St Lucia. The fish trial was performed in an eighteen 70 L tanks recirculating system using twelve tanks (Figure 6A). Each tank had its individual aeration, all tanks were connected to a 260 L sump fitted with two 300 μm socks (Figure 6B) to removed most organic matter from the incoming water before reaching a biological filter. A heater/chiller unit maintained constant water temperature at 28 +/- 1°C (Figure 6C). Water was then pumped to a pressurised micro bead filter, which served as a mechanical and biological filter (Figure 6D). Dissolved oxygen was maintained at >5 mg L^{-1} by means of an air compressor and in-tank air stones. Other water quality characteristics (ammonia, nitrite, nitrate, pH, and total hardness) were regularly checked and water exchanges performed to maintain favourable growth and health conditions.

After a three weeks acclimatisation on a commercial feed, fish were starved for 24 hours and then separated by size, with larger fish 6.11 g. $fish^{-1}$ being randomly allocated to four tanks (one per diet), and medium sized fish 4.23 g.$fish^{-1}$ being randomly allocated to 8 tanks (2 per diet). Each diet was randomly assigned to two tanks with medium fish and one tank with large fish in a balanced block design. Fish were then reared over 47 days with weight and length changes measured for individual fish, and fish losses expressed as percentage survival. Further details on feeding strategies were described elsewhere (Delamare-Deboutteville et al., 2018). All the diets were formulated to equivalent protein and energy levels. However, to isolate any particular effects caused by PPB protein, feed was provided at a reduced proportion from estimated requirements based on the diet with the lowest feed intake (usually 100% PPB diet 4).

Feed intake was recorded on a daily basis for each tank based on weight of feed provided, minus any uneaten feed collected, dried and weighed. Feed conversion ratio (FCR) was then calculated as the ratio of feed intake (dry weight basis) to fish biomass gain (live weight-gain) and weight gain was calculated from initial and final weights. At the end of the feeding trial (day 47) all the fish were humanely killed by overdose with the anaesthetic AQUI-S, prior to weight and length measurements. Five fish from each tank were then patted dry with paper towels to remove excess water and then frozen whole for total carcass composition analysis. The whole, frozen fish were passed twice through a mincer twice and a sample was taken for TS analysis and another sample freeze dried for 72 h at -106°C and 0.2 kPa for subsequent chemical analysis using the methods in Section 2.1.4 above.

Figure 6. Fish tank infrastructure used in the testing of purple phototrophic bacteria (PPB) as a replacement aquaculture feed.

2.2.2 Statistical methods

A generalized linear mixed regression model was used to assess the impact of PPB inclusion extent on feed intake, daily gain, FCR, growth rate, and mortality, besides other factors (tank number, initial fish size, age of fish etc). Limits for statistical testing were set at $p < 0.05$ (95% confidence interval). An ANOVAN (n-way) analysis of variance (ANOVA) was used to test the effects of multiple factors on each tank and sample point. Tank number was treated as categorical factor. Age of fish (time), start initial size (medium or large) and PPB fraction (diet type) were treated as primary continuous factors. Residual normality (noting $n > 36$) and heteroscedasticity (against explanatory variables) were tested. While linear modelling was used as the main statistical method for overall impact, comparison between pairs was done using an appropriate t-test.

2.3 Novel Fertilizers

2.3.1 Soil collection

A common broadacre soil (sandy duplex; sandy loam overlaying clay) was selected, and field dry soil (0–10 cm) collected from UWA Farm *Ridgefield*, Pingelly, Western Australia (Grid reference: 498504 m E, 6406727 m S). The same soil was used throughout the laboratory experiments, pot trial and field trials. This soil was previously used for cropping and pasture and had not received any lime or fertiliser amendment since 1996. The UWA Farm *Ridgefield* is situated in the Wheatbelt, which produces approximately 40% of Australia's grain and is therefore a key area to evaluate GHG abatement methods with manure application. The site receives an average annual rainfall of 445 mm (57.8 rainy days), has an evaporation rate of approximately 1700 mm and a temperature range for winter of 6 - 16˚C and summer of 15-31˚C. The native vegetation consists of White gum (*Eucalyptus Wandoo*), Salmon Gum (*Eucalyptus salmonophloia*), Red Morrel (*Eucalyptus longicornis*), Gimlet (*Eucalyptus salubris*) and Marri (*Eucalyptus calophylla*). The parent rock type is Colluvium over truncated deeply weathered lateritic profile (granite as original parent rock).

2.3.2 Manure types and collection

Manures and composts were collected for the field trial during June-July in 2015, 2016 and 2017. For the 2016 field trial fresh broiler chicken manure (litter) and semi-composted manure was collected from chicken meat enterprise in Gingin. The composted chicken manure was collected from nearby composting facility. Piggery deep-litter samples and pig compost were collected from a mixed piggery and composting enterprise. All composts were prepared using an aerated floor composting system (mobile aeration floor). During 2015-2017 we worked with our partners Richgro Ltd, Future Green solutions, The Algae Centre at Murdoch University, University of Queensland, C-Wise and a private piggery to develop and produce high quality, balance fertilisers and soil conditions including advanced composts, frass (black soldier fly castings), microalgae, PPB, pellets and digestate. The agronomic and economic value of these products was tested in the 2016 screening pot trials. The compost developed with Richgro and C-wise were evaluated during the 2016-2017 field and pot trials.

2.3.3 Screening pot experiment

Soil was collected for the experiment (0-10 cm depth) from a mixed cropping and grazing enterprise at The University of Western Australia (UWA) Future Farm near Pingelly. The soil properties are shown in Table 10, Outcome section. The soil was sieved to 2 mm before potting and 3 kg (wet weight) was added to each pot. The novel fertiliser and soil improvers were applied to the soil at an application rate of 5 t. ha^{-1}. No fertiliser treatment was added to any of the biological treatments. Control treatments included a zero-fertiliser control, and an addition of synthetic fertiliser Macropro at rates of 10, 25, 50, 75 and 100 kg/ha. District practice for this area and soil type is 100 kg. ha^{-1} (Barton et al., 2013b). Six wheat seeds (Trojan) were planted in each 2.7 L pot at a depth of 30 mm. The pots were watered to and maintained at 80 % water holding capacity. Seedlings were thinned to two per pot at the three-leaf stage, and the remaining seedlings were grown to maturity before harvest at 118 d (Mickan et al., 2018).

At the end of harvest roots were carefully lifted out of the soil and shaken vigorously to remove loose adhering soil. The tightly adhering rhizosphere soil was collected and used for subsequent soil analyses. Fresh shoot weight was taken and oven-dried at 60°C for 72 h and total shoot dry weights per pot for each treatment was calculated. The roots were washed well with water to remove the remaining adhering soil particles, blotted dry and weighed. Oven-dried shoots were ground and digested (HNO_3–$HClO_4$) and the N concentration in the digest was measured (Mickan et al., 2018).

2.3.4 Field trial 1: Evaluation of nutritive, agronomic and economic benefits of new improved fertilisers and soil improver products (2015)

A field trial examined the nutritive, agronomic and economic benefits as well as operational risks (GHG emissions, nutrient runoff and stable fly emergence) of amending of soils with high quality composts as compared to standard manures for rain-fed, cropped soils in a semi-arid region of the Western Australian Wheatbelt. The field trial was arranged in randomised block design with 4 replicates per treatment (Figure 7). The trial was planted with wheat (*Triticum aestivum cv Carnamah*) to a soil depth of 3-4 cm with no tillage during July 2015 using an airseeder. The plot size for each treatment was 10 m long by 2 m wide and separated by an unfertilised 1m buffer zone in the row. The trial comprised the following eight treatments:

1. Control: no amendment (no fertiliser)
2. Control: synthetic fertiliser at district practice (100%)
3. Control: reduced synthetic fertiliser at district practice (60%)
4. Chicken manure + synthetic fertiliser at 60%
5. Chicken compost + synthetic fertiliser at 60%
6. Pig manure + synthetic fertiliser at 60%
7. Pig compost + synthetic fertiliser at 60%
8. Mixed chicken and pig compost + synthetic fertiliser at 60%

A base fertiliser was applied at seeding (80 kg ha^{-1} 'MacroPro'®) at two rates of 100% and 60% (40 and 24 kg N ha^{-1}, respectively) in July. The manure and compost treatments (20 kg N ha^{-1}) were also applied at seeding. Manure and compost was broadcast across the surface of the soil then incorporated to a depth of 5 cm by hand.

Figure 7. The 2015 field trial at UWA Farm Ridgefield, Pingelly, Western Australia.

2.3.5 Field trial 2 (second year of crop rotation): Evaluation of the nutritive, agronomic and economic benefits of new improved fertilisers and soil improver products (2016, 2017)

A field trial examined the nutritive, agronomic and economic value of amending soils with high quality composts as compared to standard manures for rain-fed, cropped soils in a semi-arid region of the Western Australian Wheatbelt. The field trial was arranged in randomised block design with 4 replicates per treatment. The trial was planted with Kaspa Peas (100 Kg/ha seeding rate) to a soil depth of 3-4 cm with minimum tillage during June 2016 using an airseeder. The plot size for each treatment was 10 m long by 2 m wide and separated by an unfertilised 1 m buffer zone in the row. The trial comprised of eleven treatments (Table 1).

Table 1. Field trial design 2016 and 2017

Treatment Number	Description
1	Control: Nil (No Basal and no N topdressing)
2	Control: 100% Basal + N topdressing (amount of topdressing dictated by season)
3	Control: Reduced Basal (Compound fertiliser minus 15Kg p/Ha) + N topdressing
4	Dairy compost @ 15KgP/ha + 100% basal + N topdressing
5	Chicken compost @ 15KgP/ha + 100% basal + N topdressing
6	Beef compost @ 15KgP/ha + 100% basal + N topdressing
7	Pig compost @ 15KgP/ha + 100% basal + N topdressing
8	Dairy compost @ 15KgP/ha + reduced basal (standard amount minus 15Kg P/ha) + N topdressing
9	Chicken compost @ 15KgP/ha + reduced basal (standard amount minus 15Kg P/ha) + N topdressing
10	Beef compost @ 15KgP/ha + reduced basal (standard amount minus 15Kg P/ha) + N topdressing
11	Pig compost @ 15KgP/ha + reduced basal (standard amount minus 15Kg P/ha) + N topdressing

A base fertiliser was applied at seeding at two rates of 100% and 60% (25 and 15 kg P ha^{-1}, respectively) in June. The compost treatments (15 kg P ha^{-1}) were also applied at seeding. The compost was broadcast across the surface of the soil then incorporated to a depth of 5 cm by hand. All treatments except the unamended control (nil) received a further surface application of liquid N (25 L/ha UAN). Herbicides were applied both pre-emergence (1.4 kg/ha Terbyne, 1 L/ha propyzamide and 1.5 L/ha Roundup Ultramax) and post-emergence (500 mL/ha Select and 200 mL/ha Brodal). Pesticides were applied both pre-emergence (500 mL/ha chlorpyrifos) and post-emergence (100 mL/ha Transform).

2.3.6 Manure characteristics

The dry matter (DM) content of the manure was determined by drying in an oven at 105 °C. Total carbon (TC) and total nitrogen (TN) were determined by combustion analysis (vario Macro CNS; Elementar, Germany)(Jenkins et al., 2016). The ammonium (NH_4^+) and nitrate (NO_3^-) content were determined by extracting with 0.5 M K_2SO_4 and analysing on an automated flow injection Skalar Auto-analyser (Skalar San plus) (Mickan et al., 2018). The electrical conductivity (EC) and pH was measured in water or 0.01 M $CaCl_2$ mixtures, respectively, at a 1:5 soil: water ratio (w/v; g cm^{-3}) (Jenkins et al., 2016).

2.3.7 Soil measurements

Soil samples were taken at 0–100 cm at the beginning of the field trial and at 10-20 cm during key cropping (seeding, manure application, emergence, anthesis harvest) and rainfall events throughout the trial season. Micrometeorological data was collected from the field site during every sampling event. The soil moisture and water filled pore space were determined (Barton et al., 2013b). The ambient air and soil temperate and precipitation were also gathered. Bulk density, water-filled pore space (WFPS), mineral N (NO_3^- and NH_4^+), total C and N, Electrical conductivity (EC), pH and volumetric water content (VWC) were determined in the surface soil (0–30 cm) to account for changes in GHG fluxes in the soils (Barton et al., 2013b). Extractable organic C was determined by Total Organic Carbon analyser. Surface area and pore size distribution was also determined (Jenkins et al., 2016).

2.3.8 Crop productivity analysis

Crop performance was determined from a range of different indicators including grain quality, crop yield, plant biomass, plant emergence and plant nutrients. The percentage plant emergence was determined at 4 weeks by counting the number of plants in a 1m × 1m quadrat. Plant biomass and nitrogen uptake in the plant and grain were measured at selected intervals throughout the growing season. All plant material was dried in an oven at 60 ◦C for 7 days in before recording the dry mass and analysing for N using a CNS analyser (Elementar Analysensysteme GmbH, Vario Macro, Hamau, Germany)(Mickan et al., 2018). The crop yield and grain quality (protein content and screening) were determined at harvest.

2.3.9 Measurement of greenhouse gas emissions

Greenhouse gas fluxes were quantified during the wheat growing season at seeding, emergence, tillering, flowering and crop maturity using a static manual chamber system (Figure 8). At each sampling time, lids on gas chambers were sealed with an air-tight screw-top lid fitted with a septum as described in Barton et al., (2013). Gas samples were collected immediately before sealing the jars, and then at 20, 40 and 60 min, by collecting 15 mL of headspace gas and storing in 12 mL evacuated septum-sealed glass vials (Exetainer, Labco Ltd., UK) for gas analysis (Barton et al., 2013a). Headspace gas were analysed for nitrous oxide (N_2O) and methane (CH_4) using a Varian Gas Chromatograph (Model: CP-4900, Middelburg, The Netherlands) equipped with a thermal conductivity detector for N_2O analyses and flame ionisation detector for CH_4 analyses. The cumulative emissions for each GHG gas (N_2O or CH_4) were estimated and hourly N_2O (mg N_2O-N g ha^{-1} d^{-1}) fluxes were calculated from the slope of the linear increase in N_2O concentration during the enclosure period in the chamber and corrected for air temperature, air pressure and jar/chamber volume (Barton et al., 2013a).

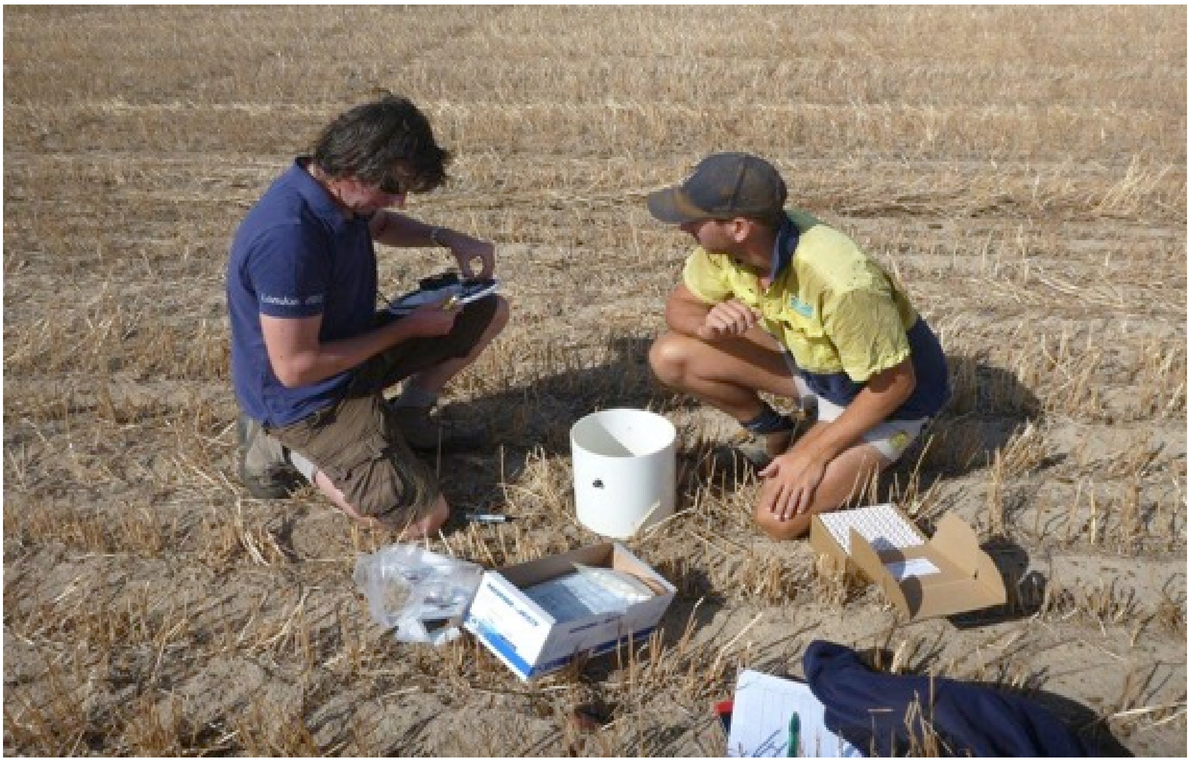

Figure 8. Taking greenhouse gas samples at the 2015 field trial site.

2.3.10 Stable fly emergence

Purpose-built fly emergence cages (0.5 m^2) were used to identify and quantify fly development after the application of different manure, compost and fertiliser treatments to broadacre farming systems (Figure 9). The fly cages were placed over the soil after crop emergence and left for 6 weeks. After 6 weeks the impact of different fertiliser treatments on oviposition (egg laying), larval development and successful adult fly emergence (stable flies and other nuisance flies) were assessed (Cook et al., Accepted).

Figure 9. The adult fly emergence trap placed over treated areas at the 2015 field trial site.

2.3.11 Cost benefit analysis

A cost benefit analysis was performed on the manure and compost products applied as fertilizers, using a modified calculator on the Grains Research and Development Corporation (GRDC) soil quality website (http://soilquality.org.au/calculators/bio product). This calculator assesses economic benefits to farmers. The cost benefit analysis assessed the use of novel fertiliser and soil improver products, if applied alone or if applied in combination with synthetic fertiliser, and either at 60 and 100 kg/ha, and compared these scenarios with the application of synthetic fertiliser at district practices. This assessment was performed for both the pot and field trials.

For the calculation we assumed the following information:

Productivity

1) On Farm Grain Price ($/tonne) - APW wheat was around $280/tonne and AGP was around $261 at port in 2015

2) Average yield - $2/tonne

Litter/manure/compost/novel fertiliser costs

1) product cost ($/tonne)- We assumed it cost about $8 to clean shed and cart on the farm, producing the semi compost and composted product costs approximately $18 and $28, respectively.

2) transport cost to farm ($/tonne) - For grain growers interested in using products on their farms we have costed at $5 and $20 per tonne freight cost within Shire (<30 km radius) and outside the Shire (<150km radius). Note if poultry litter is composted to A4544 standards then CBH may potentially back load their freight trains, thereby significantly reducing transport costs.

3) application cost ($/tonne) - $10/tonne for composted (assuming Marshall-style belt spreader is used for good quality compost), $20/tonne for raw manure product (assuming a European-style muck spreader is required for a lumpy, high moisture content product).

Synthetic Fertiliser costs

1) product cost ($/tonne) -MarcoPro at $846/tonne, topdressing with Flexi N at $409/tonne

2) transport cost to farm ($/tonne) - $20/tonne (assuming it is transported from Perth)

3) application cost ($/tonne) - if it is applied through an airseeder at seeding time, there is no additional cost, as the farmer needs to go over the paddock anyway to sow the seed. If Flexi N is top-dressed as well after emergence, we assumed $5/tonne for its application.

Other variable costs

1) Seeding cost- $0.35/kg for seed plus seed pre-treatment

2) Levies- GRDC levies @ 1% gross income

3) Chemical and pesticides-pre and post treatment $95.14/tonne

4) Lime-Ag Lime of Australia quote $10.95/tonne for under 1,000t (delivered and price excluding GST) and $10.65/tonne for >1,000tonnes (delivered and price excluding GST)

5) Grain or fertiliser freight-$20/tonne

6) Operations- fuel, oil, repairs and maintenance of equipment= $18.93/tonne

7) Contracts- labour costs for handling and application $18.50/tonne for applications made a district practice rates

8) Crop insurance- $8.50 /$1000 of crop produced

Net benefit is calculated by subtracting the total input cost from the total income. The cost benefit analysis is calculated by subtracting the net benefit for each treatment from the net benefit at 100% synthetic fertiliser (in other words, the standard fertiliser regime).

The CBA used here was a modified version of the GRDC's Gross margin template for crop and livestock enterprises (2015, In: Farm Gross Margin 2015. A gross margin template for crop and livestock enterprises. A Rural Solutions SA publication Sponsored by SAGIT and GRDC ISBN 978-1-921779-78-7).

2.3.12 Laboratory and Glasshouse trials to ascertain the Mode of Action

Evaluation of the mechanisms and mode of action for compost additions to soil (with Western Dairies and South West Catchments Council (SWCC))

The impact of dairy manure and compost on soil carbon (C) retention and bacterial communities in a dairy pasture in south-western Australia was investigated. Bacterial activities are involved in retention of soil C, and some are involved in loss of soil C during degradation of organic matter. Bacterial communities respond to addition of manure and compost and play a key role in the incorporation of these C resources into the soil matrix. In this study, bacterial communities in dairy soil amended with manure or compost in a field experiment were characterized in soil collected in 'winter' and 'summer' using community profiling of 16S rRNA genes. The soil was amended in the field with inorganic fertilizer in combination with 2t/ha dairy manure, or compost applied at 3t/ha or 6t/ha.

Evaluation of the evaluate nutritive, agronomic and economic benefits of algae by-products as an alternative fertiliser or biostimulant (with Murdoch university)

This experiment investigated the effectiveness of algal biomass produced from waste water used as a nitrogen source for plant growth. The experiment was set up in randomized design consisting of two nitrogen fertilizer treatments by five nitrogen rates (0, 25, 50, 75 and 100 kg ha^{-1}) and four replicates. The nitrogen fertilizer rates represent the ranges of rates applied to cereal crops in WA. The algal biomass was sourced from The Algae Research Centre, University of Murdoch. Wheat (*Triticum aestivum* L.) was grown in pots (1 kg soil) for 6 weeks in a glasshouse at The University of Western Australia. In addition to the nitrogen fertilizer treatments, basal nutrients were applied to ensure that there is no nutrient limitation except for N in the soil (Mulbry et al., 2005). A loamy soil with low available N was collected for the experiment (0-10 cm depth). The soil properties show that there is a low concentration of nitrogen, which makes it a suitable soil for this experiment. A cost benefit analysis of the bacterial products was performed as described above.

A second laboratory incubation study was set up to investigate the impact of different N amendments on the organic nitrogen mineralization rate in soils (Mulbry et al., 2005). A randomized block design included the two N sources (microalgae biomass and NH_4NO_3) and five N equivalent levels (0, 10, 20, 40, 80 kg N ha^{-1}) for 28 days in three replicates. The collected soil was sieved (<4 mm) and was pre-incubated at 25°C for 7 days prior to microcosm commencement. The samples were incubated at 25^{o}C, and soil moisture content adjusted to 70% of moisture-holding capacity. Each microcosm contained 40 g of soil and was amended with either the microalgae biomass or NH_4NO_3. Amended soil samples were packed into unsealed, polyethylene vials (42 mm in diameter), to the same bulk density as the soil. The water holding capacity (WHC) was adjusted to 70% prior to establishment and maintained throughout the experiment (Dempster et al., 2012). The soils were incubated at 25ºC for 28 days and changes in mineral N (NH_4^+ + NO_3^-) and CO_2 flux was measured at regular time intervals (0, 7, 14, 21 and 28 days) (Dempster et al., 2012).

2.3.13 Benefits of improved manure fertiliser products on soil biology, resistance and resilience

Evaluation of the mechanisms and mode action involved in how compost additions alter the physical properties of the soil to improve soil resilience to water stress (with Richgro)

The aim of this experiment was to determine whether compost and clay amendments in a sandy agricultural soil influenced the rhizosphere microbiome of *Trifolium subterraneum* under differing water regimes. Soil was amended with compost (2% w/w), clay (5% w/w), and a combination of both, in a glasshouse experiment with well-watered and water-stressed (70% and 35% field capacity) treatments. 16S rRNA Ion Torrent sequencing and PICRUSt analysis of functional gene prediction were used to interrogate the rhizosphere bacterial community and its functional component involved in nitrogen (N) cycling and soil carbon (C) degradation (Mickan et al., 2018).

Evaluation of the mechanisms and mode action involved in how compost additions alter the physical properties of the soil to improve soil resilience to heat stress (with Liebe Grower group)

This experiment investigated whether soils amended with manure or compost are more resilient to heat stress (Banning & Murphy, 2008; Stockdale et al., 2013). Soil samples (5 cm depth) were collected from Liebe Group Long Term Research Site (Figure 10), Buntine, which is situated in the low-rainfall semi-arid cropping zone of Western Australia.

Figure 10. The Soil Biology Trial at the Liebe Group Long Term Research Site, Buntine, WA.

A soil microcosm experiment having a 2×3×2 factorial arrangement of treatments in triplicate was conducted using 557-ml glass jars. The factors were different long-term managements (mineral fertiliser and mineral fertiliser plus organic matter), amendments (unamended, manure and compost) and heat stress factors (unheated, heated to 70°C approx. summer soil temperature in WA). Samples were monitored at regular intervals before and after stressing over a period of 100 days (Banning & Murphy, 2008). Water content, pH, CO_2 flux (respiration) was analysed at 0, 2, 6, 12, 24, 48, 72 and 100 days. Soil mineral N (NO_3^- and NH_4^+) was determined using an automated flow injection Skalar AutoAnalyser (Mickan et al., 2018). The relationship between microbial diversity, manure amendment and stress were explored using the multivariate analysis in R statistical package. The data generated was used to determine both the amplitude of the disturbance (resistance) and how long the stressed community took to recover in terms of microbial diversity and community structure (resilience) (Banning & Murphy, 2008).

3 Project Outcomes

3.1 Project level achievements

3.1.1 Novel Waste Processing Technologies

A novel waste processing technology was tested and further developed for applications in the agricultural industrial sector. The aim of such treatment is to:

1. make the wastewater inert by removing nutrients and carbon, to minimize any negative impacts when such wastewater is reused or discharged into the environment; and
2. simultaneously assimilate nutrients and carbon into high-value high-protein products, to use as novel fertilisers or novel feeds.

This alternative treatment concept is superior to traditional wastewater treatment, because traditional wastewater treatment does not recover nutrient and carbon resources.

This project tested the alternative treatment technology at small laboratory batch scale for proof-of-concept, then at larger laboratory pilot scale to get relevant design data and finally at pilot-scale on-site at an agricultural industrial site to demonstrate the concept “in the field”.

Batch screening study (Activity 2, Output 2(a); Activity 3, Output 3(a))

The below is a summary of research described in the publication; Hülsen T, Hsieh K, Lu Y**,** Tait S, Batstone DJ. (2018). Simultaneous treatment and single cell protein production from agri-industrial wastewaters using purple phototrophic bacteria or microalgae - A comparison. Bioresource Technology. 254 (2018) 214–223.

<u>Wastewater treatment performance</u>

This study, for which the methods were described in Section 2.1.1, compared the treatment of five <u>real</u> agricultural industrial wastewaters (pork, poultry, red meat, dairy and sugar) by growing purple PPB or microalgae to recover carbon, nitrogen, and phosphorous as a high-value biomass. The composition of the wastewaters from the different industry sectors were quite different (Table 2), providing opportunity to distinguish wastewaters particularly suitable for the treatment technology.

The treatment performance with PPB and microalgae was assessed based on measured removal of soluble ingredients such as sCOD, NH_4-N and PO_4-P, to produce the desired particulate microbial biomass. To quantify biomass recovery, tCOD and TN were also measured. Ideally, for a batch test, the amount of tCOD would not decrease over time, but rather increase or remain unchanged, because a decrease in tCOD indicates a loss of chemical energy rather than being diverted into microbial biomass. With microalgae it is possible for tCOD to increase over time, because new chemical energy may be added by the autotrophic photosynthesis.

Ideally, TN would not decrease over time, because a decrease in nitrogen represents a loss by volatilisation rather than being diverting into the desired microbial biomass.

Figure 11 and Figure 12 show time trends for COD (sCOD and tCOD), and ammonia and phosphate, respectively, for PPB and microalgae treatment of poultry processing wastewater. Data for the other wastewaters have been provided elsewhere (Hülsen et al., 2018a). Figure 13 compares the overall removal extents of various wastewater constituents across the test time, for the various wastewaters.

PPB converted soluble organic matter into particulate COD as microbial biomass. This is seen in the PPB results from the decrease in sCOD over time, whilst there were minimal overall losses (decreases) in tCOD over time. PPB treatment caused intermittent increases in ammonia concentration, likely due to solubilisation of proteins native to the wastewater, but this was usually followed by uptake of ammonia into microbial biomass with a subsequent decrease in ammonia concentration until the end of the test. In contrast, the microalgae tests generally showed a significant reduction in tCOD over time, indicating losses of organic matter to CO_2/H_2O via oxidation using oxygen produced by microalgae.

PPB were able to treat poultry wastewater and filtered red meat processing wastewater most effectively. Pork flush and dairy wastewaters were also treated with moderate efficiencies by PPB, with minimal losses of organic matter and nitrogen. Two sugar mill wastewaters (Sugar mill A and Sugar Mill B) were tested. Sugar mill A wastewater contained a substantial amount of ethanol, which was toxic for microalgae and PPBs and killed off the microbial population. The sugar mill B wastewater was poorly treatable by PPB, with minimal removal of sCOD, NH_4-N or PO_4-P. However, the pH in these tests decreased after 2 days of treatment to 4.0, indicating that decay and fermentation was occurring (data not shown). Accordingly, the sugar wastewater may be treatable, but may require prior dilution or mixing with other on-site wastewater streams, or pH correction, low volumetric feed rates (Hülsen et al., 2018a), or two-stage treatment with fermentation followed by assimilation. With respect to treatability using PPB, the major factor seemed to be the bioavailability of COD. PPB have a preference for uptake VFA-COD. Having said this, the pork flush wastewater had a lot of bioavailable COD, with substantial VFAs, yet only half of this VFA-COD was removed by PPB treatment. High loadings and/or ammonia toxicity may have caused this poor treatment.

The microalgae tests performed better than PPB at wastewater treatment, that is, in terms of removing sCOD, NH_4-N and PO_4-P. However, the tCOD measurements and TN losses in the microalgae tests indicated that native heterotrophic micro-organisms were oxidising organic matter and nitrogen, and that denitrification was occurring. The only exception in terms of treatability by microalgae was pork-flush wastewater, which was poorly treatable, likely due to a high NH_4-N content being toxic to microalgae growth.

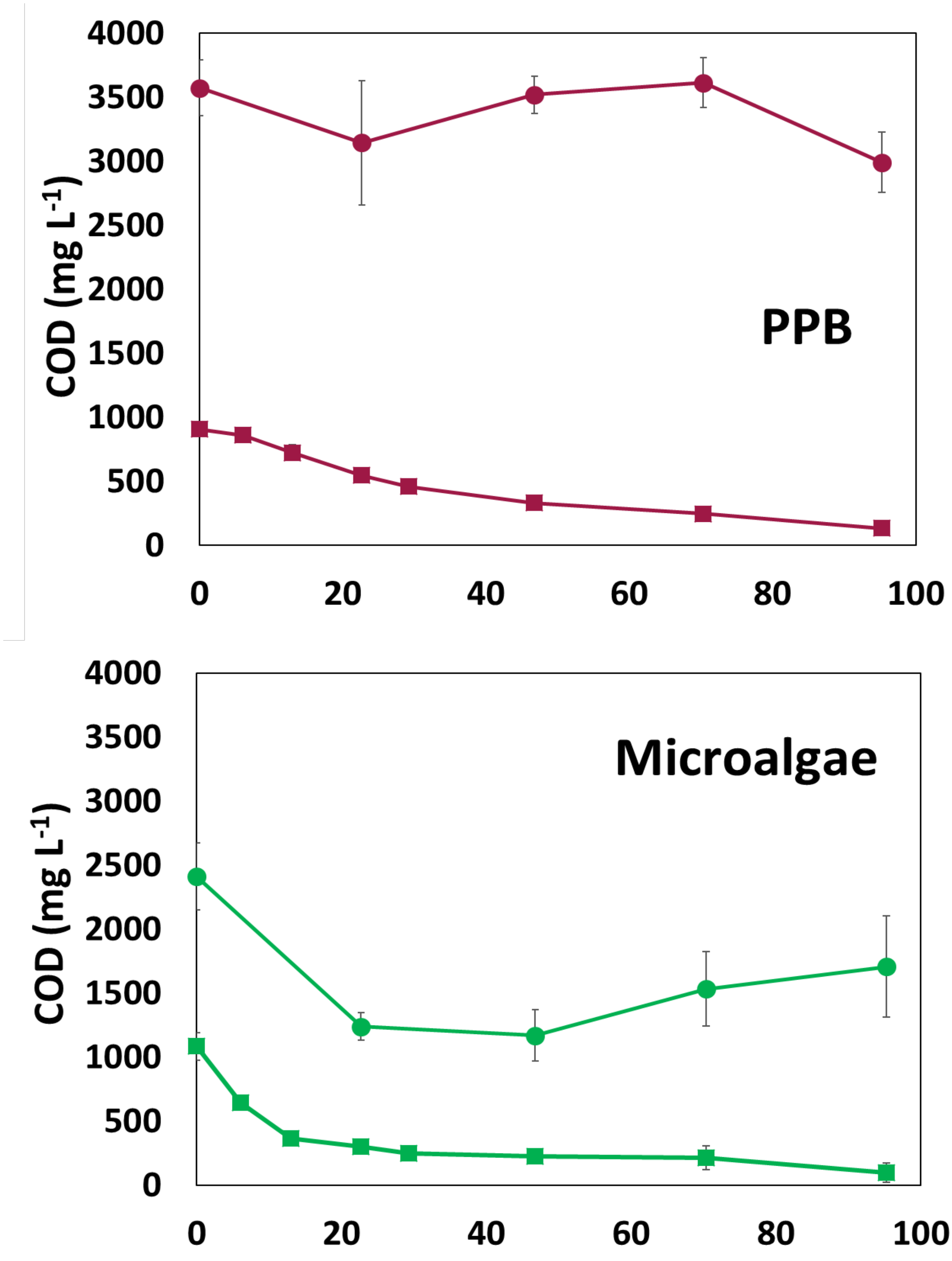

Figure 11. Time trend measurements of tCOD (●) and sCOD (■) for treatment of poultry processing wastewater by growing (top, red) PPB or (bottom, green) microalgae. Additional data are provided in Hülsen et al. (2018a).

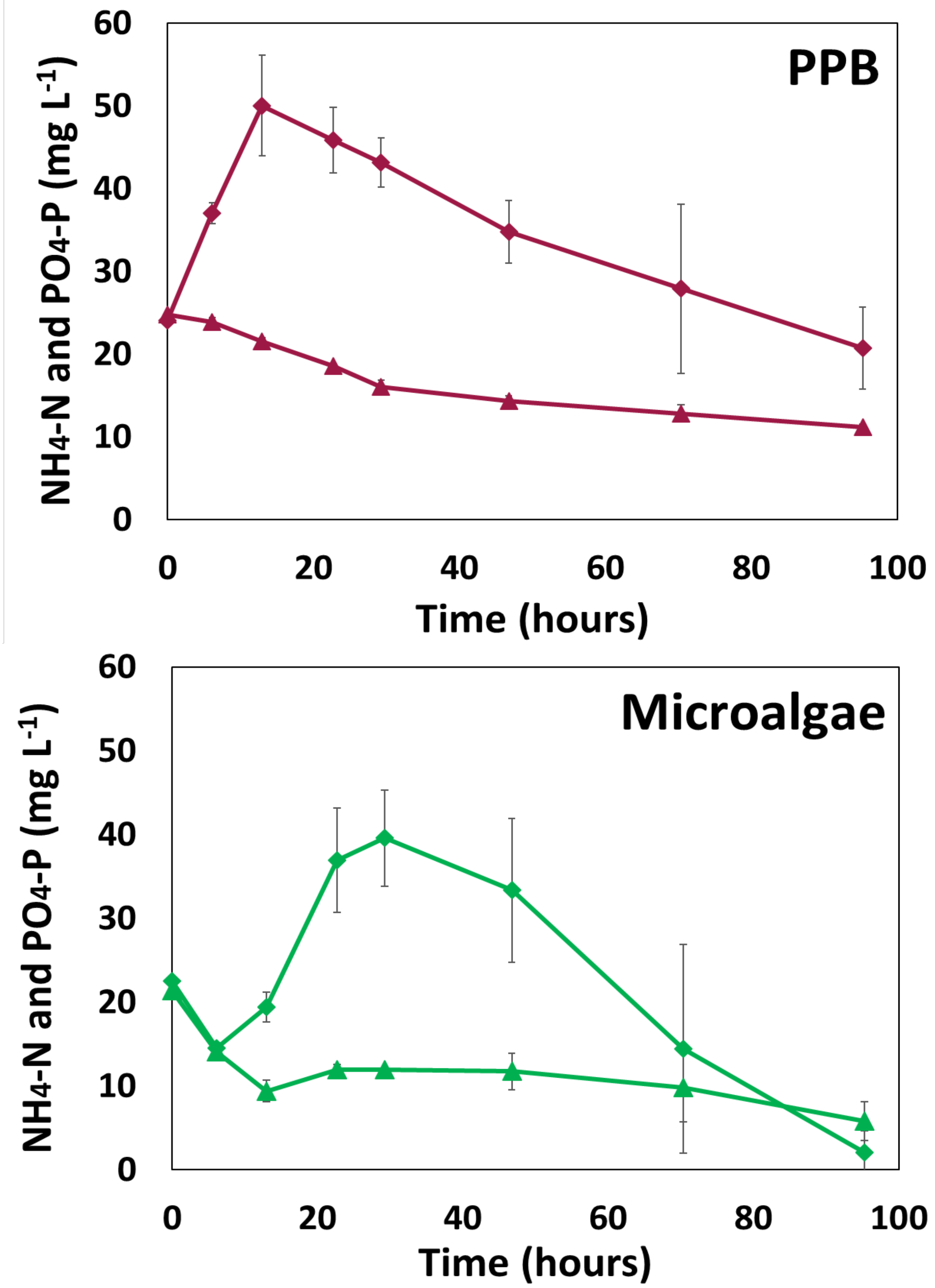

Figure 12. Time trend measurements of NH_4-N (◆) and PO_4-P (▲) for treatment of poultry processing wastewater by growing (top, red) PPB or (bottom, green) microalgae. Additional data are provided in Hülsen et al. (2018a).

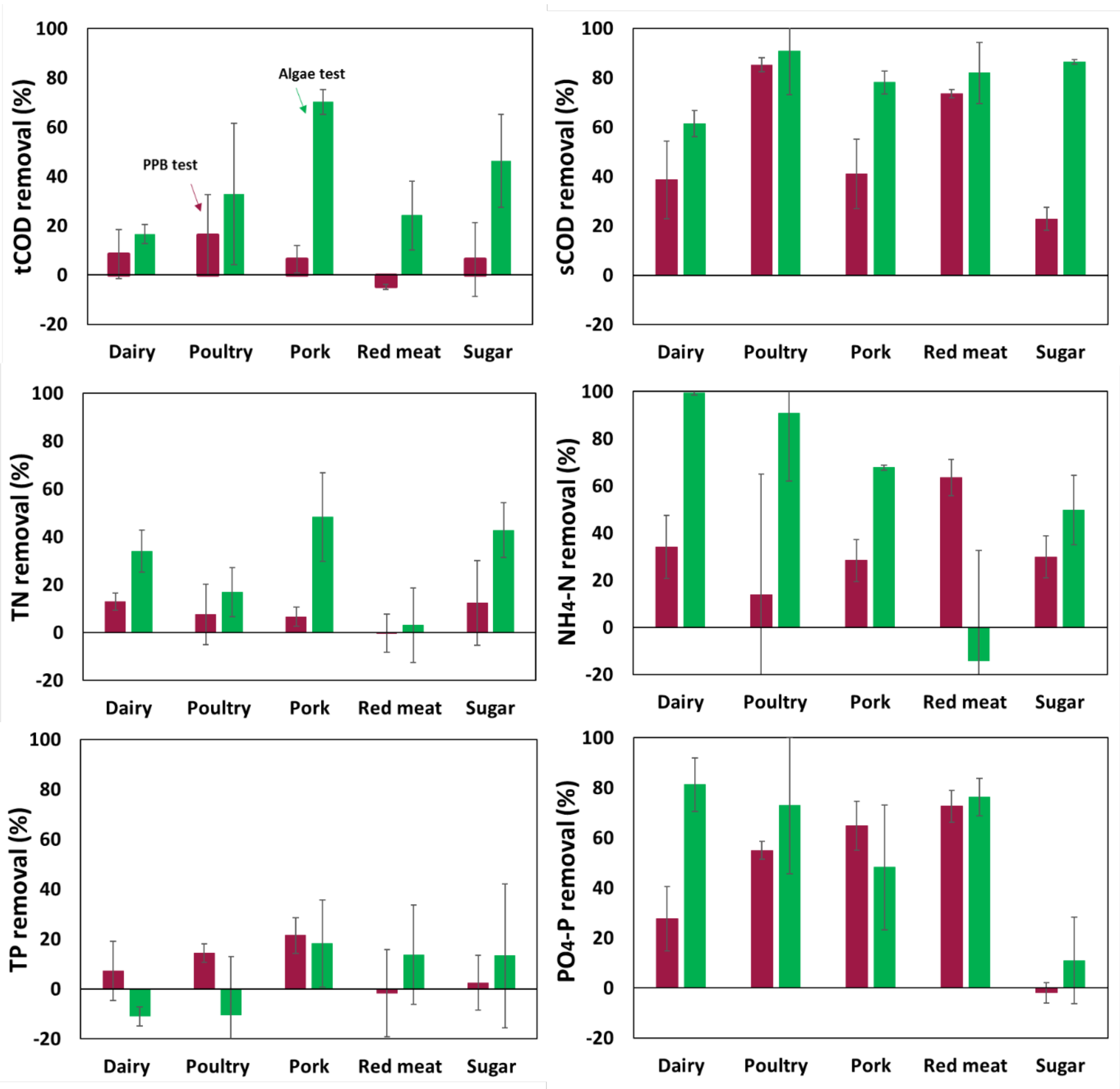

Figure 13. Removal efficiencies of PPB (purple) and algae (green) treating dairy, poultry, pork, and red meat and the dark control without biomass (hatched). Error bars represent 95% confidence regions. Additional data are provided in Hülsen et al. (2018a).

<u>Microbial biomass product</u>

The PPB biomass that formed in the tests showed a high yield and a high crude protein (CP) content, reflecting the non-destructive assimilation of carbon and nutrients by PPB. The caloric value of the PPB biomass was estimated to be higher than conventional protein meals, e.g. meat and bone meal. The algal biomass for poultry and red meat wastewater also had a high crude protein content >650 mgCP $gVSS^{-1}$, whereas protein contents for dairy and sugar tests were lower at 370 and 140 mgCP $gVSS^{-1}$, respectively. As described before, the sugar tests with PPB and microalgae were compromised by high ethanol toxicity or by low pH. A very high protein content for the pork flush tests with microalgae (1.0 gCP $gVSS^{-1}$) was believed to be mostly due to background protein from the wastewater and not actual biomass produced in the batch test.

The inocula were dominated by either PPB or algae, whilst the wastewaters were initially free of microalgae and contained only small amounts of PPB (<0.1%). In terms of microbial composition of the recovered biomass, there was a minimal shift in the overall phototroph population and limited change in the phototroph/other balance (except for sugar, where instead a substantial loss of phototrophs and photosynths occurred). Infrared illuminated systems were typically dominated by PPB, whilst the white light systems showed algae and cyanobacteria but at a lower fraction of the total population. The final relative abundance of microalgae in the microbial community was typically low (<15%, except for the dairy tests) with a diverse flanking community of aerobic bacteria and amoeba present, likely grazing on the microalgae. This mixed community is frequently reported in COD-rich algal ponds, commonly referred to as ALBAZOD (ALgae, BActeria, ZOoplankton and Detritus).

Figure 14 compares the amino acid profiles of biomass harvested from tests with red meat and poultry processing wastewater (Hülsen et al., 2018a) with that of fishmeal and soybean. The amino acid composition of the PPB biomass was similar to that of soybean meal, except for glutamic acid, being higher in soybean meal. When compared to fishmeal, the PPB biomass was deficient in all amino acids except phenylalanine. For microalgae biomass, the amino acid profiles were overall comparable to that of the PPB biomass and so showed similar potential for substituting commercial protein meals.

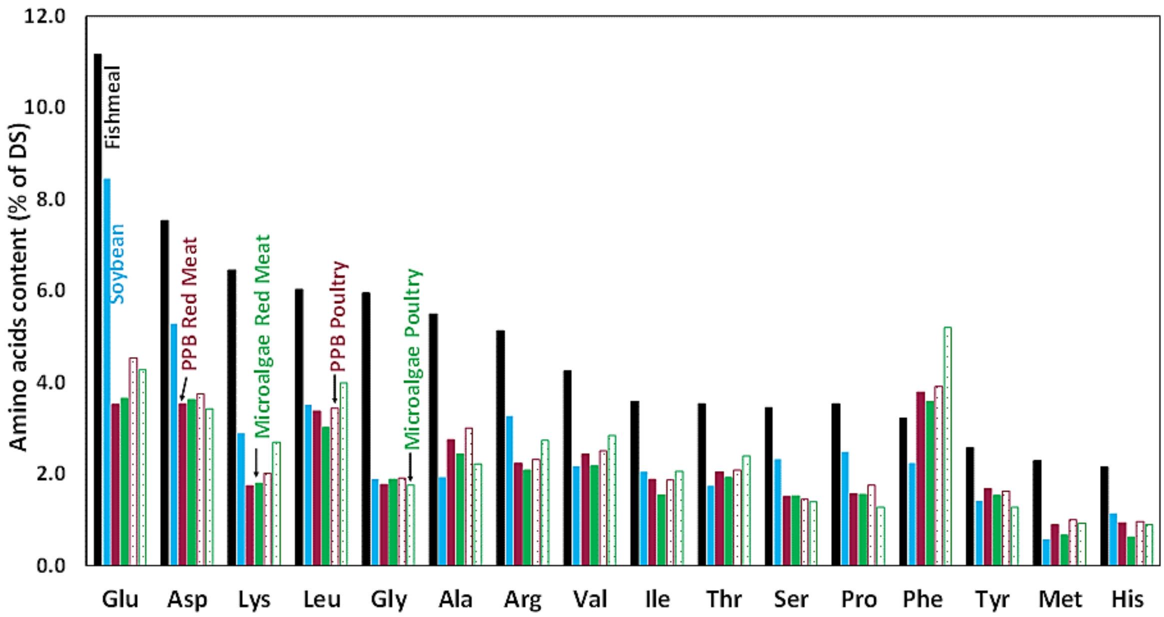

Figure 14. Amino acid composition in PPB (purple) and microalgae (green) grown on red meat (solid) and poultry (dotted) processing wastewater, being compared to amino acid compositions in fishmeal (solid, black), soybean (solid, blue) (FAO, 1970). Calculations based on amino acid residue mass in protein (molecular weight minus H_2O) (Hülsen et al., 2018a).

Summary

Both the microbial mediators (PPB and microalgae) showed good potential for future wastewater treatment, especially in light of a Nutrients-Energy-Water-Environment nexus with wastewater rather being viewed as a resource. To incorporate open algal pond systems into a common treatment configuration, the best positioning would likely be post-treatment of anaerobic effluent. This maximises energy and heat recovery from biogas by upstream anaerobic treatment, whilst assimilating residual COD and nutrients into protein rich biomass. In contrast, the PPB concept might represent a standalone technology or an upfront technology, followed by conventional treatment such as covered ponds for energy recovery. Further research below sought to clarify treatment performance in continuous and larger scale systems, also assessing aspects such as population stability and longer-term treatment performance.

Larger continuous system testing (Activity 3, Output 3(a); Activity 4, Output 4(a); Activity 5, Outputs 5(a))

The below is a summary of research described in the publication; Hülsen, T., Hsieh, K., Tait, S., Barry, E.M., Puyol, D., Batstone, D.J. 2018b. White and infrared light continuous photobioreactors for resource recovery from poultry processing wastewater – a comparison. Water Research. *In Submission*.

Based on the results above, poultry processing wastewater was selected for further testing of treatment in a larger continuous system. This research tested treatment by a novel membrane photobioreactor, with a white illuminated system (PAMBR, targeting micro-algae) and an infrared light illuminated system (PAnMBR, targeting PPB) being operated in parallel.

Wastewater treatment performance

Figure 15 show calculated wastewater treatment performance for the PAnMBR (PPB based). Figure 16 shows calculated wastewater treatment performance for the PAMBR (microalgae based) operation. Wastewater treatment by both photobioreactors was effective at removing most of the tCOD and a large proportion of TN and TP from the filtered effluent. However, the main nitrogen species in treated effluent from the PAMBR was nitrate, as opposed to ammonium in the PAnMBR effluent, suggesting that fundamentally different N removal pathways were giving similar removal performance in the two photobioreactors.

A mass balance of tCOD over period of operation showed that a major fraction of tCOD was lost from the PAMBR, likely being dissipated via aerobic oxidation to CO_2. At the same time, a substantial portion of nitrogen was also lost, probably by ammonia stripping or nitrification/denitrification. Overall, the recovery of tCOD, N and P into biomass was substantially higher in the PAnMBR than in the PAMBR. This result is consistent with the batch screening study.

To optimize the bioreactors, the hydraulic retention time was also changed from 2 days to 1 day, and encouragingly, this did not dramatically impact on performance of the two bioreactors, indicating that they are robust. A dramatic increase in the influent wastewater strength showed an increase in removal of tCOD, TN and TP, which was also encouraging, because they showed that the bioreactor concepts were resilient to changes in wastewater composition that are commonly experienced at poultry processing facilities.

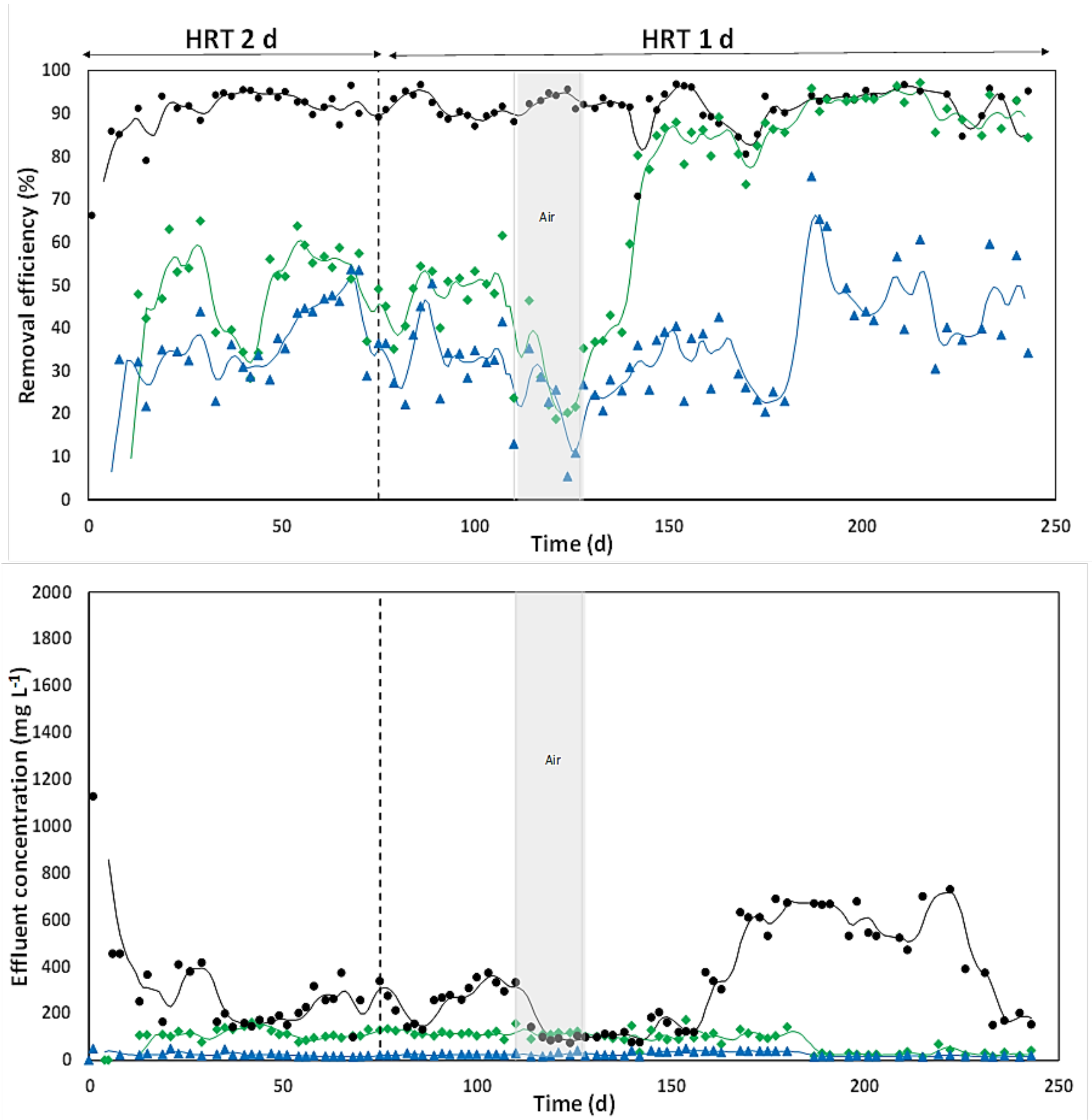

Figure 15. Performance for treatment to remove TCOD (●), TN (◆) and TP (▲), showing removal efficiencies (top) and effluent concentrations (bottom) with PPB as the biomass being grown (solids lines represent a 5 d moving average).

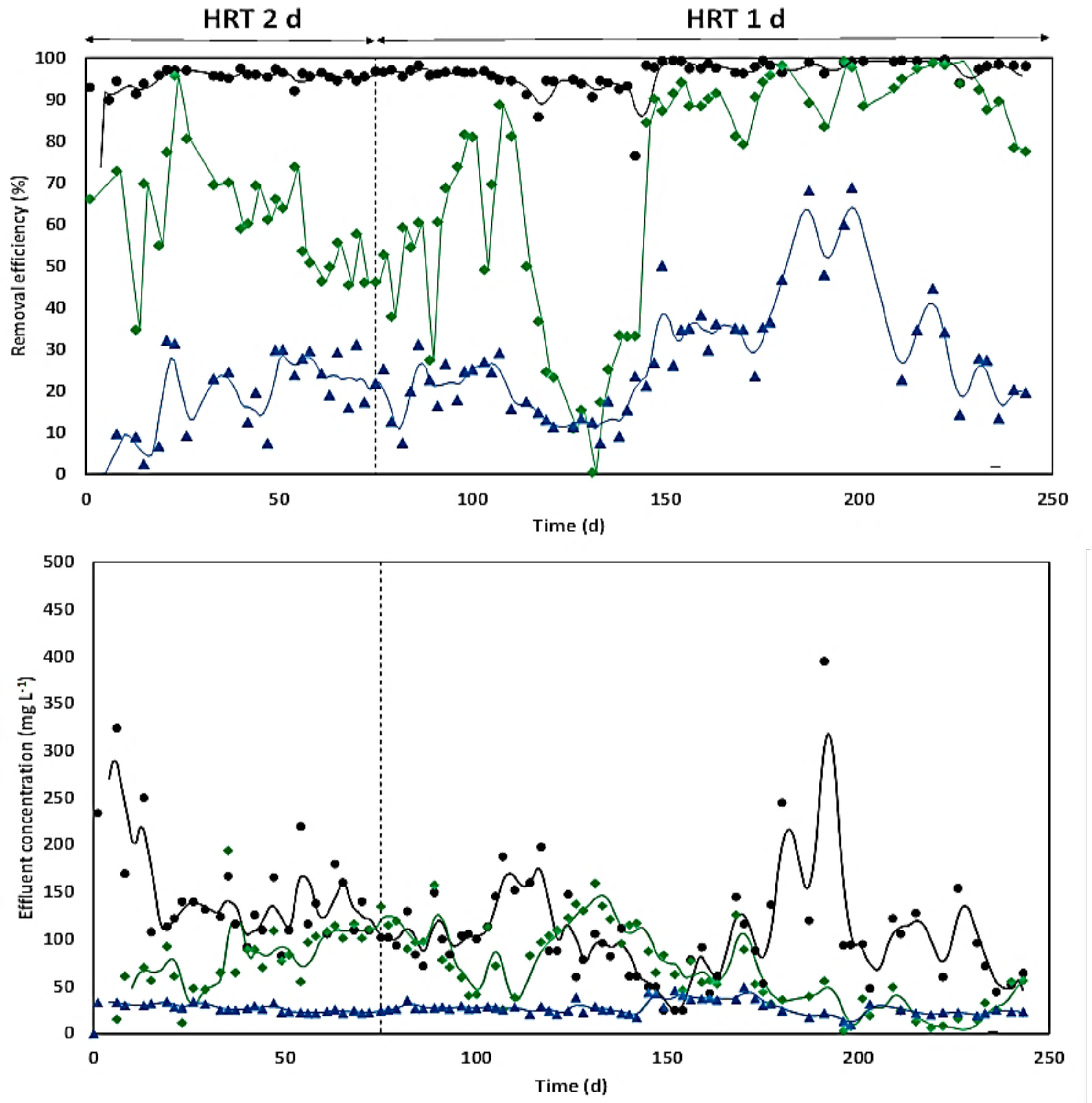

Figure 16. Performance for treatment to remove TCOD (●), TN (◆) and TP (▲), showing removal efficiencies (top) and effluent concentrations (bottom) with microalgae as the biomass being grown (solids lines represent 5 d moving average).

Microbial biomass product

Table 2 summarises the PPB biomass characteristics from the PAnMBR and Table 3 summarises the microalgae biomass characteristics from the PAMBR. For every 100 gTCOD removed by the PAnMBR (PPB based), approximately 4.1 – 8.5 gTN and 0.4 – 0.43 gTP were removed, likely being transformed into the biomass with 60% crude protein per dry mass (Table 2). For every 100 gTCOD removed by the PAMBR (micro-algae based), 7.0-9.6 gTN and 0.2gTP were. A portion of this consumed COD and TN and larger proportion of TP was transformed into biomass with 70% crude protein per dry mass.

The microbial analysis of the PAMBR (micro-algae based) showed that photosynthetic (micro-algae and others) organisms were present over the reactor operation period but did not dominate. This finding was consistent with the observations of the batch screening study above. In contrast, microbial analysis of the PAnMBR (PPB based) showed an increasing dominance of PPB driven by the infrared light illumination in combination with increased influent COD providing an increased VFA feed.

Table 2. PAnMBR bioreactor (PPB based) biomass characteristics.

	TCOD:N	TCOD:P	Protein content (g.gVS^{-1})	%P	%N
PPB - Day 10-75 – HRT of 2 days					
Average ($\bar{X}$)	4.1	0.4	0.6	1.3	10.1
Standard deviation (S_{xi})	4.6	0.6	0.2	0.4	2.5
Number of analyses (*n*)	18	19	19	19	19
PPB - Day 76 – 250 – HRT of 1 day					
$\bar{X}$	8.5	0.43	0.8	1.3	14.5
S_{xi}	10.5	0.7	0.4	1.0	11.5
n	60	60	53	57	55

Table 3. PAMBR bioreactor (Microalgae-based) biomass characteristics.

	TCOD:N	TCOD:P	Protein content (g.gVS^{-1})	%P	%N
Algae - Day 10-75 – HRT of 2 days					
Average ($\bar{X}$)	7.0	0.2	0.7	1.6	11.8
Standard deviation (S_{xi})	1.4	0.4	0.1	0.9	4.9
Number of analyses (*n*)	23	24	18	24	24
Algae - Day 76 – 250 – HRT of 1 day					
$\bar{X}$	9.6	0.2	0.7	1.8	28.1
S_{xi}	9.5	0.3	0.1	2.8	55.0
n	52	52	26	58	59

The amino acid analysis (Figure 17) showed that the PAnMBR (PPB based) biomass had slightly higher average amino acid content (340 mg gVS^{-1}) than the PAMBR (micro-algae based) biomass (230 mg gVS^{-1}), which is likely due to a mixed microbial population in the PAMBR. Both the PPB and microalgae had a balanced amino acid profile, which was lower compared to fishmeal, but was likely to add value as a fishmeal additive. For example, with a crude protein content >600mg gVS^{-1} this biomass could substitute a fraction of fishmeal. Encouragingly, the PPB biomass had higher crude protein and energy content than conventional rendering products.

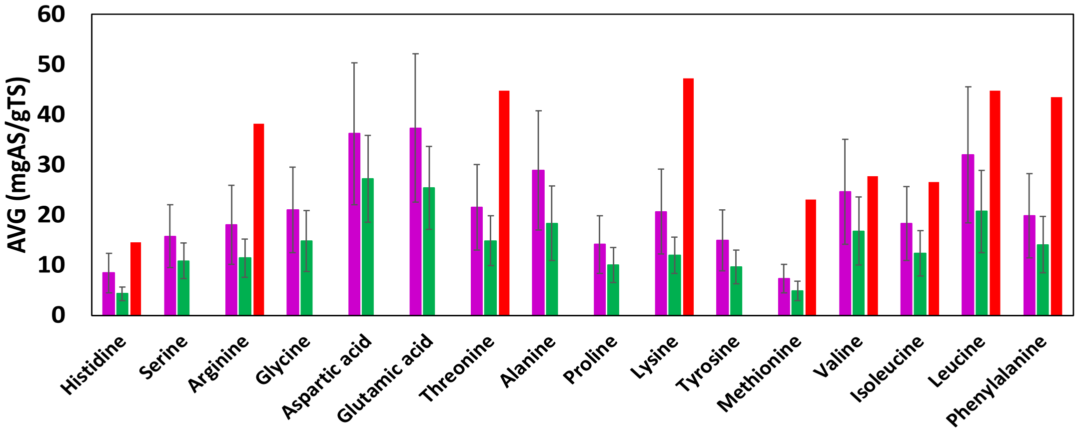

Figure 17. Average amino acid content of the PPB biomass (purple), microalgae (green) in comparison with fishmeal (red) (Miles & Chapman, 2006).

<u>Summary</u>

The treatment of poultry processing wastewater by the alternative treatment technology did not achieve typical surface water discharge limits, with the resulting quality being barely suitable for irrigation. However, the results did show substantial assimilation of carbon and nutrients, producing a high protein product instead of relatively low value biogas. Instead of aiming to reduced residual sludge production as in traditional wastewater treatment, the alternative treatment concept aims for maximum recovery of carbon and nutrients in microbial biomass as a valuable resource, whether for use as fertilizer or as a novel feed or feed additive. The robust performance of the photobioreactors over a wide variation in wastewater quality, and with an intensification step going from 2 days HRT to 1-day HRT, showed that the technology is resilient for applications. The amino acid content of the recovered product was lower than fishmeal. However, the data suggested that at least a fraction of fishmeal could be substituted with the biomass product, and relevant literature have already suggested the general applicability of phototrophic bacteria grown in artificial media (Al Azad et al., 2002; Banerjee et al., 2000; Kim & Lee, 2000). It therefore seemed justified to test PPB biomass in feed trials, as described below.

Key challenges that remain are:

1. the cost of illumination, and the limits of light penetration being attenuated by mobile biomass concentration in the bioreactor liquid;
2. the stability of the microbial population and predation is still a key concern, especially with micro-algal systems, although infrared light and VFA laden wastewater appeared to favour increased dominance of PPB;
3. the dilute low concentration of PPB and micro-algal biomass in suspended photobioreactors, requires separation and drying steps at additional cost that is difficult to economically justify; and
4. whilst the micro-algal systems appeared to provide superior wastewater treatment performance, this also came at the expense of carbon and nitrogen dissipation, instead of recovery. In this regard, the PPB technology appeared to be of greatest interest, and so was further explored at larger scale at the UQ Gatton piggery.

Large demonstration scale field reactor testing (Activity 4, Output 4(b); Activity 4, Output 4(a); Activity 5, Outputs 5(a))

The operation of the continuous photobioreactors on poultry wastewater, as described above, provided a successful proof-of-concept of the wastewater treatment technology in terms of longer term and robust performance. These results justified the up-scaling of the technology to a larger field pilot (500-1000L), with the main aims to provide reasonable treatment efficiency, producing significant quantities of consistent quality biomass, and to reduce operational costs. This was done in a Gatton trial, where instead attached growth and manual harvesting as a means to pre-concentrate and separate the biomass from a recalcitrant wastewater background. The Gatton trial also prioritised the use of sunlight, as a means to further reduce operational costs. Based on a current 1000 L pilot unit in domestic wastewater (See Section 3.1.2 below), the project team designed a flat panel flow cell to grow PPB attached to screened sunlight illuminated surfaces.

Based on the batch screening study and the continuous bioreactor operation we concluded that PPB can effectively treat poultry processing wastewater, but that dairy and piggery flush manure could also be treated with moderate efficiencies. There were a number of unsuccessful attempts to secure a third-party trial site with a poultry processor. Unfortunately, our contact at the poultry processing company in Brisbane left and the implementation of the onsite pilot unit was delayed and eventually suspended. Instead, the project team decided to move forward and install and operate the pilot system at a piggery at UQ Gatton campus. This pilot system was to treat piggery flush manure as-is from the pig sheds prior to onsite treatment in existing anaerobic ponds. The wastewater at this site is typical of conventional pork production. This integrates very well with covered pond technologies in pork production (Figure 18). The larger pilot trials were not aiming for complete treatment of the wastewater but rather to maximise biomass growth with minimal energy inputs.

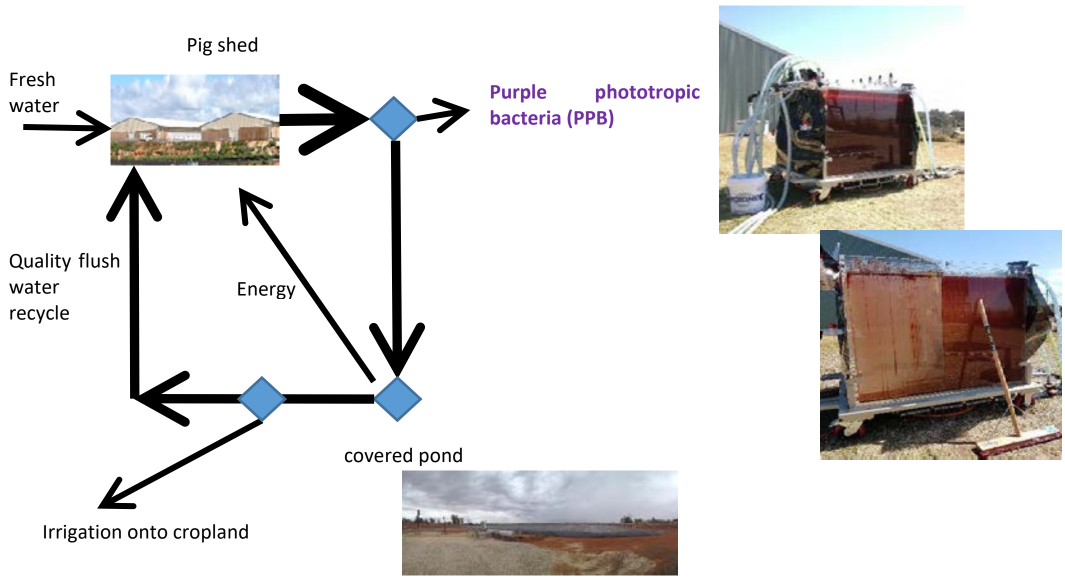

Figure 18. Integration of purple phototrophic bacteria treatment with other conventional manure wastewater handling in conventional pork production.

Wastewater treatment performance

The pig manure flush wastewater, contained faeces, urine, wash water, undigested feed, and other gritty particulates (e.g. pig hair, sand). The untreated wastewater loaded into the photobioreactor cartridges contained on average: 4.1 g/L tCOD, 1.42 g/L sCOD, 860 mgN/L NH_4-N, 69 mgP/L PO_4-P, and 116 mg/L acetic acid. The average TSS and VSS were 2070 mg/L and 320 mg/L, respectively. The pH was on average 7.95. Figure 19 shows tCOD and sCOD measurements for a 100 L and 80 L bioreactor cartridge.

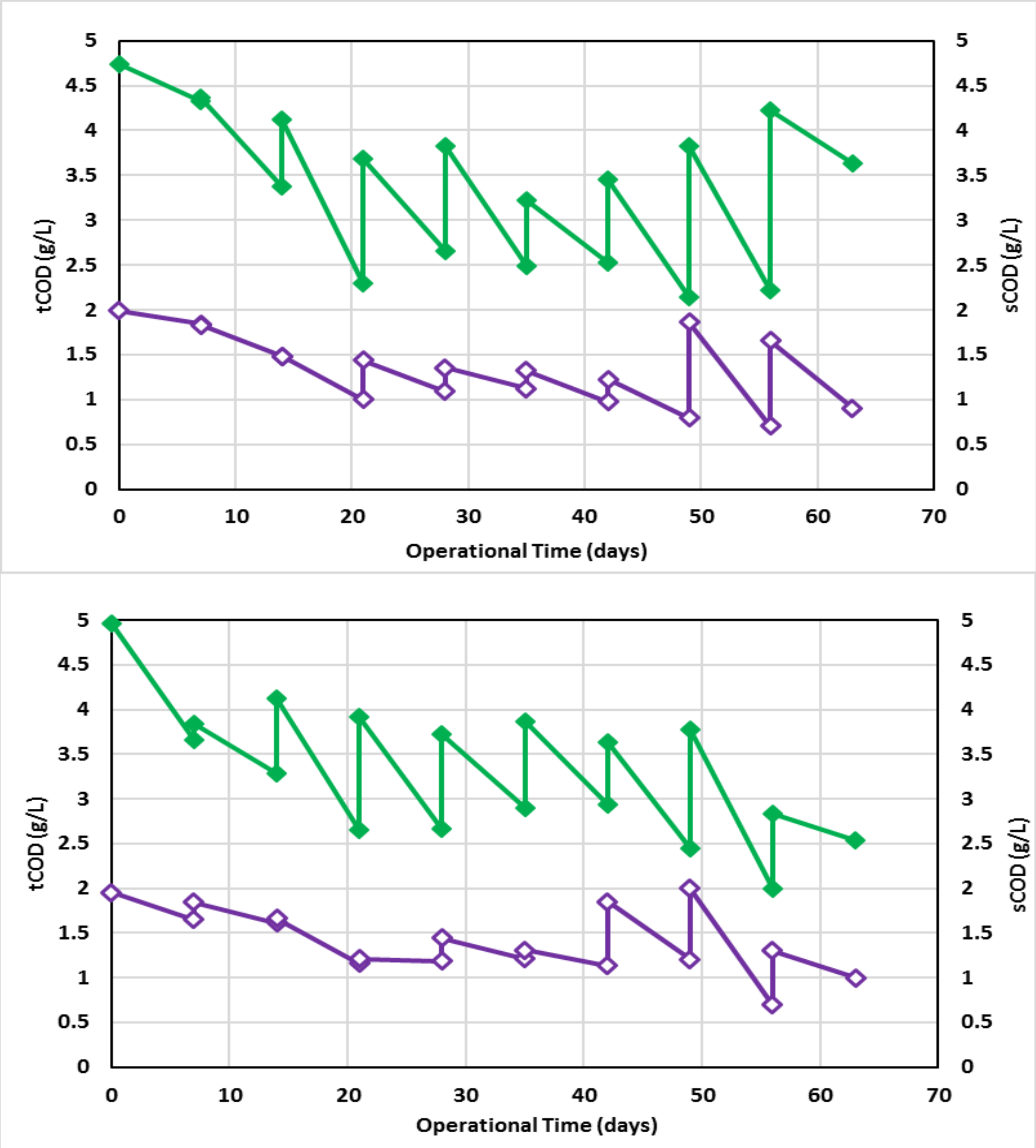

Figure 19. Measurements of tCOD (closed symbols) and sCOD (open symbols) over time for (top) the 100 L photobioreactor cartridge and (bottom) the 80 L photobioreactor cartridge.

The initial start-up of the system occurred during the first 30 days or so, after which PPB was clearly visible attached (covering the entire surface) to the photobioreactor cartridge walls (Figure 4). This is encouraging, because no inoculum was added and the system self-inoculated, and yet grew PPB within a short start-up period of 15 days. The start-up of the system was therefore quite simple as compared to other anaerobic systems like covered anaerobic ponds, which can take months to commission.

The multiple step-wise increases in tCOD or sCOD concentration shows when fresh wastewater was intermittently added at the start of each operational batch, and the downward sloping lines between these step-wise increases represent removal of tCOD and sCOD by the treatment of wastewater by the cartridge bioreactor. A decrease in tCOD shows deposition of COD as microbial biomass grows onto the illuminated walls or settling of organic solids (despite intermittent mixing, Section 2.1.3). Removal of sCOD is expected to be mostly due to microbial biomass growing onto the illuminated cartridge walls. From the results in Figure 18, it is clear that the cartridge bioreactors only removed part of the organic matter present in the wastewater, with a minimum of 0.65 g/L sCOD at the end of an operational batch.

Figure 20 below presents aerial productivities during the various batches of operation of the pilot, and separately for the 100 L and 80 L cartridges, and for each of these, the productivities for the western and eastern illuminated walls. The areal productivity is measured as dry biomass weight recovered per illuminated surface area per day (gTS.m^{-2}.day^{-1}). Areal productivities are very important for determining the efficiency and feasibility of a photobioreactor. For reference, with a higher productivity, a smaller system size (lower capital cost) is required to produce a certain quantity of biomass. The results in Figure 19 show that biomass productivity was consistently as high as 5.5 - 10 gTS.m^{-2}.day^{-1}. It was noted that when the cartridge was free drained too rapidly, a considerable proportion of the attached biomass sloughed off and so was lost. These biomass productivities are comparable to those observed elsewhere for micro-algal growth on anaerobically digested piggery flush manure (Moheimani et al., 2017). These areal productivities are also high compared to immobilised/attached algae biofilm productivities (e.g. 0.71. (Ozkan et al., 2012) and 0.58 - 2.57 g m^{-2} d^{-1} (Johnson & Wen, 2010)).

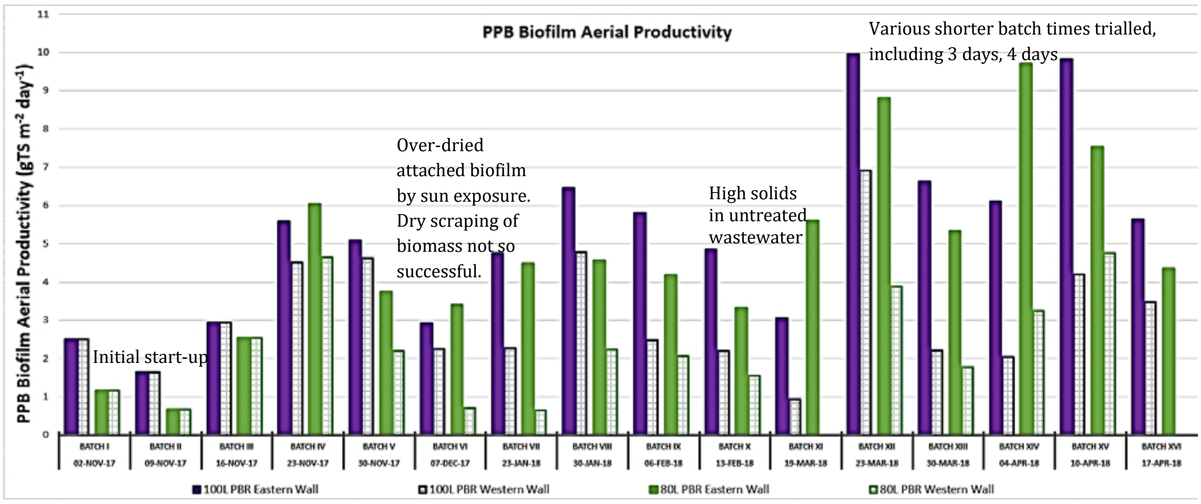

Figure 20: PPB Biofilm Areal Productivity. Note in batches I, II, and III, the cartridges were harvested together.

Important, because the raw piggery effluent was uncharacteristically low in VFAs, from batch VII onwards, acetic acid was added to the cartridges at 200-500mg/L to test the impact on biomass productivity. This was done because VFA levels in wastewater at the Gatton piggery were characteristically low, thought to be due to relatively high flush water volumes used in the piggery. For example, the pork flush manure for which the batch screening tests were performed contained >1000 mg/L VFAs (Hülsen et al., 2018a). The addition of VFA in this case, made the wastewater treated here similar to the pig flush manure treated in the batch screening tests. Importantly, the biomass productivity increased substantially when acetic acid was added, again showing the strong preference of PPB to grow on VFAs in wastewater, as noted in the batch screening tests above. Other challenges with operation (Figure 19) included difficulty to sustain a PPB culture when the TSS in the wastewater was high at 3% or above. This could have been due to excessive light attenuation at the higher solids concentrations (albeit that the harvested PPB biomass grew directly on the illuminated surface) or due to shear sloughing of microbial biomass from the cartridge walls during intermittent mixing.

It was encouraging that microbial biomass production improved when the batch time was shortened to 4 days and even 3 days in the later batches (down from the typical 6-day batch time in the earlier batches). This could impact on the extent of wastewater treatment but could mean that the system could be intensified to save on capital costs.

Figure 21 below shows measured temperature data for liquid in the 60 L cartridge. From these results, it is noteworthy that the cartridges operated at temperatures higher than 40°C for up to half of their operational life. This is a very encouraging observation, because it showed that the mixed culture PPBs present in the photobioreactors were extremely robust, withstanding regularly switching from mesophilic to thermophilic ranges, whilst maintaining productivity. In this regard, growing microalgae can require cooling to maintain productivities (Mata et al. (2010); Tredici and Materassi (1992)) (Béchet et al., 2010; Schenk et al., 2008), adding to cost and complexity of microalgal cultivation systems.

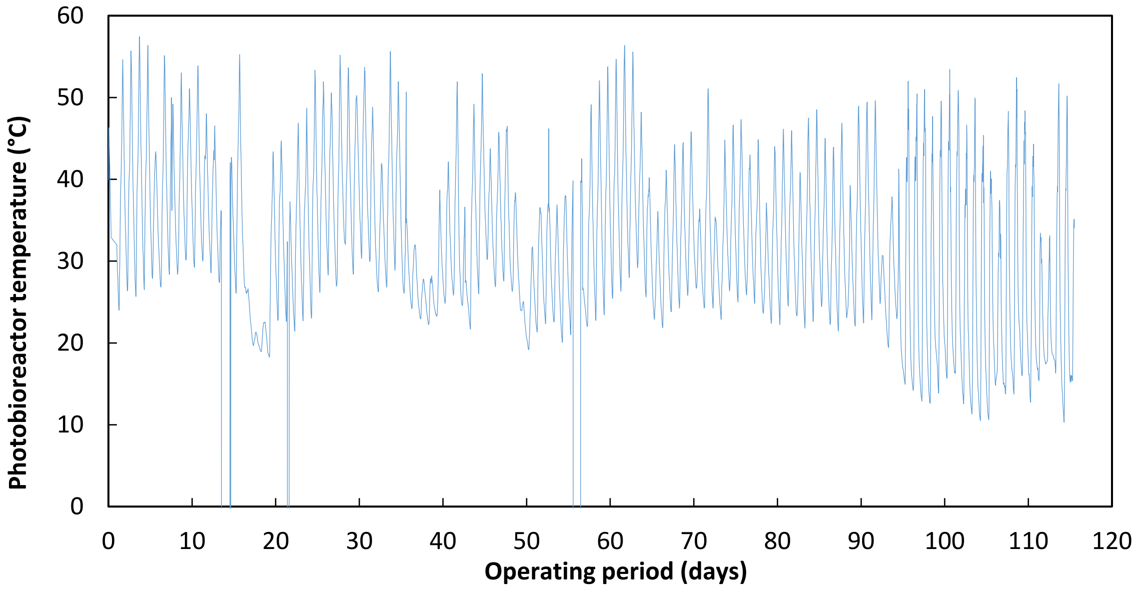

Figure 21: Temperature within the 60L photobioreactor over the trial period. Note that the incidents where temperature dropped to zero, is when the sensor was intermittently switched off to extract logged data.

Dissolved oxygen measurements on the cartridge reactors showed that they were operating essentially at 0 mg/L dissolved oxygen, with any dissolved oxygen intermittently introduced when wastewater was loaded, being rapidly consumed by biological activity in the cartridge.

<u>Microbial Biomass</u>

The microbial biomass collected from the cartridge walls was scraped upwards using a rubber Squeegee. This was effective to recover the biomass separate from the contaminated wastewater matrix and was expected to improve product quality. Importantly, the harvested biomass had a high protein content (588 mg CP.g VS^{-1}) and was at a high solids (TS) concentration of 10% with a high organic matter (VS) proportion at 73% of TS. These were very encouraging observations, because;

1. such a concentrated product would require much less energy for drying, and
2. the typical challenges of harvesting associated with microalgal systems and suspended biomass systems. Substantial energy is typically required for post treatment steps to up-concentrate suspended microalgae from 0.5 g.L^{-1} (0.05% DW) in open ponds and 2-6 g.L^{-1} (0.2-0.6% DW) in closed systems (PBRs) (Brennan & Owende, 2010; Davis et al., 2011) up to a reasonable dry product for applications. It has been reported that the harvesting and dewatering of suspended growth systems can contribute as much as 30% of the biomass production costs (Molina Grima et al., 2003).

The nutrient content of the biomass was 0.084 g N.g TS^{-1} and 0.049 g P.g TS^{-1}, which may be interesting from a slow release fertilizer perspective. Heavy metals in the product included copper at 1.18 mg.g TS^{-1} and Zinc at 1.18 mg.g TS^{-1}. Other heavy metals were at or below detection levels by ICP analysis.

Future application of wastewater treatment technology (Activity 5, Output 5(b); Activity 6, Output 6(a); Activity 7, Output 7(a), Output 7(b))

A life cycle analysis generally indicates that algae in closed photobioreactors would be cost-prohibitive, unless high-value products could be produced in a sterile medium with axenic cultures (Resurreccion et al., 2012). Although areal productivities can be high and control of predation and culture composition is increasingly being achieved with microalgal systems (Taiganides, 1992b), product inhibition and cooling requirements with microalgal systems are on-going challenging, also in terms of overall operating cost. Regardless, where space, sunlight and temperature are not limiting, open raceway ponds or high rate algal ponds could still be viable options. Microalgae seemed to outperform PPB system in terms of wastewater treatment.

Patenting of attached growth based on a tubular reactor design was pursued with funding partners Water Sensitive Cities CRC and the Smart Water Fund. The bioreactor designs used in these domestic projects were different from the Gatton pilot set-up of the present project. The outcome was that patenting potential of the attached growth concept was weak, because of prior art and material available in the public domain. An integrated position paper was published from this parallel domestic wastewater work (Matassa et al., 2015) (Batstone et al., 2015), **not** funded by the present project.

The scale up and deployment of the current cartridge system seems feasible. This would require a commercialisation partner and a major primary industry partner. From the testing in the present project, it seems most feasible to deploy the technology in the pork, poultry or red meat processing sectors, because the baseline data from this project provides some certainty about the treatability of wastewaters from these sectors. Sunlight instead of active illumination ensures cost-feasibility, and wastewater derived nutrients circumvents nutrient costs while this technology removes carbon and nutrients from the wastewater, which save significant discharge costs.

PPB appears to be the preferred platform for recovery of microbial biomass, with PPB sourcing its chemical energy from the wastewater. This means that PPB prefers rapidly bioavailable VFAs, which must be present in the raw wastewater, or can be sourced from fermentation of other biodegradable organic matter. A reduced light energy input in combination with a high yield and high crude protein content could balance capital and harvesting costs. A value proposition comparable to soybean and poultry by-product meals (370 and 550 USD tonne^{-1}, prices at 09-2016) may be feasible (Hülsen et al., 2018a). However, PPB biomass could be targeted at supplementing a part of fishmeal in conventional aquaculture feeds (>1400-1800 USD tonne^{-1}) as was trialled in the study reported below. We note that high grade feed mixtures might be downgraded to lower categories if co-mixed with animal by-products (e.g. protein from animal-derived wastewaters). This may lead to a preference for non-animal derived wastewaters, e.g. sugar mill, and these aspects are worthy of further exploration.

3.1.2 Novel Feeds

The activities described in this section tested as an aquaculture feed, the purple phototrophic bacteria (PPB) biomass produced by the alternative wastewater treatment technology (Section 3.1.1). This section summarises a full journal paper (Delamare-Deboutteville et al., 2018). The co-authors of the journal paper (Delamare-Deboutteville et al., 2018) are acknowledged and thanked for allowing inclusion of this work in the project report. The tests described here were carried out under approval granted by the University of Queensland Native and Exotic Wildlife and Marine Animals (NEWMA) Ethics Committee (permit SBS/130/17).

Fish feed trials (Activity 6, Output 6(a); Activity 7, Output 7(c); Activity 8, Output 8 (a)))

<u>Feed characteristics</u>

Table 4 summarises characteristics of the PPB used in the feed of the fish trials. Because this PPB was produced on synthetic wastewater, and for comparison purposes, Table 4 also presents measured characteristics for PPB produced on poultry processing wastewater. In comparison, the PPB used in the fish trials had a comparable protein content of 58% (w/w), a lower fat content of 3.4% (w/w) and a lower carbohydrate content of 18.8% (w/w).

Table 4. PPB biomass composition grown on different sources.

		Synthetic wastewater	Poultry*
TKN	g N kg^{-1}	121	93
TP	g P kg^{-1}	19	11
Protein	w/w%	58	58
Fat	w/w%	3.4	24.9
Carbohydrates**	w/w%	18.8	29.9
Ash and minerals	w/w%	7.8	5
Gross Energy	MJ kg^{-1}	21.3	22.4
Total Carotenoids****	g kg^{-1}	6.8± 0.3	Not measured
Alpha + beta carotene	ug 100g^{-1}	<5	Not measured
Total BChlorophyll****	g kg^{-1}	11.3 ± 0.8	Not measured
Amino acids***			
L-Alanine	g kg^{-1}	43.9	25.4
L-Arginine	g kg^{-1}	28	20.7
L-Aspartic Acid	g kg^{-1}	42.6	32
L-Cystine	g kg^{-1}	n/a	
L-Glutamic Acid	g kg^{-1}	49.9	37.3
L-Glycine	g kg^{-1}	28.4	16.2
L-Histidine	g kg^{-1}	13.5	8.2
L-Isoleucine	g kg^{-1}	24.9	16.7
L-Leucine	g kg^{-1}	45.9	30.8
L-Lysine	g kg^{-1}	28.4	18
L-Methionine	g kg^{-1}	9.1	8.9
L-Phenylalanine	g kg^{-1}	28.9	19.9
L-Proline	g kg^{-1}	22.1	14.6
L-Serine	g kg^{-1}	20.6	13.8
L-Threonine	g kg^{-1}	28.1	18.6
L-Tyrosine	g kg^{-1}	18.4	15.1
L-Valine	g kg^{-1}	33.4	22.5
Total amino acids	g kg^{-1}	466.1	318.7

*based on gTSS rather than gTS adapted from Hülsen et. al. (2018) **calculated (100-protein+fat+ash+moisture). ***Amino acids from PPB biomass grown on synthetic wastewater (WW) analysed by Symbio and from domestic wastewater by Ridley. ****Total carotenoids and Bchlorophyll analysed at UQ. TKN: total Kjeldahl nitrogen, TP: total phosphorous, PHB: polyhydroxybutyrate.

Total carotenoid content in the PPB was 6.8 g kg^{-1}. Concentrations of alpha- and beta- carotene were below 5 µg $100g^{-1}$. Total bacteriochlorophyll was 11.3 g kg^{-1}. Microbial composition analysis confirmed dominance of PPB (>70% of sequences). Moisture, crude lipid, crude protein and crude ash ranged from 3.0-5.2%, 11.8-13.3%, 55.5-57.3% and 8.0-9.4%. Diets 3 and 4 containing the two highest inclusions of PPB and were much darker in colour than the diets with lowest inclusions (Figure 22). The pellets, regardless of the diet, retained structural stability after 10 min immersion in water, albeit that water receiving diets 3 and 4 were slightly more turbid at 10-15 minutes after feeding.

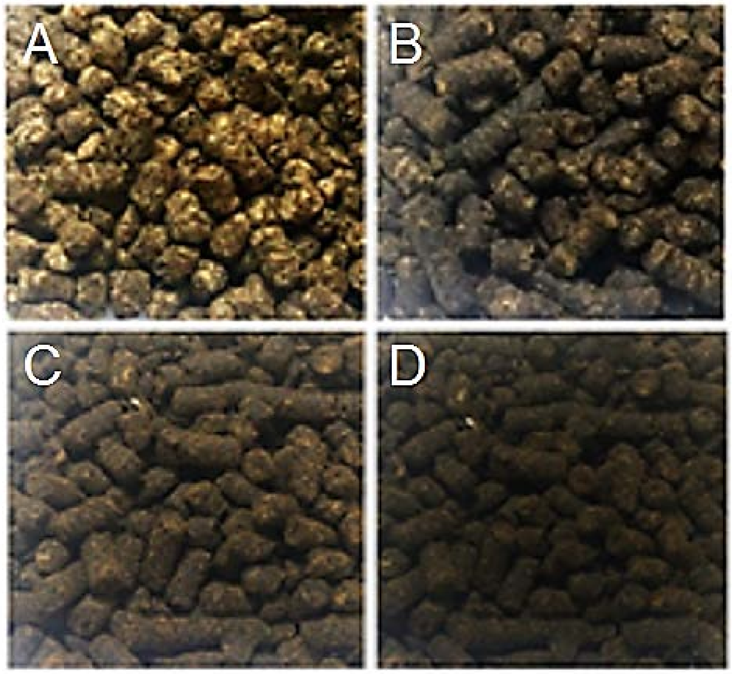

Figure 22. Diet pellets. A. diet 1 (0% PPB); B. diet 2 (33% PPB); C. diet 3 (67% PPB); D. diet 4 (100% PPB).

Fish growth performance

Figure 23 summarises growth performance measures for the fish feed trial (daily growth rate, feed intake, feed conversion ratio (FCR), weight length gain and survival). Overall, the fish readily ate the feed provided even with 100% PPB inclusion (daily feed intake not impacted by diet) and fish survival was high (94%-100%) and not significantly affected by PBB inclusion ($p > 0.05$). However, growth and length and weight gain were negatively affected by PPB inclusion (Figure 23).Figure 24 presents photos, showing the typical appearance of fish at the end of the fish trial.

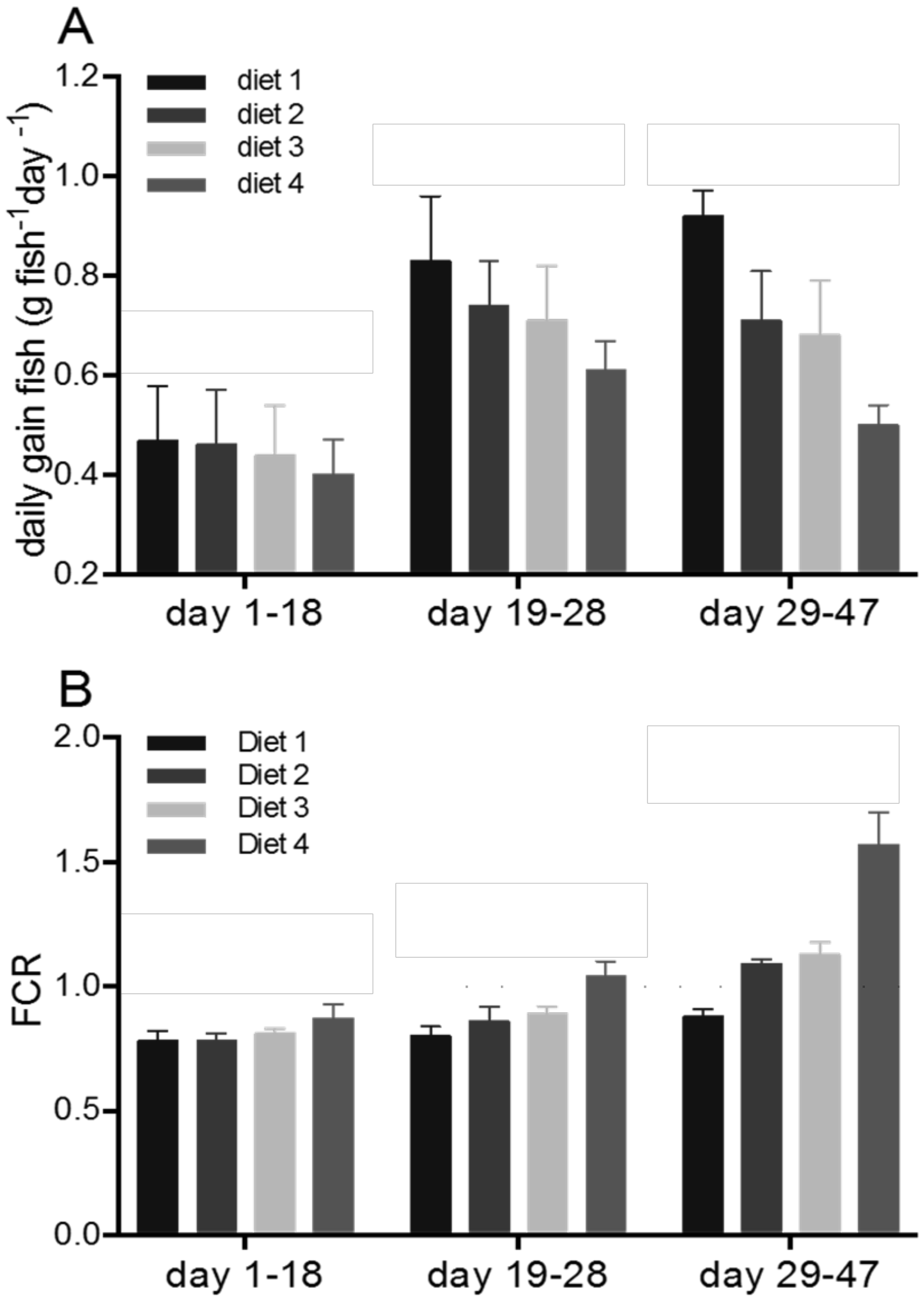

Figure 23. Performance measures for Lates calcarifer juveniles fed different diets formulated with varying inclusions of fishmeal (FM) and purple phototrophic bacteria (PPB) (diet 1: 30%FM-0%PPB, diet 2: 20%FM-10%PPB, diet 3: 10%FM-20%PPB, diet 4: 0%FM-30%PPB). Error bars show 95% confidence intervals on calculated mean values for three replicate tanks.

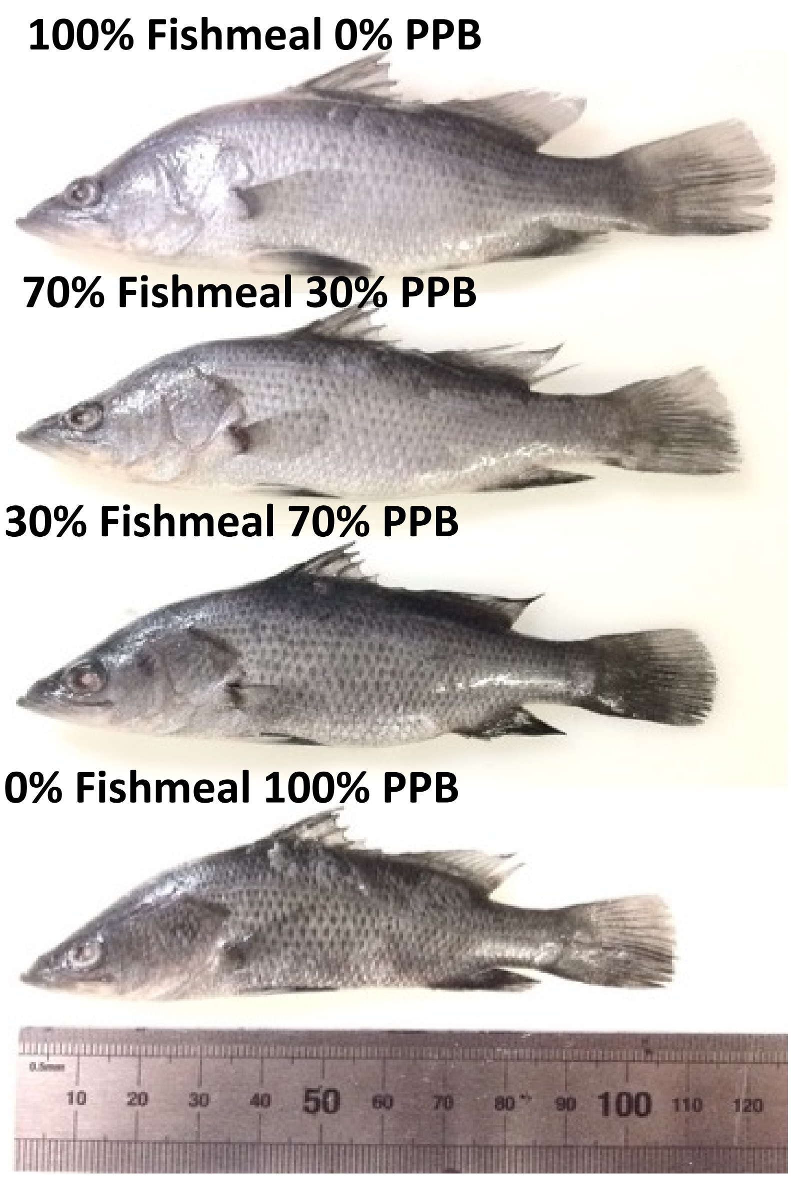

Figure 24. Example photos of the physical appearance of fish at the end of the trial. Please note that these fish were frozen and then defrosted prior to taking this photo.

Applications (Activity 8, Output 8(b))

Aquaculture has been the fastest growing animal food-protein industry, representing 50% of all food fish (Tacon & Metian, 2013). Considering its importance and growth, replacement or reduction of the main feed protein source in aquaculture feeds, namely fishmeal, is critical to the continued future growth of fish farming (Naylor et al., 2009; Troell et al., 2014). Microbial protein is particularly interesting, because it is said to have a range of advantages as a feed ingredient (Matassa et al., 2016). There has been substantial interest in proteins from algae and yeasts in animal feeds (Matassa et al., 2015; Øverland & Skrede, 2017; Yaakob et al., 2014), as well as bacterial biomass (Pikaar et al., 2017) generally characterised by a protein content of 50-65% (Anupama & Ravindra, 2000).

There has been a number of studies investigating PPB as feed supplement at very low inclusion rates in economically important species such as Nile tilapia, marble goby, barramundi and *Tor tambroides* (Banerjee et al., 2000; Chowdhury et al., 2016; Loo et al., 2015; Shapawi et al., 2012), however PPB has not been previously trialled as a bulk ingredient. This was done in the present fish feeding trials, showing for a popular tropical aquaculture fish barramundi (Asian sea bass, L. calcarifer), that fishmeal can be bulk substituted with PPB without compromising survival rate, feed intake or carcass composition, albeit with moderate negative effects on fish growth and feed conversion. The outcomes were commercially viable, given that this was initial study, with feed formulation not yet optimised. In terms of commercial viability, up to 66% of the fishmeal could be substituted with PPB without major impact on fish performance for the first 28 days (noting that the impact is progressive after 28 days). 33% fish meal replacement with PPB had little or no impact. It is noted that PPB used in this study was produced from synthetic wastewater, however, the comparison showed that it was broadly comparable to PPB derived from poultry processing wastewater. Consistent ingredient quality is very important for commercial feed formulation (Tangendjaja, 2015). PPB from different sources has been found to be reasonably consistent (Hülsen et al., 2018a). The value of bulk protein in fishmeal has ranged from 2.5–3 US$ per kg crude protein (assuming 65% CP). In addition, the treatment of wastewaters for PPB recovery adds further value, potentially lowering wastewater discharge costs whilst simultaneously transforming wastewater nutrients and carbon into protein-rich biomass at high yields.

3.1.3 Evaluation of the nutritive, economic and agronomic value of new fertilizer products against conventional products

Plant growth screening of novel fertiliser products (Activity 2, Output 2b) and product specification (Activity 8, output 8a)

A pot trial was conducted over 80 days to screen the novel fertilisers and soil conditioners developed in collaboration with our industry partners against other livestock waste by-products and in comparison, to a range of conventional synthetic fertiliser applications. Plant growth (as inferred by the total plant biomass of above-ground parts at harvest) was quantified at the end of the growing period (Mickan et al., 2017). The nutritive value (both macro and micronutrients) was also determined so that a cost benefit analysis could be performed to ascertain the nutritive, economic and agronomic value of the novel and improved by-products. Table 5 shows the nutrition profile for piggery manure and compost and the novel fertilisers developed as part of this project. Representative synthetic fertilisers commonly used in cropping, horticulture, turf, pasture and top dressing were also included in the comparison.

The majority of the novel by-products had the potential to enhance soil quality and crop productivity. The frass, advanced compost and pellets have the highest percentage dry matter content, which could enhance soil stability and structure leading to greater water holding capacity and nutrient cycling (Abbott et al., 2018). The novel fertilisers and soil conditioners contained a high concentration of carbon that provides energy for the soil microbial community and their activities (Jenkins et al., 2010; Mickan et al., 2017). The carbon to nitrogen (C:N) ratio was highly variable between the different manures types with the lowest C:N ratio found in the microalgae and PPB products. C:N ratio can affect the rate of decomposition of wastes to release nutrients for plant uptake (Hadas & Portnoy, 1997; Ren et al., 2014). Optimal waste degradation occurs at a C:N ratio of around 20:1 but can occur efficiently between 12:1 to 30:1 (Fisk et al., 2015a). The majority of the manures in this study fell within this C:N range and the manures with the highest C:N ratios (i.e. composts) were expected to degrade slowest.

Table 5 shows that the manure by-products contained a range of nutrients (NPK) that could improve soil fertility and partially replace the synthetic fertilisers. However, to avoid any potential runoff and leaching, the N, P and K supplied by these products should be matched to the crop demands (Abbott et al., 2018). Nutrient removal by crops is dependent on both the crop and soil type (Hawkesford, 2014). For instance, on the dry semi-arid soils of Western Australian's (WA) Wheatbelt, wheat removes nutrients at a 4:1:1 ratio, approximately utilising 40, 7 and 10 $kg.ha^{-1}$ of N, P and K, respectively. Commercial fertiliser companies try to match this demand in their fertiliser preparation and formulations. In WA, the standard district practice when growing wheat consists of applying a starter fertiliser, usually MacroPro, at seeding, followed by a liquid application of Flexi-N after seedling emergence. Similarly, a good compost would blend different waste by-products to create a nutritively balanced, organic fertiliser product or soil improver (Abbott et al., 2018). Overall, the novel fertilisers and soil conditioners contain a higher concentration of macronutrients compared to the alternative products on the current market and their formulations are generally more balanced and closer to the desired 4:1:1 ratio of NPK.

Table 5. Macronutrients profile for novel fertiliser products compared to commercial fertilisers and current manures and composts on the market

		Nutrient content % (w/w)					
Production system	Products	DM	C:N	C	N*	P	K
Conventional							
Synthetic fertiliser	Cropping				9.7	11.2	11.2
	Horticulture				4	7	7
	Tuft				12.8	2.3	6.2
	Pasture					7.6	8.4
	Top dressing				32		
Current market	Fresh manure	66.7	14.4	47.6	3.3	0.7	0.5
	Stockpiled manure	47.3	18.5	38.9	2.1	0.8	0.1
	Low grade compost	42.7	14.8	28.1	1.9	0.6	0.5
	High grade compost	47.3	19.7	40.3	2.1	1.0	1.7
Alternative							
Soil conditioners	Pellets	84.1	10.5	41.1	3.9	0.9	1.1
	Advanced compost	73.6	12.3	46.7	3.8	1.0	1.4
Novel fertilisers	Frass	66.2	11.5	45.9	4.0	1.1	1.3
	Algae	15.1	6.3	40.1	6.3	1.5	0.6
Slow release	PPB*	10.0	4.9	42.1	8.7	0.5	0.1
	Digestate	37.4	9.4	36.1	3.8	1.0	2.5

*N, P and K is nitrogen, phosphorus and potassium; PPB is purple phototrophic bacteria

The conventional synthetic fertiliser applications resulted in a typical fertiliser response for grain yield (Figure 25). Yield at district practice (100 kg. ha^{-1}) was 2.635 ± 0.063 g $stem^{-1}$ (mean ± SEM), while yield from the zero-fertiliser control treatment was lower (0.889 ± 0.095 g $stem^{-1}$). There was a lot of variability in plant response between the different amendments, with the majority having a yield slightly above that of the control (nil amendment). The results indicate that some of the novel fertiliser products (frass, advanced compost and microalgae) performed at a level similar to that achieved with conventional fertiliser addition and this has been observed previously for modified manure by-products (Abbott et al., 2018). This implies that the application of these products on cropping enterprises has the potential to reduce synthetic fertiliser inputs without affecting yield, thereby increasing the on-farm productivity and profitability. This was an encouraging finding, but full agronomic benefits would need to be demonstrated and verified in future studies under field conditions.

A significant decrease in yield was observed for the standard manure and compost amendments with the exception of the fresh chicken manure treatment that had a yield equivalent to 50 kg.ha^{-1} of synthetic fertiliser. This suggests that the application of chicken manure to cropping could potentially provide half of the crop's nutritional requirements, meaning that the grower could reduce their synthetic fertiliser inputs. The effectiveness of fertiliser placement will need to be evaluated in future studies in the field, also considering a full cost benefit analysis.

Despite having the highest N content of all the waste derived by-product amendments, PPB did not perform well in this study, with a yield equivalent to 10 kg. ha^{-1} of synthetic fertiliser. This is plausibly due to the high crude protein of PPB, which would degrade slowly in soil, resulting in slower more controlled release of nutrients. This makes PPB potentially interesting as a slow release fertilizer (Mulbry et al., 2005) for horticulture (particularly citrus and shrub production) and rehabilitation of disturbed land. However, slow release applications were not the focus of the present study, so subsequent testing of PPB shifted towards use as an alternative feed. It is recommended that future studies test PPB as a slow release fertilizer amendment.

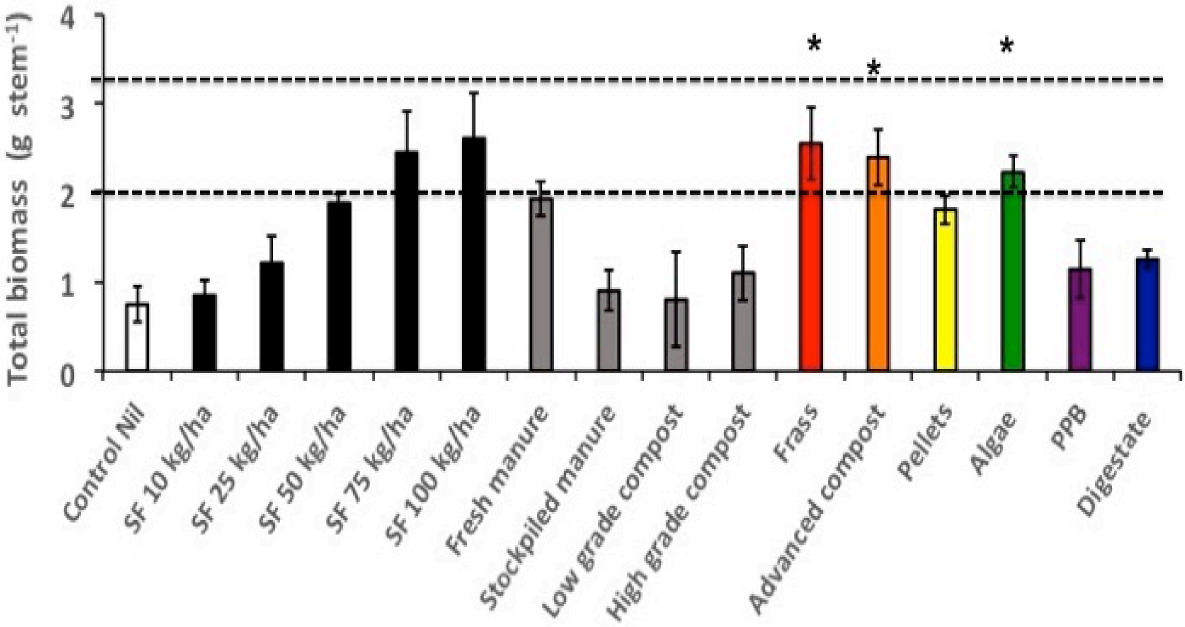

Figure 25. Total biomass production of wheat, in response to 5 rates of synthetic fertiliser (applied at 10, 25, 50, 75 and 100 kg/ha), four standard waste by-products currently on the market (fresh and stockpiled manure, low and high-grade composts). The control was no addition of fertiliser. Error bars represent standard error (n=4). Amendments not significantly different from the 100kg/ha synthetic fertiliser treatment are highlighted (*).

3.1.4 Cost benefit analysis of novel fertiliser and soil conditioner products (Activity 4, output 4c; Activity 8, output 8c) and identifying markets (Activity 8, output 8b)

Producers and growers looking to use novel fertilisers and soil conditioners are advised to do a full nutritive, economic and agronomic cost benefit analysis and soil analysis, to identify which products and nutrients would be most beneficial for their soils and crops.

The following calculations were performed using a modified calculator based on the Australian Pork Limited (APL) *Piggery manure and effluent and soil quality* website and the *Farm Gross Margin 2015* published on the GRDC website.

Nutritive cost benefit

The cost benefit analysis in Table 6 shows that the novel fertiliser products have a higher nutritive value compared to the standard manure and compost products, specifically, almost double the value. The microalgae, PPB and digestate contain the highest concentration of nutrients.

Table 6. Cost benefit analysis to determine nutritive value of novel fertilisers in relation to current manure and compost products on the market

	Values of nutrients applied ($/t)			
Products	N	P	K	Total
Fresh manure	39.7	28.0	8.0	75.7
Stockpiled manure	25.3	32.1	1.4	58.8
Low grade compost	22.8	24.0	8.0	54.8
High grade compost	24.6	40.0	27.2	91.8
Pellets	51.1	36.0	17.6	104.7
Advanced compost	46.0	40.0	22.4	108.4
Frass	48.2	44.7	21.1	114.0
Algae	76.1	58.0	10.2	144.3
PPB	103.8	21.8	1.0	126.6
Digestate	46.0	41.5	39.4	126.9

Agronomic and economic cost benefit

A full cost benefit analysis (CBA) was performed on the yield data from the screening trial (Figure 25) and assumptions detailed in the method (Section 2.3.11), using a modified calculator published on the GRDC website and grower guidelines.. The calculations are based on a grain grower specific and assume they will cover the cost of freight from the livestock enterprise (poultry, piggeries on feedlot farms) either within the shire (<30km) or outside the shires (>150 km). For the high-quality composts, frass and novel fertilisers (PPB, microalgae, pellets), CBH a major grain freight operator, will consider back loading the freight trains if the product is shown to be safe to handle with a low pathogen and biosecurity risk. Under this scenario, it is assumed that the cost of freight will be greatly reduced to 'within' shire costs providing the producer is within 30km radius of the nearest CBH collection point.

The CBA in Table 7 shows that with the exception of frass and advanced compost, using the novel fertilisers in wheat cropping did not result in a net benefit when compared to applying the synthetic fertiliser at 100 kg/Ha (district practice). However, there was increased financial gain in terms of both increased crop productivity and reduced input (variable) costs as a result of applying frass ($76.72) or advanced compost ($ 20.16) compared to the synthetic fertiliser treatment. The next best novel fertiliser relative to the untreated manure was the microalgae, whose application resulted in a loss of $39.52 compared with a loss of $79.61 from the manure application. Turning livestock manures and effluents into pellets, PPB and digestate resulted in a more stable by-product where nutrients were released gradually into the soil over time. Consequently, application of these products resulted in the large loss when compared to standard practice of applying synthetic fertiliser.

Table 7. Cost benefit analysis to determine the agronomic and economic value of novel fertilisers in relation to current manure and compost products on the market when applied within a 30 km radius from source. Where FM=fresh manure, SM= stockpiled manure, LGC = low grade compost, HGC = high grade compost, Frass, AC = advanced compost, P= pellets, AL = microalgae, Dig = digestate, SF = synthetic fertiliser applied at 100 Kg/ha and Con = control (nil amendment).

$/ha	FM	SM	LGC	HGC	Frass	AC	P	Al	PPB	Dig	SF	Con
Grain quality	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1
Grain price (AGP1)	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0
Yield (mean)	1.9	0.9	0.8	1.1	2.5	2.4	1.8	2.2	1.1	1.3	2.6	0.7
Gross income	503.7	234.9	208.8	287.1	665.2	625.1	472.4	582.0	297.5	326.7	680.7	195.6
Levies	5.0	2.3	2.1	2.9	6.7	6.3	4.7	5.8	3.0	3.3	6.8	2.0
Seed	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4
Chemicals	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1
Fertiliser + freight	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	118.9	0.0
Grain freight	30.9	14.4	12.8	17.6	40.8	38.3	29.0	35.7	18.2	20.0	41.7	12.0
Manure cost	8.0	12.0	18.0	30.0	25.0	30.0	30.0	50.0	50.0	25.0	0.0	0.0
Application	20.0	20.0	10.0	10.0	10.0	10.0	0.0	10.0	10.0	20.0	5.0	0.0
Freight inside the Shire (<30 km)	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	0.0	0.0
Operations (fuel & maintenance)	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9	10.5
Contract (fertiliser application)	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	18.5	0.0
Crop Insurance	4.0	1.9	1.7	2.3	5.3	5.0	3.8	4.7	2.4	2.6	5.4	1.6
Variable Costs	224.8	207.5	201.4	219.6	244.6	246.4	224.3	263.0	240.5	227.8	336.9	147.5
Gross Margin	278.9	27.4	7.4	67.5	420.6	378.7	248.1	319.0	57.1	98.9	343.8	48.1
Profit relative standard practice	-64.9	-316.4	-336.5	-276.4	76.7	34.9	-95.8	-24.8	-286.8	-244.9	0.0	-295.8

Table 8 shows that the use of manures, composts and novel fertilisers outside the shire are less financially rewarding when compared to using these by-products within the Shire in close vicinity to the livestock enterprises. This is due to the high cost associated with transporting by-products long distances. Nevertheless, the frass and advanced compost treatments were still profitable.

Table 8. Cost benefit analysis to determine the agronomic and economic value of novel fertilisers in relation to current manure and compost products on the market when applied up to 150 km away from source. Where FM=fresh manure, SM= stockpiled manure, LGC = low grade compost, HGC = high grade compost, Frass, AC = advanced compost, P= pellets, AL = microalgae, Dig = digestate, SF = synthetic fertiliser applied at 100 Kg/ha and Con = control (nil amendment).

$/ha	FM	SM	LGC	HGC	Frass	AC	P	Al	PPB	Dig	SF	Con
Grain quality	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1
Grain price (AGP1)	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0
Yield (mean)	1.9	0.9	0.8	1.1	2.5	2.4	1.8	2.2	1.1	1.3	2.6	0.7
Gross income	503.7	234.9	208.8	287.1	665.2	625.1	472.4	582.0	297.5	326.7	680.7	195.6
Levies	5.0	2.3	2.1	2.9	6.7	6.3	4.7	5.8	3.0	3.3	6.8	2.0
Seed	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4
Chemicals	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1
Fertiliser + freight	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	118.9	0.0
Grain freight	30.9	14.4	12.8	17.6	40.8	38.3	29.0	35.7	18.2	20.0	41.7	12.0
Manure cost	8.0	12.0	18.0	30.0	25.0	30.0	30.0	50.0	50.0	25.0	0.0	0.0
Application	20.0	20.0	10.0	10.0	10.0	10.0	0.0	10.0	10.0	20.0	5.0	0.0
Freight outside the Shire (<150 km)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	0.00	0.00
Operations (fuel & maintenance)	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9	18.9	10.5
Contract (fertiliser application)	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	18.5	0.0
Crop Insurance	4.0	1.9	1.7	2.3	5.3	5.0	3.8	4.7	2.4	2.6	5.4	1.6
Variable Costs	239.5	222.2	216.1	234.3	259.3	261.1	239.0	277.7	255.2	242.5	336.9	147.5
Gross Margin	264.2	12.7	-7.3	52.8	405.9	364.0	233.4	304.3	42.4	84.2	343.8	48.1
Profit relative standard practice	-79.6	-331.1	-351.2	-291.1	62.0	20.2	-110.5	-39.5	-301.5	-259.6	0.0	-295.8

Identifying market opportunities (Activity 8, Output 8c)

Table 9 shows the predicted cost of product under different markets based on surveys of the current market for biological products. The CBA revealed that the frass and advanced composts were both economically viable when compared to the standard fertiliser practice. This means these products could exploit a whole range of different markets from conventional markets (comparable to synthetic fertilisers) to high end and specialist markets like hydroponics, with a retail value of between $8-80. The microalgae fertiliser is probably better suited to a niche market or organically certified market where is can retail anywhere from $50 up to $100. This will help recover some high input cost from microalgae production ($2000/t). The PPB and pellets also have higher associated costs of production and would be better developed as slow release fertilisers and tailored to a range of commercial markets ($30-100+) from larger retailer (e.g. Bunnings) to the niche and organically certified specialist markets. Finally, the digestate is probably most suitable to local or direct grower markets owing to its restrictive use in Western Australia. Currently, growers need to hold a licence to apply digestate to their land. Also, the large cost associated with the transportation of digestate prevents it from being transported long distances.

Table 9. Predicted cost of products under different markets based on current biological, manure and compost products on the market. Where FM=fresh manure, SM= stockpiled manure, LGC = low grade compost, HGC = high grade compost, Frass, AC = advanced compost, P= pellets, AL = microalgae, Dig = digestate, SF = synthetic fertiliser applied at 100 Kg/ha and Con = control (nil amendment).

Target Market ($/t)	FM	SM	LGC	HGC	Frass	AC	P	Al	PPB	Dig
Direct to grower	8	12	18	30	25	30	30	30	30	25
Local markets	5	5	10	15	12	15	15	15	15	12
Composting facilities	8	8	8	8	8	8	8	8	8	8
Larger retailers	10	10	15	20	20	25	35	35	35	20
Organically certified	15-30	15-30	20-30	20-50	20-50	20-50	30-60	30-60	30-60	25-40
Niche and specialist	0	0	0	20-60	20-80	20-80	50-100	50-100	50-100	20-50

3.1.5 Overcome barriers to adoption by involving primary producers during field trials to assist early adoption.

Farmer surveys (Activities 2-4, Output 2c, 3c, 4d)

Primary industries were consulted early on the project, and this highlighted a number of barriers to adoption for the use of waste by-products, including:

1. Knowing how to decide whether soil needs amendments and which amendment
2. Economic feasibility of novel fertilisers, composts and manures and their sustainable supply
3. Logistical problems of utilising organic matter (e.g. transportation, application rates and requirements for additional machinery or equipment),
4. Validation and cost benefit analysis of novel fertilisers to determine their agronomic and economic value,
5. Evaluation and quantification of their benefits (soil health and resilience) and risks (GHG emissions)
6. Continuity and quality control of organic inputs and biological products,
7. Assessment of a variety of products available and tailored to end-users.
8. Legalisation and regulation restricting their use in agriculture (e.g. Stable fly Ban Act)

The pot screening trial identified several promising novel fertilisers and soil improvers including frass, microalgae and advanced compost. The cost benefit analyses and farmer surveys revealed that the advanced compost was most cost-effective amendment, could be applied across the multiple agricultural industries and was most likely to be adopted.

Testing novel fertilisers amendments under field conditions (Activity 2, Output 2 b and c)

A series of field trials were conducted in order to assess the effects of advanced on crop performance and yield as well as the soil biology and chemical fertility. A full cost benefit analysis was performed to determine whether compost amendments i) increase productivity and profitability; ii) improved the soil health, resilience and production in semi-arid soils; iii) utilise waste by-products in such a way that it is economically feasible, with reduced operational cost and has a viable market and iv) will overcome barriers to adoption such as greenhouse gas (GHG) emissions and stable fly emergence. Farming practices that harness the soil biology and improve the soil's resilience to climate change, heat stress and drought will increase sustainability, profitability and productivity.

Site characteristics for all field trials

All trials were conducted on the same paddock at the UWA future farm in Pingelly, although crops varied according to the cropping rotation. Table 10 shows the soil is a typical acidic sandy duplex common in the Western Australian Wheatbelt with a sandy loam texture (0-30 cm) overlying deeper clayey soil (30-100 cm). The bulk density ranged from 1.22 g cm-3 in the topsoil to 1.83 g cm-3 at the bottom of the soil profile. Soils with a bulk density higher than 1.6 g cm-3 tend to restrict root growth. Hence the deeper clayey soil could impact on crop productivity. The moisture content (%) and water filled pore space (%) was greater at depth in the clayey textured soil which could mean the soil is more susceptible to waterlogging and GHG emissions during rainfall events.

Table 10. Physicochemical parameters (mean values) down the soil profile

Soil depth (cm)	pH	Clay (%)	Silt (%)	Sand (%)	Texture	Bulk density (g cm-3)	Moisture content (%)	WFPS* (%)
0-10	4.74	9.18	6.01	84.81	Sandy Loam	1.22	6.77	5.99
10-20	4.83	9.75	5.90	84.35	Sandy Loam	1.42	8.86	28.74
20-30	5.36	9.57	6.27	84.16	Sandy Loam	1.64	8.89	19.63
30-60	5.74	31.97	8.95	59.08	Clay	1.54	13.09	22.27
60-80	5.93	40.89	7.70	51.41	Clay	1.66	14.17	30.79
80-100	6.08	54.64	7.92	37.44	Clay	1.83	17.06	31.85

*water filled pore space (water filled pore space)

Soil improver and fertiliser product analysis used in the field trials

The pH of the compost samples were more neutral to alkaline, as compared to the manures, which were acidic (Table 11). With the exception of the chicken manure (4.14%), most of the manures and composts had a low percentage N content ranging from 1.05-2.53%. Care needs to be taken when applying chicken manure to soil to avoid ammonia volatilisation, which can lead to indirect nitrous oxide emissions and damage to the germinating seed, resulting in reduced crop yields (VanderZaag et al., 2011). In light of the high N content in the chicken manure it may be advisable to incorporate the manure immediately after spreading to reduce both indirect and direct N_2O emissions (VanderZaag et al., 2011). The percentage C content was highest in the pig and chicken manures (42.29 and 40.79 %, respectively) and lowest in the dairy compost (12.49% The C:N ratio of manures has a big impact on its GHG potential. The C:N ratio affects the rate of decomposition with optimal waste degradation occurring around carbon: nitrogen ratio of 20:1 but it can occur efficiently between 12:1 to 30:1 (Fisk et al., 2015a; Fisk et al., 2015b). Majority of the manures and composts fall within this range and the pig manure with the highest C:N ratio (28:1) are expected to degrade more slowly. However, the dairy compost had a low C: N ratio (12:1) implying it probably needed more time to mature. For manures with low C:N ratio (e.g. chicken manure had a low C:N ratio of 10:1), co-composting them with a rich carbon source such as crop residues, green waste or composted municipal waste can reduce their GHG potential whilst improving their degradation and release of nutrients into the soil (Abbott et al., 2018). The percentage moisture was lowest in the feedlot compost and highest in the pig manure.

Table 11. A comparison of physicochemical parameters (mean values) between different manure and compost types used in the field trials.

Soil improver	pH	Total N (%)	Total C (%)	C:N	Moisture content (%)
Chicken manure	6.74	4.17	40.79	10:1	32.81
Chicken semi-compost	7.39	1.58	21.87	14:1	30.93
Dairy compost	7.86	1.05	12.49	12:1	28.82
Feedlot compost	8.52	1.65	23.19	14:1	16.34
Pig manure	6.98	1.53	42.29	28:1	60.07
Pig compost	7.67	1.62	20.62	13:1	38.31
Chicken compost	7.49	2.53	38.00	15:1	43.73

Field trial 1: evaluate and quantify the benefits and risks (GHG emissions and stable emergence) of applying manures and composts to cropping (Activity 4, output 4d), mode of action (Activity 6, outputs 6b) and assess whether they can partially replace the synthetic fertiliser (Activity 6, outputs 6b and c)

Land application of manure and spent poultry litter can improve crop performance, carbon storage and soil quality but can also increase the risk of stable fly (*Stomoxys calcitrans*) outbreaks and greenhouse gas emissions (GHG) (Cook et al., 1999; VanderZaag et al., 2011). Stable fly outbreaks are becoming an increasing economic burden to livestock industries and rural communities of Western Australia through reduced production, recreation and tourism. Due to the stable fly's continual negative impact, the fly officially became a Declared Pest under the Biosecurity and Agricultural Management Act in 2013 (Cook et al., 1999). Under this Act, the use of raw poultry manure is banned in 12 Shires surrounding Perth. Increasing quantities of broiler litter are being produced annually and its disposal needs a sustainable management strategy. The regulation of manure disposal has led to the loss of important spent litter markets causing significant cost increases to West Australian (WA) broiler growers.

A field trial consisting of eight different amendments in triplicate (unamended; synthetic fertiliser applied at 60 and 100 kg/ha; fresh, semi-composted or composted chicken manure applied with synthetic fertiliser at 60 kg/ha; pig manure or compost applied with synthetic fertiliser at 60 Kg/ha) was established with wheat as the crop. The chicken manure (litter) and semi-compost were sourced from a poultry producer. The chicken compost (raw materials from same farm) were source from the local compost facility. The pig manure and composts were sourced from a farm and composting enterprise. Both composts were generated using mobile aerated floor composting units.

There was no significant difference in grain yield between the manure and composts treatments and 100% and 60% synthetic fertiliser treatment (Figure 26), due to the large variation of yields between replicates. However, there is a trend towards higher yields in the plots receiving the chicken litter, semi compost and compost treatment as well as the synthetic fertiliser applied at 100kg/ha. Although, this is only one year of harvest, if the trend continues, farmers on mixed cropping and livestock enterprises could potentially increase their productivity and profitability by utilising their manure fertiliser products and reducing their fertiliser input by 40%.

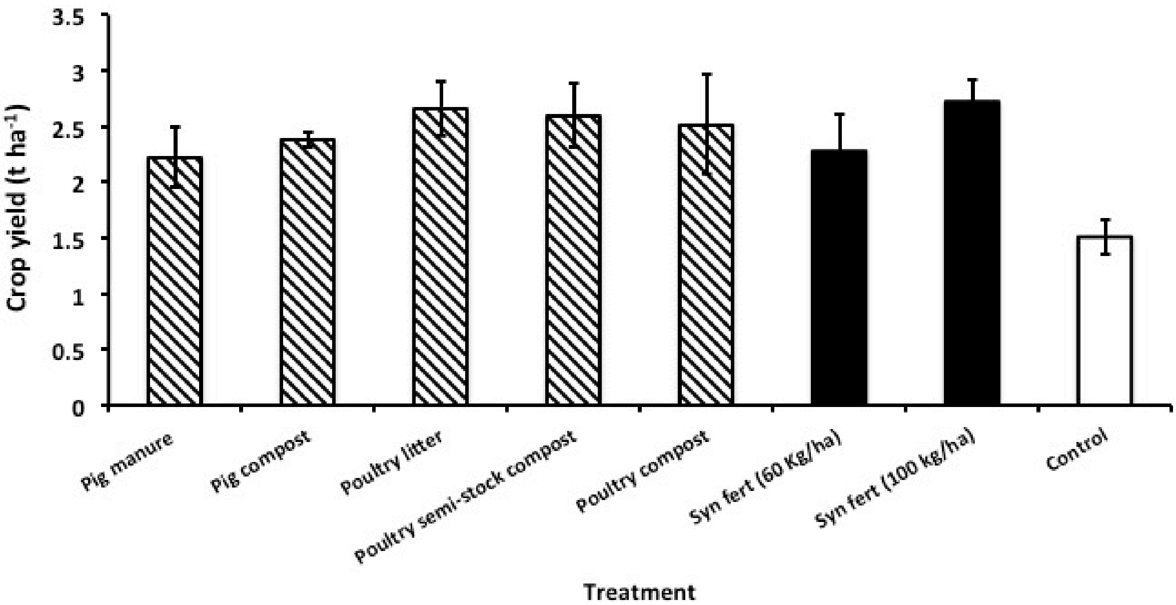

Figure 26. Wheat yield data for soil receiving different fertiliser and manure inputs at the field trial 1 site, UWA Future Farm, Pingelly, Western Australia. All treatments were done in triplicate. Error bars indicate the standard error where n=3.

A cost benefit analysis (Table 12) of the manure/compost +60% synthetic fertiliser vs synthetic fertiliser at 60% and 100% distinct practice was performed on the data from the field trial using a modified calculator from GRDC soil quality website. The calculations were done from a grain grower specific, assuming they are picking up the compost/manure from a broiler farm and transporting it to their farm, either within the banned shires (i.e., Gingin, Chittering, Swan, Kwinana, Murray, Wanneroo, Harvey, Capel, Armadale, Cockburn, Kalamunda) or outside the banned shires.

The cost for a grain grower picking up chicken litter and compost from a broiler farm and taking it outside the banned shires incurs a loss of between $6.71/ha and $10.49/ha compared to adding 60% and 100% synthetic fertiliser singly. However, the use of chicken litter and compost combined with a reduced application of synthetic fertiliser in comparison the synthetic fertiliser applied singly at district practice (100%) within the banned shires results in a profit of $8/ha and $4/ha, respectively. This illustrates how prohibitive transporting chicken litter and its by-products outside the banned shires is to broiler growers. It costs a grain grower about $16-20/t to have spent litter or compost delivered a distance of 150km, which is the typical minimum distance to cart litter from the cluster of poultry broiler farms in Gingin, WA to the closest non-prescribed Shire (e.g. Dandaragan, Victoria Plains).

Table 12. Cost benefit analysis to determine the agronomic and economic value of manure and compost products applied to cropping.

$/ha	Pig manure	Pig compost	Poultry litter	Poultry semicompost	Poultry compost	Syn fert (60 Kg/ha)	Syn fert (100 kg/ha)	Control
Grain quality	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1	AGP1
Grain price (AGP1)	261.0	261.0	261.0	261.0	261.0	261.0	261.0	261.0
Yield (mean)	2.22	2.38	2.66	2.47	2.68	2.36	2.72	1.51
Gross income	580.13	621.83	693.21	644.62	699.97	615.96	710.30	393.95
Transported within the shire (<30 km)								
Variable Costs	305.23	308.54	314.20	310.35	324.73	274.82	339.27	163.27
Gross Margin	274.90	313.30	379.01	334.28	375.23	341.14	371.03	230.68
Profit relative standard practice	-96.13	-57.73	7.99	-36.75	4.21	-29.88	0.00	-140.35
Transported outside the shire (<150km)								
Variable Costs	319.93	323.24	328.90	325.05	339.43	324.70	274.82	339.27
Gross Margin	260.20	298.60	364.31	319.58	360.53	374.78	341.14	371.03
Profit relative standard practice	-110.83	-72.43	-6.71	-51.45	-10.49	3.76	-29.88	0.00

Reduced GHG potential from applying novel fertilisers (Activity 3, output 3b)

To ensure these next generation biological products are environmentally sustainable, their ability to mitigate GHG emissions will also be evaluated by measuring GHG fluxes. Analysis of the gas composition revealed that soils amended with manure and compost were overall low GHG emitters, with only a small amount of nitrous oxide (N_2O) being produced and this has been observed before in this region (Barton et al., 2016; Barton et al., 2013b; Barton et al., 2010). Moreover, these amended soils are a major sink of methane (CH_4) (Figure 27). The greatest N_2O fluxes occurred during the seedling emergence phase of the wheat life cycle and this probably reflects an increase in water filled pore space following a significant rainfall event (25 mm) on the previous day of sampling. The anaerobic environment created by the rapid increase in water filled pore space following a rainfall event has previously been shown to favour nitrified-derived N_2O emissions (Barton et al., 2013a; Harris et al., 2013). There was a marked increase in N_2O flux when the rainfall exceeded 30 mm.d^{-1} for all soils.

In contrast, the N_2O flux was very low during the other stages of wheat development. With the exception of plots receiving chicken manure, all other the manure treatments were greater than the 60% fertiliser treatment but lower than the 100% fertiliser treatment. This implies a significant reduction in N_2O emissions relative to synthetic fertiliser applied at distinct practice (100%). Previous work has revealed that N_2O emissions from these soils were likely to be attributed to nitrifying bacteria, either through direct nitirification or via nitrifier denitrification pathways and not denitrification (Barton et al., 2013a).

There was a significant methane (CH_4) sink when amended with manures. Methane uptake was greatest in the samples taken at crop maturity just before harvest. Methane uptake was also observed in soils amended with composted manures (pig, beef and dairy) with the exception of chicken manure that performed better when untreated. Methane oxidation by soil methanotrophic bacteria is the only biological sink for CH_4 but the microorganisms and mechanisms involved are not fully understood (Stiehl-Braun et al., 2011). Applying N fertilizer and soil amendments has been shown to either inhibit or promote soil CH_4 oxidation by methanotrophs (Acton & Baggs, 2011; Stiehl-Braun et al., 2011). However, the key microbiological populations and processes responsible for methane oxidation in soils, the factors regulating their growth and whether can they be optimised is largely unknown (Acton & Baggs, 2011). Methane uptake could lead to significant reductions in GHG emissions by negating on-farm N_2O and CH4 emissions (Barton et al., 2016).

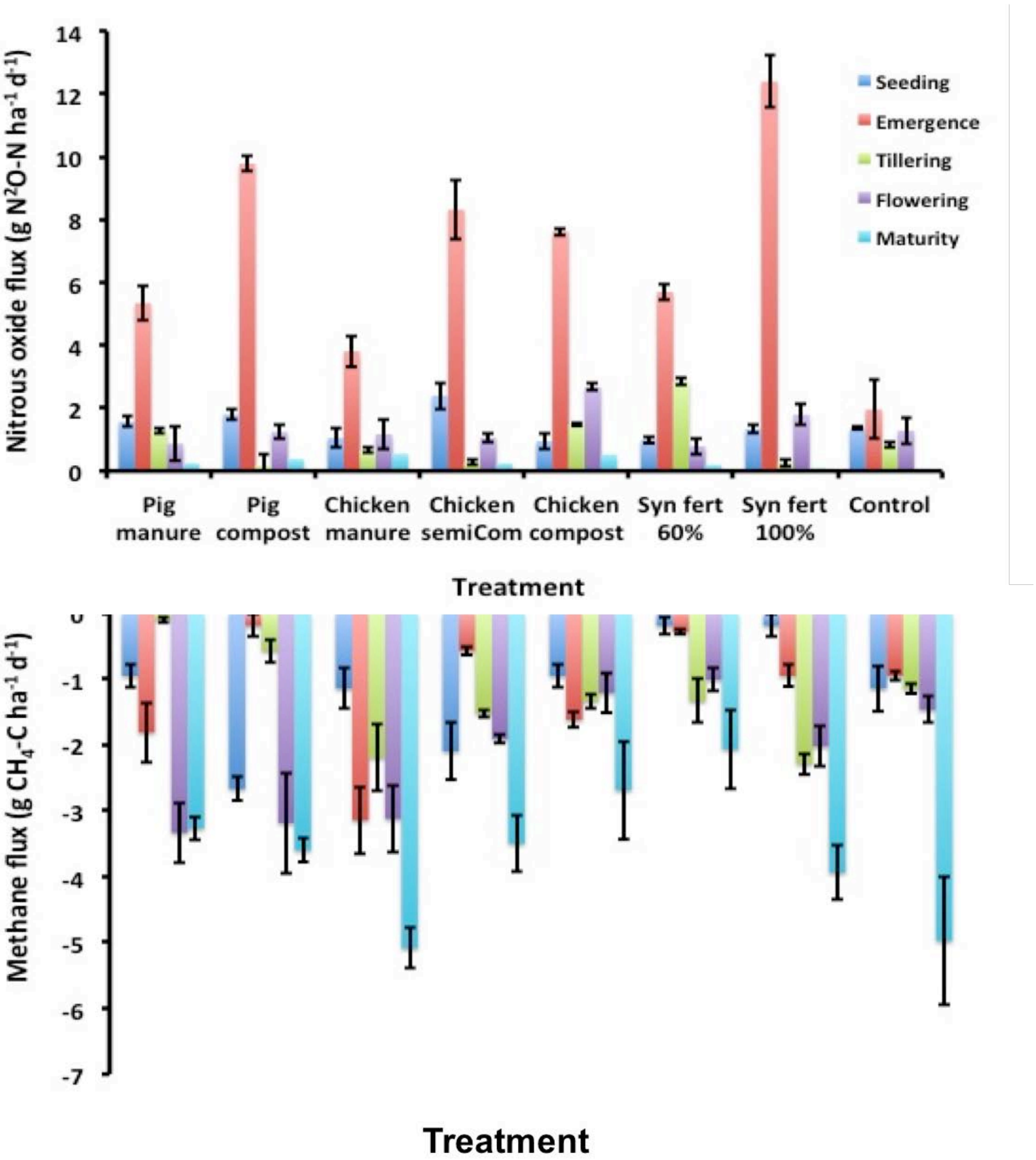

Figure 27. Cumulative nitrous oxide (N_2O) and methane (CH_4) flux at seeding, emergence, tillering, flowering and maturity during the growing season for soil amended with different manure, compost and synthetic fertiliser inputs at the 2015 field trial at UWA Farm Ridgefield, Pingelly, Western Australia. All treatments were done in triplicate. Error bars indicate the standard error where n=4.

Stable fly emergence (Activity 3, output 3b)

In Sept 2012, the Stable Fly (SF) was declared a pest under the Biosecurity & Agricultural Management Act 2000. Consequently, stockpiling, land application and movement of manure was banned in 13 Shires around Perth (Cook et al., 2011). Transporting the litter outside the banned Shires is very costly. Regulation of manure disposal options have led to loss of important manure marketing options causing significant cost increases (> 4 million), currently it costs producers $8 per m^3 to dispose of litter.

Overall, the research findings showed that there was no stable fly emergence from any of the treatments (Cook et al., Accepted). The emergence of other nuisance was also very low from broadacre soils amended with manure and compost (Table 13). The application type (stockpiled manure versus composted manure) had no significant influence on stable fly development and emergence. Moreover, stable fly and nuisance fly emergence from soils amended with chicken manure or compost (which has been identified as a major breading ground of stable flies) was comparable to soils amended with pig manure and compost.

Table 13. The mean (± standard error) number of flies emerging from soil amended with different fertiliser, manure and compost treatments at the field trial 1 site (Pingelly, WA) prior to the sowing of a cereal crop.

	CV	ANTH	PFF	RHIN	CA	SAR	SF
Pig manure	5.1±0.9	20.4±3.6	0.9±0.4	0.9±0.3	0	0	0
Pig compost	4.8±1.0	23.3±4.4	0.9±0.3	1.4±0.4	0	0.1±0.1	0
Chicken litter	2.8±0.4	24.1±5.1	0.8±0.2	0.9±0.2	0.1±0.1	0	0
Chicken semi compost	4.4±0.9	26.2±5.7	1.3±0.4	1.1±0.3	0	0	0
Chicken compost	5.3±1.7	28.6±4.4	0.8±0.2	0.6±0.2	0	0	0
Synthetic fert low	6.4±1.1	16.2±3.8	0.6±0.2	0.7±0.3	0	0	0
Synthetic fert high	5.4±1.2	18.6±4.1	1.3±0.4	1.1±0.4	0	0	0
Unamended (nil)	2.8±1.6	12.6±4.0	0.1±0.1	0.9±0.3	0	0	0

CV = Calliphora vicina (European blue bottle blowfly); ANTH=Anthomyiid flies (flower flies); PFF = Pepper Fruit Fly (Atherigona orientalis); RHIN = Rhiniinae (sub-family of calliphoridae blowflies); CA = Calliphora albifrontalis (western golden-haired blowfly); SAR=Sarcophagidae (flesh flies); SF = Stomoxys calcitrans (stable flies)

Soil benefits and mode of action of the composts (Activity 6, outputs 6b)

Manure and composts can alter chemical properties of soil such as organic matter content, pH and cation exchange capacity (CEC). Matured composts can have strong buffering capacities, and are mainly alkaline or neutral (Abbott et al., 2018). Raising the pH can increase the availability of macro-nutrient and micro-nutrient availability to plants and soil microorganisms (Abbott et al., 2018; Walker et al., 2004).

The soil pH was measured at the beginning and end of the field trial to determine whether manure and compost amendments could raise the pH of the semi-arid soil. The results show a marked increase in pH in the soil amended with chicken compost compared to the other amendments at the end of the field trial (Figure 28). Agricultural manures and wastes may have a liming effect since they usually contain basic cations such as Ca^{2+} or Mg^{2+} which serve to neutralize soil acidity (Walker et al., 2004). Furthermore, both chicken manure and compost are more alkaline than the other livestock by-products and this is correlated with a high concentration of ammonium. The addition of chicken compost could be used to increase the pH of the soil, reducing the amount of liming required especially in acidic, semiarid areas such as Western Australia. Soil pH has been identified as a key driver of microbial community structure and function (Fierer & Jackson, 2006; Jenkins et al., 2016; Jenkins et al., 2009). Through the increase in organic matter predominantly through treatment with compost or manure CEC is increased, resulting in increased availability and reduced leaching of nutrients, also improving crop nutrition (Walker et al., 2004).

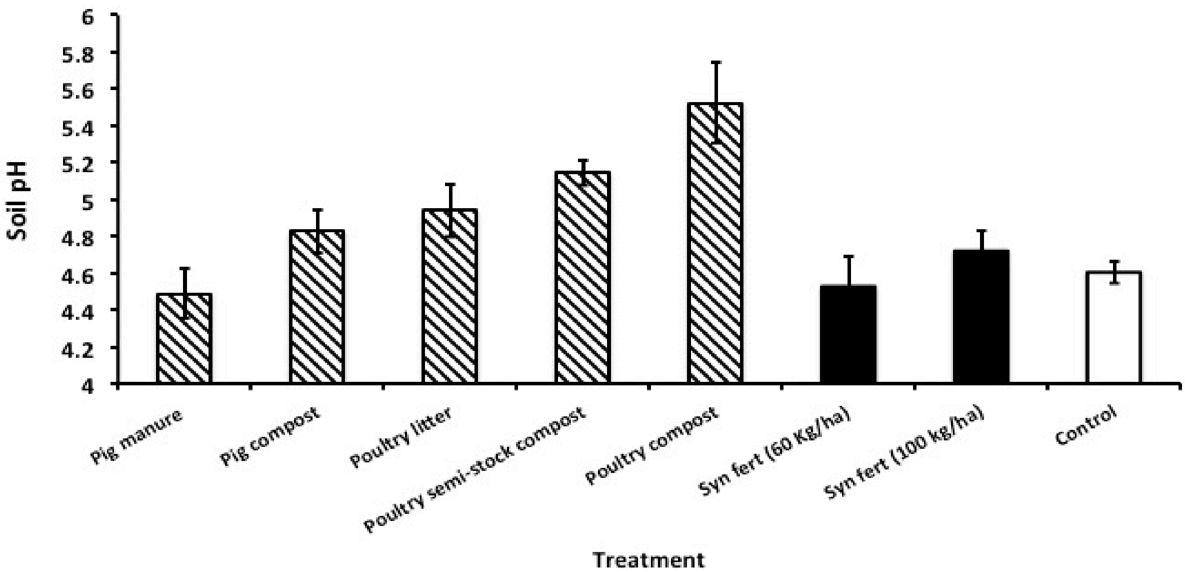

Figure 28. Soil pH in soils amended with different composts and fertilisers pellets after 128 days incubation study. Error bars represent standard error (n = 3).

Overall, the findings very clearly showed that stable flies (and other nuisance flies) are unable to develop from spent litter applied directly to non-irrigated pastures and cropping land at rates up to 10t/ha. In addition, land application of spent litter improved soil quality, crop productivity and did not have any significantly contribute to greenhouse gas emission. This research shows that spent litter can be used in agriculture without being associated with fly breeding. This will enable the chicken meat industry to overcome environmental issues associated with litter disposal, gain community and planning support, develop new markets and revenue and increase production to meet expected growth in demand.

Broiler growers will save a minimum of \$8/m3 of litter (≈\$1,000 per batch) by enabling its use in broadacre farming, composting operations and potentially irrigated horticulture (with defined amendments). Currently broiler growers must store their litter on site, which represents a biosecurity hazard, raises social concerns around odour and the perception that the stacks of litter are promoting fly breeding. Some chicken meat processors have already begun implementing a ban on the storage of spent litter on farm due to biosecurity risk.

Field trial 2: Cost benefit analysis on whether the application of composts (prepared using the advanced method) to cropping can partially replace the P synthetic fertiliser (Activity 8, outputs 8c)

A two-field trial (2016, 2017) consisting of eleven different amendments in triplicate (unamended; synthetic fertiliser applied at 60 and 100 kg/ha; piggery, dairy, chicken, feedlot or compost applied with synthetic fertiliser at 60 or 100 Kg/ha) was established with wheat as the crop. All composts were generated using mobile aerated floor composting units.

Crop productivity for field trial 2016

There was a significant difference between the control (no fertiliser inputs) and the plots receiving 100% synthetic fertiliser, dairy compost + 100 % synthetic fertiliser, pig compost + 100 % synthetic fertiliser, beef compost + 60 % synthetic fertiliser and pig compost + 60 % synthetic fertiliser (Figure 29). Interestingly, the yields were comparable between the plots receiving 100 % synthetic fertiliser, beef compost + 60 % synthetic fertiliser and pig compost + 60 % synthetic fertiliser which means livestock enterprises could potentially increase their productivity and profitability by reducing their fertiliser input and utilising their manure fertiliser products.

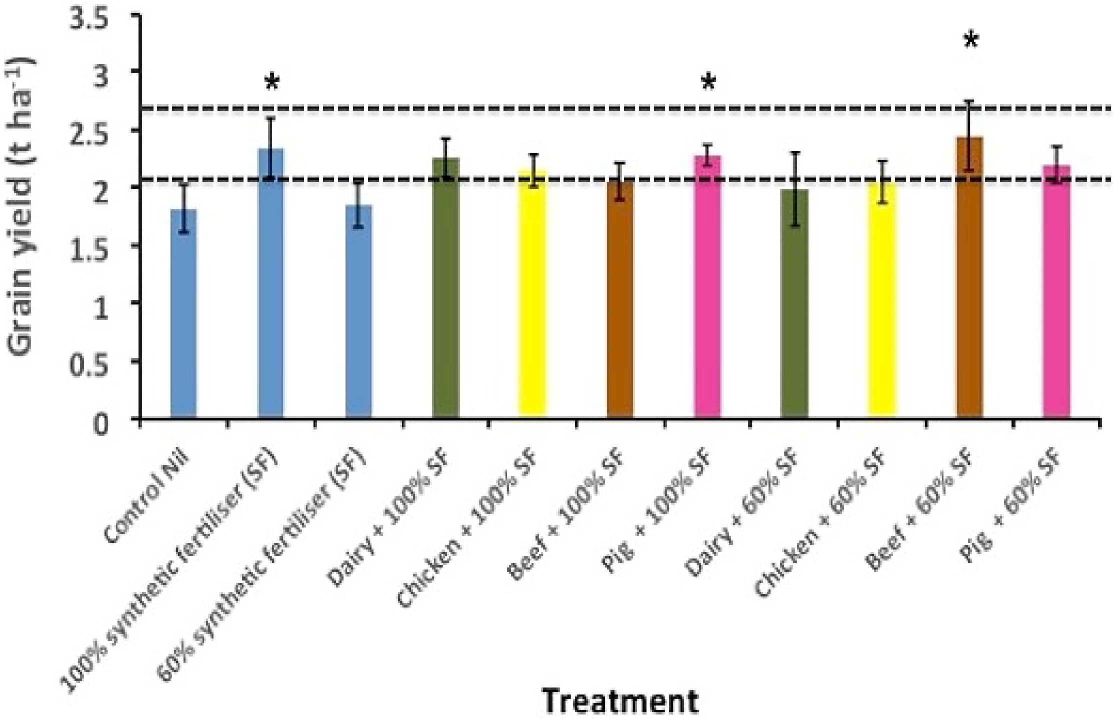

Figure 29. Pea yield data for soil receiving different fertiliser and manure inputs at the field trial 2 site (2016), UWA Future Farm, Pingelly, Western Australia. All treatments were done in triplicate. Error bars indicate the standard error where n=3. Amendments that are significantly different from the control treatment are highlighted (*).

Crop productivity for field trial 2017

The field trial design from the trial in 2016 was repeated in 2017. There was a significant difference between the control (no fertiliser inputs) and the other treatments (Figure 30) but no significant difference between the treatments. This is partly due to the late season rain that promoted rapid growth and consequently the treatments evened out. This resulted in record grain yields and large profits gains across the region. Overall, reducing synthetic fertiliser inputs and partially replacing it with compost did not impact on the crop productivity.

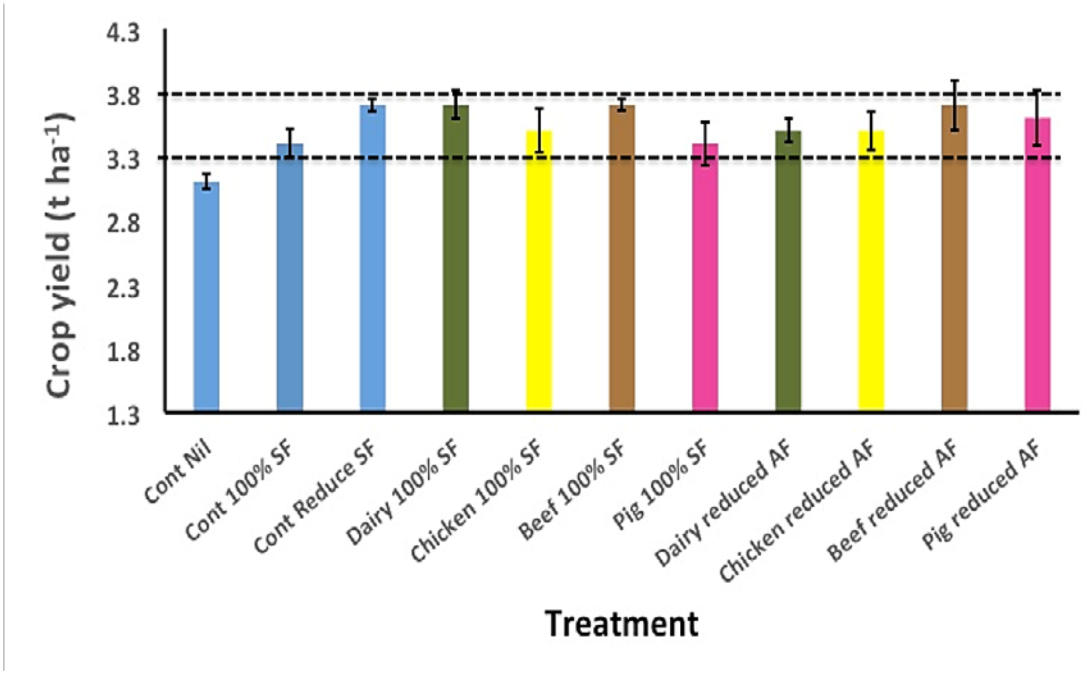

Figure 30. Oat yield data for soil receiving different fertiliser and manure inputs at the field trial 3 site (2017), UWA Future Farm, Pingelly, Western Australia. All treatments were done in triplicate. Error bars indicate the standard error where n=3. Amendments that are significantly different from the control treatment are highlighted (*).

Cost benefit analysis for composts applied to Peas (Activity 8, Output 8c)

The following calculations are based on the field trial of 2016 after a year of compost application. The cost benefit analysis in Table 14 shows that applying beef chicken compost in combination with synthetic fertiliser at the reduced rate of 60% resulted in net profit inside the shire relative to the synthetic fertiliser applied at 100%. Furthermore, application of pig and dairy compost (within the shire) in combination with synthetic fertiliser almost resulted in a positive (loss of $38 and $48, respectively). Perhaps further applications of compost will increase the yield in subsequent cropping year resulting in positive gains from these compost sources.

Table 14. Cost benefit analysis of compost products applied in combination with synthetic fertiliser at 60 and 100% rate of district practice to Peas within and outside the shire. Where SF = synthetic fertiliser applied at 60 or 100 Kg/ha and control = nil amendment.

$/ha	Dairy 100% SF	Chicken 100% SF	Beef 100% SF	Pig 100% SF	Dairy reduced SF	Chicken reduced SF	Beef reduced SF	Pig reduced SF	SF (100 kg/ha)	SF (60 kg/ha)	Control
Grain quality	Peas	Peas	Peas	Peas	Peas	Peas	Peas	Peas	Peas	Peas	Peas
Grain price (AGP1)	330.0	330.0	330.0	330.0	330.0	330.0	330.0	330.0	330.0	330.0	330.0
Yield (mean)	2.3	2.2	2.1	2.3	2.0	2.0	2.4	2.0	1.8	2.3	1.8
Gross income	745.8	709.5	676.5	755.7	654.5	675.4	806.3	657.8	608.3	772.2	599.5
Transported within the shire (<30 km)											
Variable Costs	355.4	353.0	350.8	356.0	301.7	303.1	311.8	302.0	268.4	334.2	171.8
Gross Margin	390.4	356.5	325.7	399.7	352.8	372.3	494.5	355.8	339.9	438.0	427.7
Profit relative standard practice	-47.5	-81.4	-112.2	-38.3	-85.2	-65.7	56.5	-82.1	-98.0	0.0	-10.3
Transported outside the shire (<150 km)											
Variable Costs	380.1	377.7	375.5	380.7	326.4	327.8	336.5	326.7	268.4	334.2	171.8
Gross Margin	365.7	331.8	301.0	375.0	328.1	347.6	469.8	331.1	339.9	438.0	427.7
Profit relative standard practice	-72.2	-106.1	-136.9	-63.0	-109.9	-90.4	31.8	-106.8	-98.0	0.0	-10.3

Cost benefit analysis for composts applied to Oats (Activity 8, Output 8c)

The following calculations are based on the field trial of 2017 after two years of compost application. A cost benefit analysis of the compost +60% or 100% synthetic fertiliser vs synthetic fertiliser at 60% distinct practice (since this was higher than the 100%) was performed on the data from the 2017 field trial using a modified calculator from GRDC soil quality website. The cost benefit analysis in Table 15 shows that with the exception of chicken and pig compost + 100% synthetic fertiliser, using livestock composts in combination with synthetic fertiliser at both the 60 % and 100 % rate resulted in a net benefit when compared to applying 100% synthetic fertiliser singly (district practice). Interestingly, applying synthetic fertiliser at 60% district practice resulted in a greater yield and profit when compared the synthetic fertiliser applied at 100% treatment. Furthermore, the use of compost outside the shire was also financially rewarding for majority of the compost treatments.

Table 15. Cost benefit analysis of compost products applied in combination with synthetic fertiliser at 60 and 100% rate of district practice to Oats within and outside the shire. Where SF = synthetic fertiliser applied at 60 or 100 Kg/ha and control = nil amendment.

$/ha	Dairy 100% SF	Chicken 100% SF	Beef 100% SF	Pig 100% SF	Dairy reduced SF	Chicken reduced SF	Beef reduced SF	Pig reduced SF	SF (100 kg/ha)	SF (60 kg/ha)	Control
Grain quality	Oat1	Oat1	Oat1	Oat1	Oat1	Oat1	Oat1	Oat1	Oat1	Oat1	Oat1
Grain price (AGP1)	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Yield (mean)	3.7	3.5	3.7	3.4	3.5	3.5	3.7	3.6	3.7	3.4	3.1
Gross income	**740.0**	**700.0**	**740.0**	**680.0**	**700.0**	**700.0**	**740.0**	**720.0**	**740.0**	**680.0**	**620.0**
Transported within the shire (<30 km)											
Variable Costs	**378.3**	**374.4**	**378.3**	**372.4**	**326.8**	**326.8**	**330.7**	**328.8**	**300.4**	**349.5**	**192.7**
Gross Margin	**361.7**	**325.6**	**361.7**	**307.6**	**373.2**	**373.2**	**409.3**	**391.2**	**439.6**	**330.5**	**427.3**
Profit relative standard practice	**31.2**	**-4.9**	**31.2**	**-22.9**	**42.7**	**42.7**	**78.8**	**60.8**	**109.1**	**0.0**	**96.8**
Transported outside the shire (<150 km)											
Variable Costs	**407.1**	**402.9**	**407.1**	**400.9**	**355.4**	**355.4**	**360.8**	**357.4**	**345.9**	**311.9**	**196.1**
Gross Margin	**558.6**	**510.6**	**558.6**	**486.5**	**558.1**	**558.1**	**675.2**	**582.2**	**541.5**	**653.8**	**613.0**
Profit relative standard practice	**-95.2**	**-143.2**	**-95.2**	**-167.3**	**-95.7**	**-95.7**	**21.4**	**-71.6**	**-112.3**	**0.0**	**-40.8**

3.1.6 Specific mode of action of the new fertilizer/compost products so that they can be tailored to end-users (Activities 5-7, Outputs 5c, 6b, 7c)

Livestock by-product amendments (manure, compost, pelletised manure, algae and bacteria biomass) are reported to influence the plant-soil system by altering the biological, chemical, and physical properties in a number of ways (Abbott et al., 2018). Understanding the modes of actions of how each by-product operates will assist producers, growers, agronomists, and consultants in matching the best by-product to overcome the main environmental constraints on production in specific regional, farm and paddock scenarios.

Adding manures and composts to pasture improve microbial N and C cycling and pasture productivity

Manures, composts and other biological amendments possess a suite of soil and plant health attributes, such as increased soil carbon storage, N cycling and improved crop tolerance to adverse seasonal conditions (Abbott et al., 2018). Although the mechanisms and modes of actions are not fully understood, biological amendments interact with microbial communities in soil in a number of ways. They may stimulate microbial growth either directly by providing nutrients or indirectly by increasing plant growth and enhancing root carbon flow leading to an increase in the size of the microbial biomass (Ahkami et al., 2017; Jenkins et al., 2009; Stockdale et al., 2013). The quality and quantity of the biological amendment can further alter the biota and microbial community diversity and function.

Bacterial activities are involved in retention of soil C, and some are involved in loss of soil C during degradation of organic matter (Stockdale et al., 2013). Bacterial communities respond to addition of manure and compost and play a key role in the incorporation of these C resources into the soil matrix (Zhen et al., 2014). In this study, bacterial communities in dairy soil amended with manure or compost in a field experiment were characterized in soil collected in 'winter' and 'summer' using community profiling of 16S rRNA genes. The soil had been amended in the field with inorganic fertilizer in combination with 2t/ha dairy manure, or compost applied at 3t/ha or 6t/ha.

The dominant bacterial phyla were copiotrophic bacteria Proteobacteria, Actinobacteria and Firmicutes (Mickan et al., 2018). There were effects of manure and compost, both within and between sampling times, on the relative abundance of soil bacteria. The occurrence of C degrading functional genes and N functional genes were predicted using PICRUSt (Figure 31) (Langille et al., 2013). Predicted gene counts associated with breakdown of hemicellulose, cellulose and chitin were dominant in winter soil samples with application of manure, which were higher than for compost.

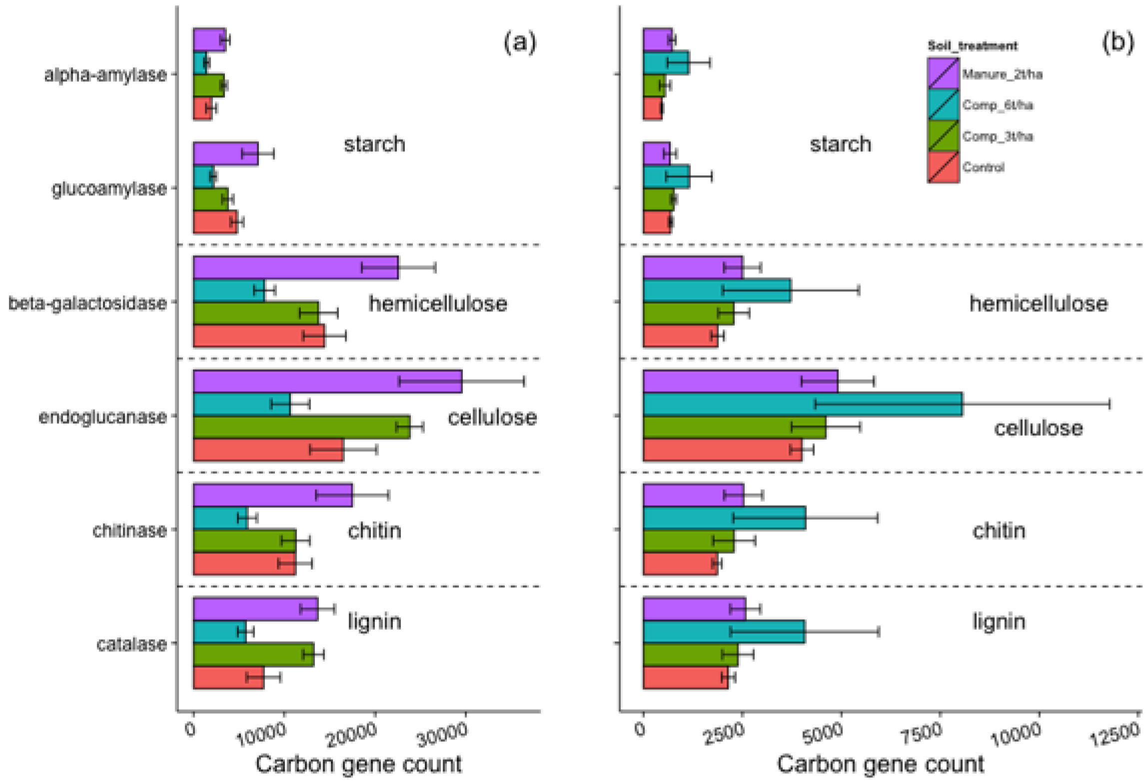

Figure 31. Predicted carbon gene count (PICRUSt) for the most abundant detected carbon degrading genes for winter (a) and summer (b) soil samples from dairy pastures amended with compost and manure across the spectrum of labile to recalcitrant soil carbon degrading enzymes. Soil treatments were the addition of compost (3t/ha, 6t/ha), manure (2t/ha) and control (unamended soil). Bars represent the mean of each treatment and error bars are the standard error of the mean (n=3).

Predicted N gene count for amoB associated with nitrification was lowest for soils treated with 6t/ha compost in winter samples (Figure 32). At higher application rates of compost, the C:N ratio of the soil is increased resulting in net N immobilisation over nitrification (Chaves et al., 2005; Fisk et al., 2015a). This study illustrates the complexity of soil bacterial community responses to manure and compost applied to dairy pasture. One feature of this dynamic was reduced potential for degradation of soil C and mineralization of N and therefore higher C and N retention in soils when 6t/ha compost was applied compared to application of 3t/ha compost and non-composted manure. Management practices that enhance C sequestration and N retention in agricultural soils may enhance crop productivity whilst limiting C and N losses via greenhouse gas emissions and leaching (Barton et al., 2016).

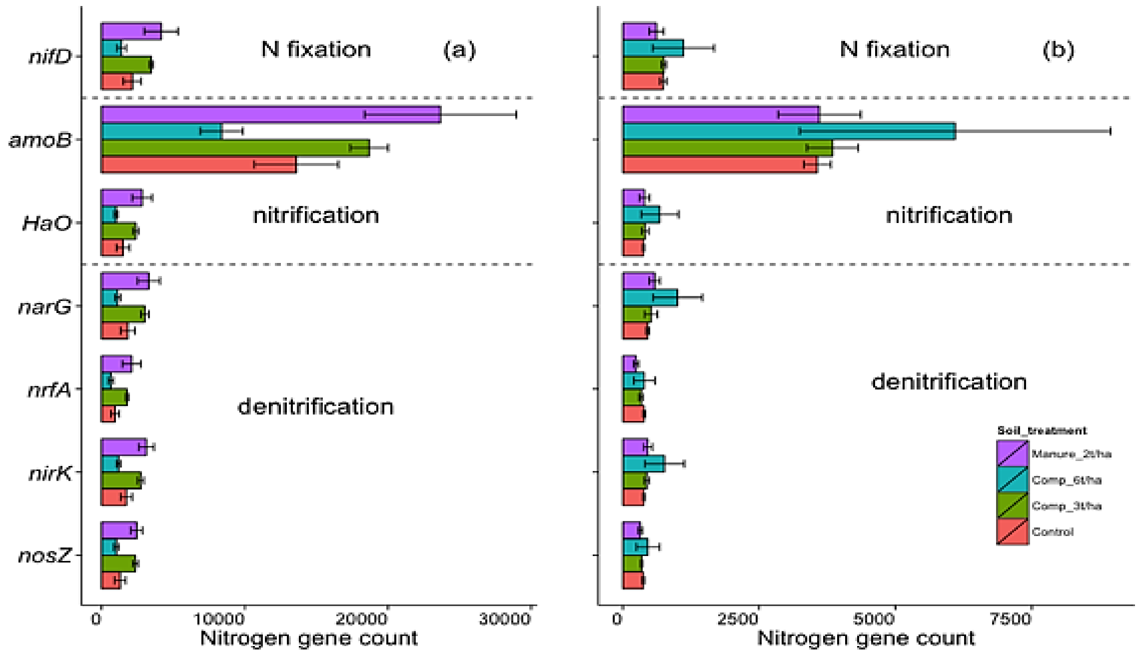

Figure 32. Predicted carbon gene count (PICRUSt) for the most abundant detected nitrogen cycling genes for winter (a) and summer (b) soil samples from dairy pastures amended with compost and manure across the spectrum of labile to recalcitrant soil carbon degrading enzymes. Soil treatments were the addition of compost (3t/ha, 6t/ha), manure (2t/ha) and control (unamended soil). Bars represent the mean of each treatment and error bars are the standard error of the mean (n=3).

Assessing whether microalgae can behave as a biostimulant (Output 7c)

Micro-algae can contain a range of plant-based biostimulant including phytohormones, betaines, oligosaccharides elicitors, micro-algal protein hydrolysates, which may aid N uptake into the plant biomass (Garcia-Gonzalez & Sommerfeld, 2016; Mulbry et al., 2005). A series of experiments were conducted to better understand the mode of action and mechanisms involved.

Specific objectives were to:

i. investigate the effectiveness of microalgae biomass to supply N to wheat in comparison with a synthetic N fertilizer, ammonium nitrate (NH_4NO_3), and

ii. assess the release of inorganic N (potential N mineralization rate) following the application of microalgae biomass to soil when compared with a synthetic N fertilizer.

Firstly, a pot experiment was carried out with the following treatments: microalgae biomass and NH_4NO_3 at five N equivalent levels (0, 10, 20, 40, 80 kg N.ha^{-1}) for 6 weeks. Wheat (*Triticum aestivum L.)* was used as the host plant in a randomized block design in three replicates. Utilization of the two N sources significantly improved the dry-harvest for wheat plants at the greatest application level compared with the control. The N sources and levels significantly affected the N uptake in shoots (Figure 14). The application of microalgae biomass was able to support up to 85% of the shoot production when compared to the synthetic fertiliser (NH_4NO_3).

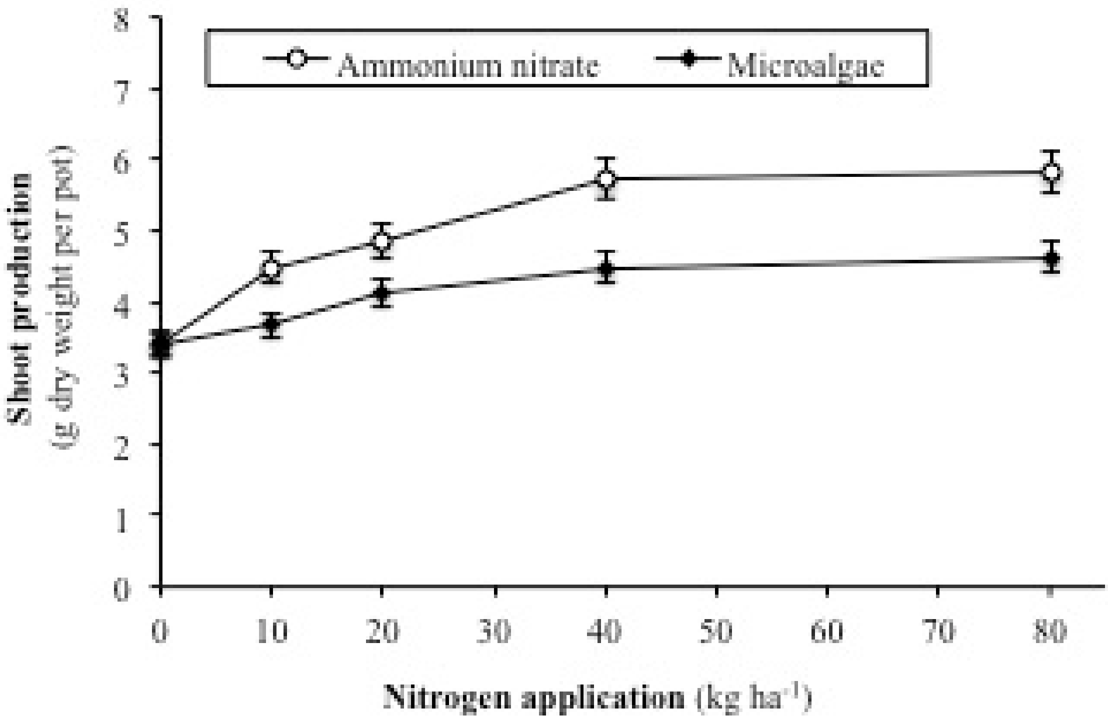

Figure 33. Effect of different N sources (NH4NO3 and microalgae biomass) on shoot production (mean ± SEM, where n=3) for wheat when applied at different levels (equivalent to 0 to 80 kg N. ha^{-1})

The greatest total N uptake (g N per pot) and N concentration (g.kg^{-1}) were observed for NH_4NO_3 at an application level of 80 kg. ha^{-1} (P<0.001; Table 16). The total N uptake and N concentration in the shoots increased when the N levels were increased in both N sources (Table 16). Despite, the good response of shoot production following the application of microalgae, the N concentration for all the N levels was significantly lower in the microalgae biomass when compared with NH_4NO_3 (P<0.001). An explanation for this anomaly is that the microalgae contain biostimulant substrates where the positive effects (shoot production) extend beyond direct nutrient addition effects (i.e. N uptake) (Garcia-Gonzalez & Sommerfeld, 2016). In fact, seaweed extracts have been shown to contain plant hormones and growth-promoting substances that stimulate root growth and increase nutrient uptake (Saa et al., 2015). They can also change the soil microbial structure and function including nutrient provision and C sequestration, leading to improved soil resilience to seasonal and soil stresses (Calvo et al., 2014; Haslam & Hopkins, 1996). One mechanism by which biostimulants may facilitate increased plant growth is through increasing the rate of processes involved in the breakdown and release of plant-available nitrogen from soils and litters (Van Oosten et al., 2017).

Table 16. Effect of different N sources on measured wheat properties [shoot dry weight (DW), shoot N concentration, and total N uptake per pot] when applied at different levels (equivalent to 0 to 80 kg N. ha^{-1}). Values presented are means (± standard error), n = 3. Three plants per pot were grown for 6 weeks.

Source	Nitrogen application level (kg N.ha^{-1})	Shoot DW (g per pot)	Shoot N concentration (g.kg^{-1} shoot DW)	N uptake (g N per pot)
Ammonium	0	3.40 ± 0.21	3.12 ± 0.28	10.61 ± 0.37
Nitrate	10	4.49 ± 0.21	4.13 ± 0.20	18.54 ± 0.93
	20	4.83 ± 0.05	4.88 ± 0.14	23.57 ± 0.22
	40	5.73 ± 0.22	6.85 ± 0.07	39.25 ± 0.64
	80	5.80 ± 0.08	7.52 ± 0.64	43.62 ± 0.03
Microalgae	0	3.38 ± 0.09	3.00 ± 0.21	10.14 ± 0.05
Biomass	10	3.70 ± 0.17	3.01 ± 0.11	11.14 ± 0.11
	20	4.28 ± 0.26	3.24 ± 0.34	13.87 ± 0.41
	40	4.57 ± 0.10	3.36 ± 0.66	15.36 ± 0.05
	80	4.61 ± 0.08	4.40 ± 0.11	20.29 ± 0.22
P-value	Source	***	***	***
	N-level	***	***	**
	Source x N-level	***	***	***
LSD	Source x N-level	1.31	3.05	21.99

*significant at P=0.05; **significant at P=0.01; ***significant at P=0.001.

Secondly, the mineralization of organic nitrogen from microalgae biomass in laboratory conditions was investigated. A randomized block design included the two N sources (microalgae biomass and NH_4NO_3) and five N equivalent levels (0, 10, 20, 40, 80 kg N. ha^{-1}) for 28 days in three replicates. The amount of mineral N produced varied depending on the N source and the N level applied (P<0.001; Table 17). Increasing the amount of N applied increased the amount of mineral N for both N sources, but to a greater extent for NH_4NO_3 than for microalgae biomass. The concentration of NH_4^+ and NO_3^- following the application of microalgae biomass was significantly lower for all N application levels compared to NH_4NO_3 (P<0.001).

Table 17. Changes in mineral N (NH_4^+ + NO_3^-) with time for two N sources (microalgae biomass and NH_4NO_3) applied at different levels (n=3), incubated at 25°C, and soil moisture content adjusted to 70% of moisture-holding capacity.

Nitrogen levels (kg ha-1)	Mineral N (mg kg^{-1} dry soil)				
	Day				
	0	7	14	21	28
Microalgae biomass					
0 (Control)	1.10 ± 0.07	1.04 ± 0.04	1.06 ± 0.05	1.08 ± 0.04	1.06 ± 0.08 [c]
10	1.24 ± 0.04	10.37 ± 0.02	13.25 ± 0.89	14.14 ± 1.01	17.33 ± 0.26 [bc]
20	1.22 ± 0.02	11.03 ± 0.38	11.70 ± 0.37	14.16 ± 0.41	18.50 ± 0.14 [bc]
40	1.15 ± 0.09	11.50 ± 0.47	13.91 ± 0.18	15.42 ± 0.82	19.49 ± 0.20 [b]
80	1.34 ± 0.05	15.25 ± 0.96	17.92 ± 1.07	17.95 ± 0.20	21.07 ± 0.63 [a]
Ammonium nitrate					
0 (Control)	1.03 ± 0.01	0.94 ± 0.02	1.16 ± 0.17	1.28 ± 0.20	1.19 ± 0.04 [c]
10	0.98 ± 0.03	30.87 ± 0.67	32.26 ± 1.75	31.81 ±0.52	32.73 ± 0.69 [bc]
20	1.05 ± 0.01	61.14 ± 1.12	61.86 ± 2.12	59.71 ± 3.10	70.55 ± 2.23 [bc]
40	1.05 ± 0.02	119.32 ± 2.49	132.49 ± 0.65	124.25 3.40	130.90 ± 3.74 [b]
80	0.97 ± 0.03	218.85 ± 0.98	241.10 ± 5. 09	236.59 ± 0.42	255.26 ± 3.52 [a]

Values with the same letter have no significance difference based on 95% confidence intervals.

Increasing soil N sources increased carbon dioxide (CO_2) production and soil mineral N but only with microalgae biomass at higher N rates (Figure 34). Adding microalgae biomass also increase the CO_2 production by 30% when compared with the synthetic control. This is because microalgae mainly contains organic forms of N that needs to be first mineralised to inorganic N by microorganisms before it can be utilised by plants and the increase in heterotrophic microbial activity is accompanied with an increase in respiration (CO_2) (Edmeades, 2003; Fisk et al., 2015a).

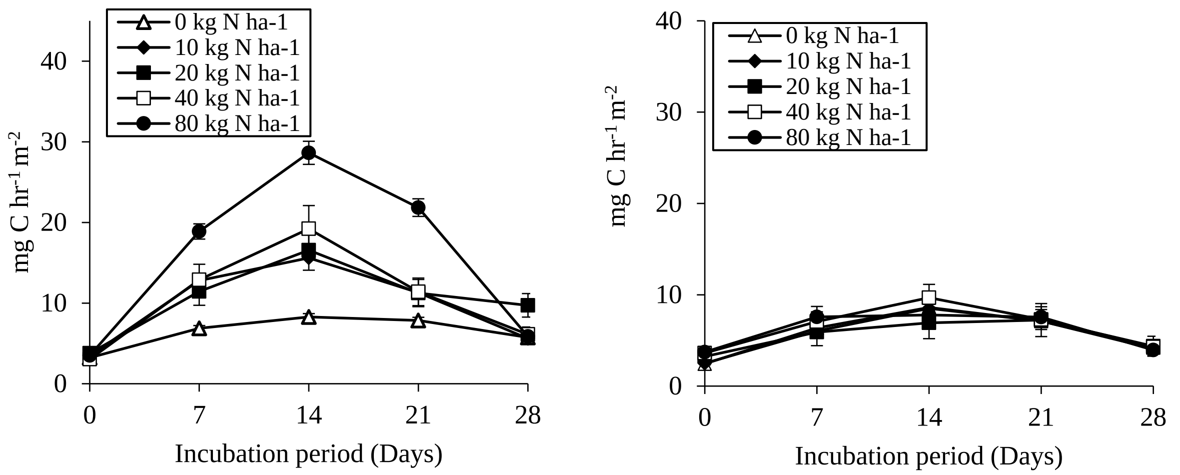

Figure 34. Effect of different N sources: (a) microalgae biomass, and (b) NH_4NO_3, on CO_2 (mg C hr-1 m-2) when applied at five N application levels in the soil. Bars indicate SEM; n=3.

Results of this study indicated that microalgae biomass is an available nitrogen source for plants but not to the same extent as NH_4NO_3. Management practices that combine the use of microalgae biomass and synthetic N fertilizers should be encouraged as they can improve dry-matter production, improve microbial activities in soil, and help with the reduction of nitrous oxide and methane emissions.

3.1.7 Extent to which improved composts enhance soil biology, resistance and resilience to heat and water stress so that the end-users can adapt to climate change (Output 8d)

Impact of composts on soil resilience to water stress (Output 8d)

Water repellent sandy soils in rain-fed agricultural systems of Australia limit crop production, and have negative environmental effects associated with leaching and soil erosion (Roper et al., 2015). Applications of compost either with or without clay have been shown to ameliorate soil constraints such as water-stress and are therefore potential management strategies for sandy agricultural soils (Giusquiani et al., 1995; Hirich et al., 2014; Mickan et al., 2018). Compost amendments influence the physical structure of soil changing the pore space for water/gas storage and movement, the rooting matrix for plants, and the habitable space and proximity to resources for biological communities (Giusquiani et al., 1995; Zebarth et al., 1999). The resulting improved structure enhances biological processes that support plant growth such as C and N cycling (Mickan et al., 2018). Consequently, these amendments may improve crop growth and the resistance and resilience of production systems to stress and perturbation (Stockdale et al., 2013). However, the mechanisms and mode of action involved are poorly understood.

A pot trial was established to determine whether compost and clay amendments in a sandy agricultural soil influenced the rhizosphere microbiome of Trifolium subterraneum under differing water regimes. Soil was amended with compost (2% w/w), clay (5% w/w), and a combination of both, in a glasshouse experiment with well-watered and water-stressed (70% and 35% field capacity) treatments. 16S rRNA Ion Torrent sequencing and PICRUSt analysis of functional gene prediction were used to interrogate the rhizosphere bacterial community and its functional component involved in nitrogen (N) cycling and soil carbon (C) degradation.

Under both well-watered and water stressed conditions, shoot mass was increased with application of compost singly whilst root mass and P uptake were unaffected (Figure 35). In contrast, the application of clay either singly or combined with compost led to a reduction in root, shoot and P uptake. Interestingly, AM fungal colonisation was greatly reduced in soil receiving compost applied singly.

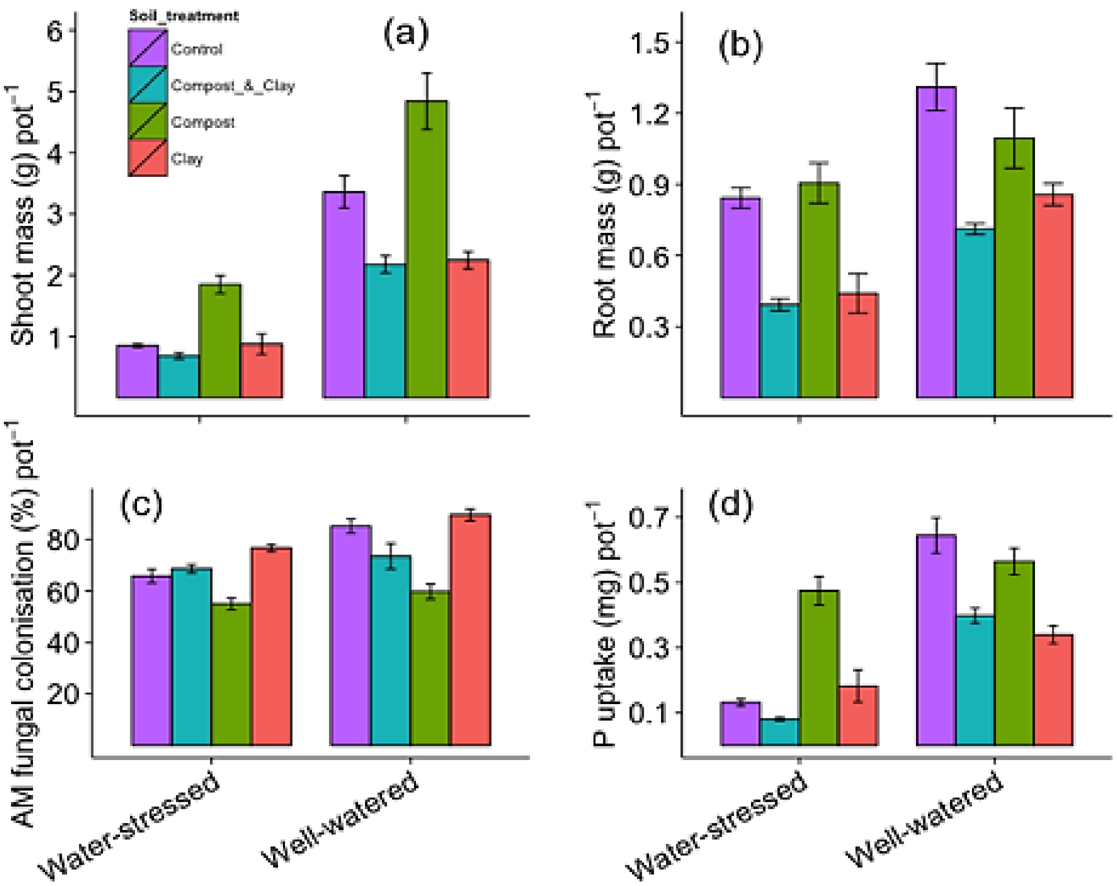

Figure 35. Dry shoot (a) and root (b) mass of Trifolium subterraneum, AM fungal root length colonisation (%RLC) (c), and P uptake (d) in response to soil amendments of compost 2%w/w, clay 5% w/w, and the combination of compost 2%w/w + clay 5% w/w. The control was no addition of compost or clay. Water treatments were well-watered (75% FC) and water-stressed (35% FC). Bars represent standard error (n=4).

Analysis of the bacterial community structure revealed that the relative abundance of phyla varied with both different soil amendments and with water treatment (Figure 36). Under well-watered conditions there was a marked increase in the relative abundance of gram-negative bacteria Chloroflexi, Proteobacteria and Bacteroidetes for all treatments and a reduction in the relative abundance of Verrucomicrobia, Acidobacteria, Planctomycetes, Firmicutes and Actinobacteria. Under water-stressed conditions the relative abundance of Firmicutes increased for all treatments whereas Actinobacteria increased for all treatments except for the singly applied compost. Also, the relative abundance of other Phyla were less impacted when soil was amended with compost alone.

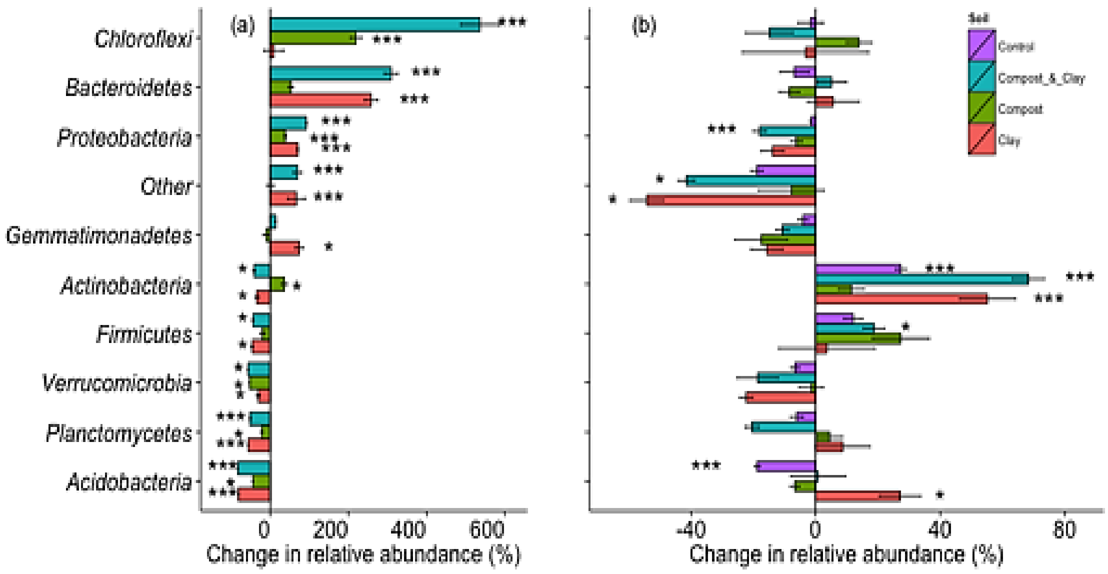

Figure 36. Variation in rhizosphere bacterial relative abundance from soil treatments compared to unamended soil control at the Phylum level for (a) within the well-watered treatment (70%FC), and (b) water-stress alterations (35% FC) in relative abundance of bacteria within each soil (soil control inclusive) as compared to the well-watered control (70%FC). Soil treatments consisted of amending soil with; compost 2%w/w, clay 5% w/w, and the combination of compost 2%w/w & clay 5% w/w. Post Hoc Tukey HSD significant P values are indicated by * and * corresponding to P < 0.05 and < 0.001, respectively. Bars represent the mean value for each treatment and error bars are the standard error of the mean (n=4).**

Further analysis of predicted functional genes showed there was a marked increase in the relative abundance of copiotrophic bacteria (Fierer et al., 2007; Jenkins et al., 2010) associated with recalcitrant C degrading genes and a reduction in bacteria associated with labile C pathways (e.g. glucoamylase, beta-galactosidase) in the compost only treatment (Figure 37). Soil amendment with compost has been shown to enhance hydrolytic activities enzyme activities involved in C cycling previously (Das et al., 2017). Some of these microorganisms secrete polysaccharides that help bind soil particles and improve soil structure (Young & Crawford, 2004). Water stress impacted on the rhizosphere bacterial community in all soil treatments except the compost only amended soil, indicating that the compost increased the resistance of rhizosphere microbiome to water-stress (Mickan et al., 2018).

Application of compost decreased the abundance of the nrfA gene within the rhizosphere of the well-watered soil. The nrfA gene catalyzes the reduction of nitrite to ammonia a key step in the dissimilatory nitrate reduction to ammonium (DNRA) pathway that promotes the retention of N in soils (Welsh et al., 2014). In contrast, soils receiving clay either singly or in combination with compost had a higher abundance of the denitrifying nitrous oxide reductase gene (nosZ) that catalyzes the reduction of nitrous oxide to dinitrogen (Giles et al., 2017) . Since denitrification leads to N loss whilst respiratory ammonification retains N, manipulating these pathways via soil amendment (clay and compost) have implications for N retention, plant productivity and climate change (Abbott et al., 2018; Mickan et al., 2018). Thus, compost and clay amended soil under water-stress influence the rhizosphere bacteria and their N cycling and C degradation pathways.

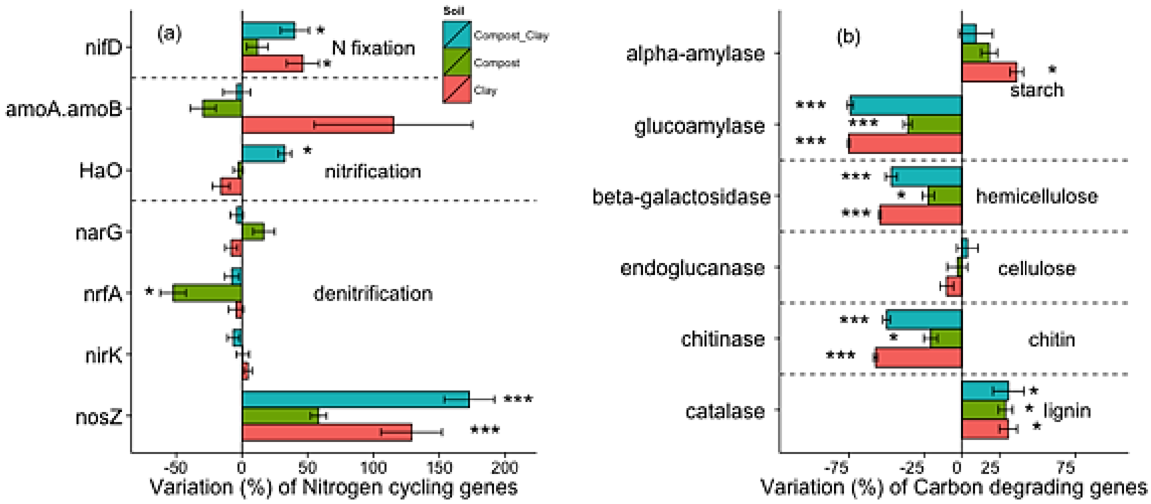

Figure 37. The percentage variation of genes for soil treatments as compared to the unamended control soil within well-watered conditions (70%FC) of the most abundant PICRUSt predicted N cycling genes (a), and C degrading genes (in order of complexity from labile to recalcitrant) (b). Soil treatments consisted of amending soil with; compost 2%w/w, clay 5% w/w, and the combination of compost 2%w/w & clay 5% w/w. Post hoc Tukey HSD significant P values are indicated by * and * corresponding to P < 0.05 and < 0.001, respectively. Bars represent the mean value for each treatment and error bars are the standard error of the mean (n=4).**

Impact of composts on soil resilience to heat stress (Output 8d)

In a final lab incubation experiment, we investigated how heat-disturbance affects soil resilience in response to the addition of short-term fertilization (manure and compost) and long-term organic matter inputs in a semi-arid area of Western Australia. The results suggest temperature disturbance in this semi-arid soil has a critical effect on soil microbial activity (SIR-CO_2) and microbial population size (16S rRNA gene copy number) of total bacteria. Long-term organic matter amendment increased the microbial activity and bacterial abundance but there was no effect on resistance and resilience actually decreased in these soils. For soil with no OM history adding short-term manure or compost decreased the soil's resistance and resilience. For soil with history of OM application, adding manure or compost had no effect on the resistance, however, adding manure increased the soil's resilience. Manure contains a variety of decomposable carbon forms that increases soil microbial activities (Abbott et al., 2018; Edmeades, 2003; Hopkins et al., 2016). Compost, on the other hand, contained more stable, complex forms of carbon meaning soil microorganisms degrade it more slowly (Farrell & Jones, 2009). Considering the on-farm management practice, the agricultural soils were well adapted to the high temperatures climate in WA, thus neither long-term wheat chaff inputs and short-term manure or compost application is beneficial for soil resilience in response to heat shock.

Overall, soil receiving long-term mineral + OM had a lower resilience to heat stress than the soil receiving long-term mineral only (Figure 38). For soil with history of mineral fertiliser only, adding compost or manure lowered the resistance compared to the unamended treatment (Figure 38a). For soil with history of mineral fertiliser + OM, adding manure or compost had no significant effect on resistance (Figure 38b). For soil with history of mineral fertiliser only, adding compost or manure lowered the resilience (Figure 38c). For soil with history of mineral fertiliser + OM, adding manure but not compost increased the soil's resilience (Figure 38d).

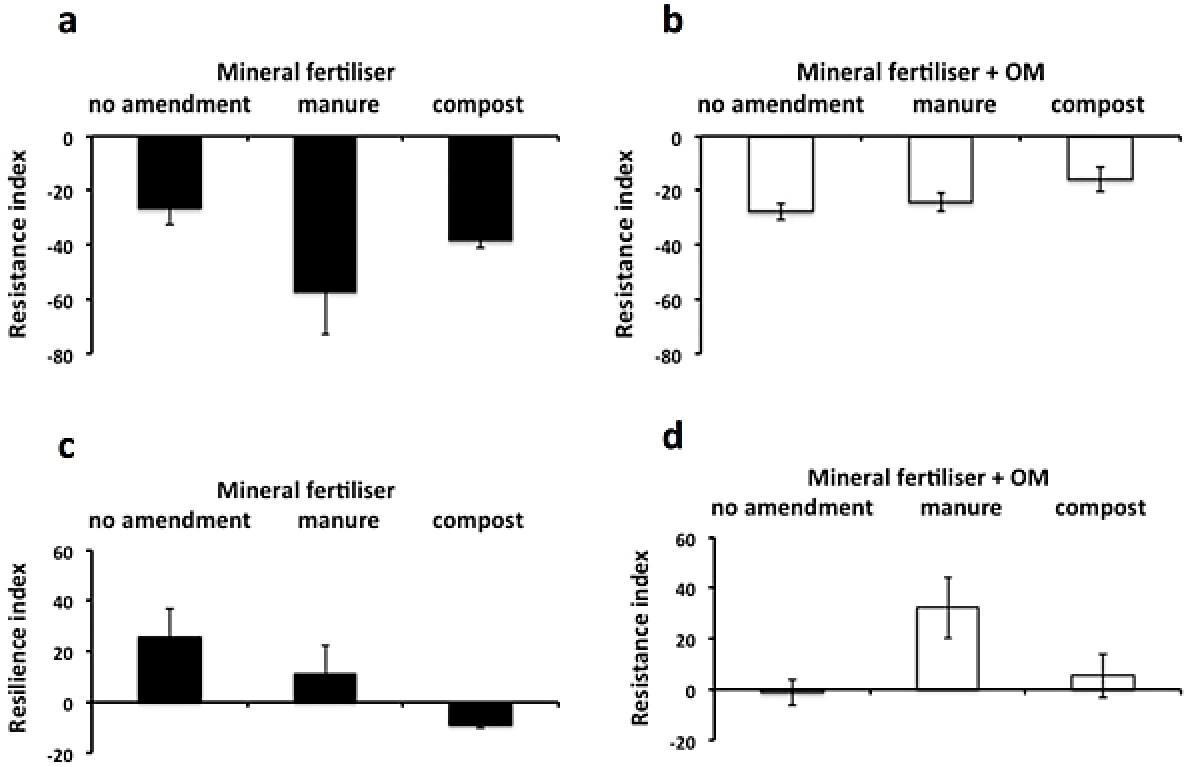

Figure 38. Resistance and resilience of SIR-CO2 production in unstressed and heat stressed soils. The mean resistance index for manure, compost and unamended heat stressed soils compared to the unheated treated soils for mineral fertilizer (a) and mineral fertilizer plus organic matter (b) soils (mean ± SEM; n = 3). The mean resilience index for manure, compost and unamended heat stressed soils compared to the unheated treated soils for mineral fertilizer (c) and mineral fertilizer plus organic matter (d) soils (mean ± SEM; n = 3).

Soil bacterial abundance resistance and resilience

For the long-term mineral fertilizer soil adding manure lowers the resistance and the compost improves the bacterial resistance (Figure 39aa). For mineral fertilizer + OM soil adding manure or compost had no significant difference on resistance (Figure 39bb). Overall the mineral fertilizer +OM soil is more resistant than the mineral fertilizer only soil. For mineral fertilizer soil adding compost lowers the resilience whereas adding manure has no effect (Figure 39cc). For mineral +OM soil adding manure decreased the soil's bacterial population size resilience whereas adding compost has no effect (Figure 39dd). Interestingly, none of soil receiving long-term mineral + OM treatments recovered and had a lower resilience to heat stress than the soil receiving long-term mineral only.

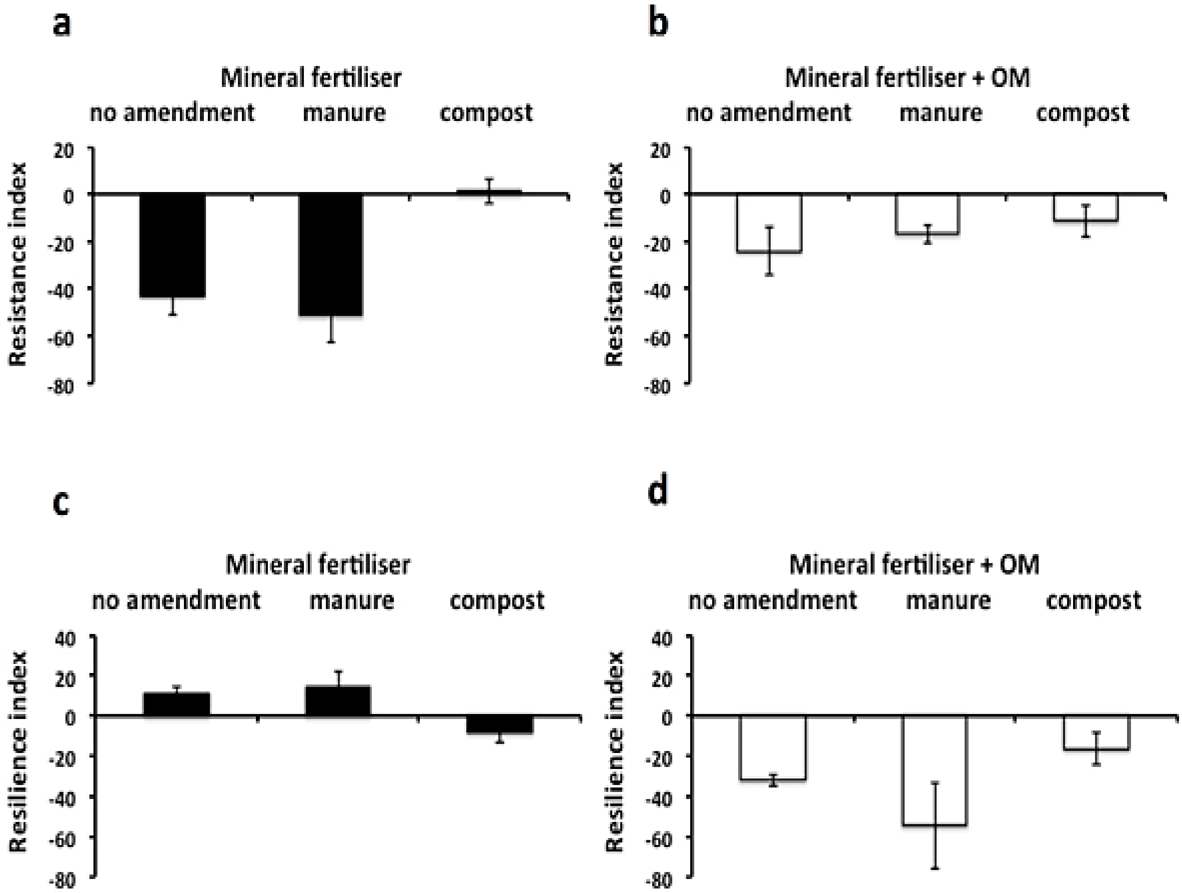

Figure 39. Resistance and resilience of bacterial abundance (16S rRNA gene copies) in unstressed and heat stressed soils. The mean resistance index for manure, compost and unamended heat stressed soils compared to the unheated treated soils for mineral fertilizer (a) and mineral fertilizer plus organic matter (b) soils (mean ± SEM; n = 3). The mean resilience index for manure, compost and unamended heat stressed soils compared to the unheated treated soils for mineral fertilizer (c) and mineral fertilizer plus organic matter (d) soils (mean ± SEM; n = 3).

Generalised linear models for SIR-CO_2 and bacterial abundance

Generalised linear models in Table 3 show the relationship between SIR-CO_2 and bacterial abundance (16S rRNA) and management treatments heat stress, long-term organic matter short-term OM and manure. The results show that the SIR-CO2 and bacteria populations were significantly influenced by heat stress and short-term manure amendment.

Table 18. Generalised linear models (GLMs) exploring the relationship between SIR-CO2 and bacterial abundance (16S rRNA) and management treatments heat stress, Long-term organic matter and short-term OM and manure. These GLM models explained (61.2%, and 48.1%, respectively) of the total variance in the data and had an intercept of 0.0263 and 1.687E+08, respectively. Values represent the estimate, standard error (SE), T value and P value. Relationships shown to be significant are reported at the 0.001 to 0.05 levels (*P<0.05, **P<0.01, *** P<0.001)

Model	Variable	Estimate	SE	T value	P value
SIR-CO_2	Heat stress	-0.00685	0.00298	-2.300	0.0226*
	Long-term OM	-0.00579	0.00298	1.942	0.0537
	Short-term OM	0.00613	0.00365	0.170	0.8654
	Manure	0.00817	0.00365	2.237	0.0265*
Bacterial abundance	Heat stress	6.522E+07	1.952E+07	3.342	0.0010**
	Long-term OM	1.971E+07	1.127E+07	1.01	0.3138
	Short-term OM	1.137E+07	1.012E+07	0.476	0.6349
	Manure	4.91E+07	2.390E+07	2.054	0.0415 *

3.1.8 Findings/Conclusion

The cost benefit analysis showed increased profitability as a result of applying beef compost (on-farm) in combination with synthetic fertiliser at the reduced rate of 60% following 2 years of application. The implication of this is that beef feedlot farmers can turn their manure into high quality compost and reduce their input costs by using less synthetic fertiliser without affecting yield. Long-term field trials have shown it usually takes 3-5 years of repeated manure or compost application before significant gains in crops yields are reported. It is hoped that after three years of compost application we will see further profit gains in the amended soils. Long-term field trials are recommended to eliminate seasonal variation from the trends. Interestingly, the crop quality (grain grade) was also better in the soils receiving feedlot compost. According to the 2015/16 season pricing, the price of grain varied considerably across the different grain grades [APW1 (base top grade), ASW (-$15), AGP1 (-$70) and Feed (-$100)]. Thus, a farmer producing a slighter lower yield but at higher grain quality will get a better financial return from their investment.

We found that applying compost to dairy pastures in WA reduced the C and N rate of mineralization and retained higher levels of C and N in soils leading to better soil function, structure and stability. The implication of this is that enhanced C sequestration and N retention in agricultural soils may increase crop productivity whilst limiting C and N losses via greenhouse gas emissions and leaching.

The addition of chicken compost to acidic soils could overcome constraints on grain production due to acidity in this region since our pot trials showed it raised the pH of the soil and improved biological function and nutrient availability. The implication of this is that farmers will be able to use less lime thereby reducing their input costs. Also, improved biological function and nutrient availability may increase crop yields.

The application of compost in our pot trials showed increased shoot growth under both well-watered and water stressed conditions. Furthermore, the application of compost increased the resistance of rhizosphere microbial community to water-stress by favouring microbes involved in C and N cycling pathways that led to C and N retention. This will have implications for plant productivity and climate change by increasing the availability of nutrients and minimising their loss from agricultural systems.

A pot trial assessing the suitability of microalgae biomass produced from piggery waste as a fertilizer found that the microalgae biomass was able to support up to 85% of shoot production when compared to the synthetic fertiliser. The algae also behaved as a biostimulant by facilitating plant growth through increased breakdown and release of plant-available nitrogen from the soil. The management implications of applying algae in combination with synthetic N fertilizers are improved microbial activities in soil, release of plant-available nitrogen and reduced dependency on synthetic fertilisers.

The emergence of stable fly and other nuisance was very low for broadacre soils amended with manure and compost under field conditions. The application type (raw manure versus composted manure) had no significant influence on stable fly development and emergence. This trial demonstrated that the timing of manure application did not have an impact on stable fly emergence which reduces the need to stockpile manure until seeding (winter), a practice, which is currently banned in WA. Moreover, stable fly emergence from soils amended with chicken manure or compost (which has been identified as a major breading ground of stable flies) was comparable to that in soils amended with pig manure and compost. This work in part has enabled the chicken meat industry to overcome environmental issues associated with litter disposal, gain community and planning support, develop new markets and revenue and increase production to meet expected growth in demand.

Overall, the total GHG emissions from the soils amended with manure or compost were relatively low and similar to the synthetic fertiliser treatments and other studies done in the semi-arid, rain-feed cropping regions of Western Australia. The soils were both a minor source of nitrous oxide (N_2O) and sink of methane (CH_4). Majority of the composted manures resulted in a significant increase in CH_4 uptake implying that more balanced fertiliser products could stimulate methanotrophic microorganisms and methane uptake leading to significant reduction in GHG emissions.

Improving soil resilience plays an important role in crop production, in particular for semi-arid soil to meet future climate change. For all treatments, heat shock caused a significant decrease in microbial activity and bacterial abundance. This shows that prescribed burning has the potential to negatively impact microbial community structure and function and that other farming practices for weed and pest control should be used.

However, the heated treatment recovered quickly within 50 days. The results indicated that long-term OM addition of wheat chaff reduces the resilience of the microbial activity and bacterial population. Therefore, adding wheat chaff is not a good management option for improving soil resilience to heat stress. Also, the application of 20 t/ha of organic matter (chaff) is pricey and not practical in a commercial farming business.

Interestingly, the results indicated that soil receiving the short-term manure amendment had a higher microbial activity and was more resilient to heat stress especially in the long-term OM soils. However, manure amendments could increase denitrification and nitrous oxide emissions if not applied appropriately by matching N availability to N requirement of the crop. In contrast, short-term compost amendment was found to reduce the microbial activity, population size and resilience. Therefore, adding compost does not appear to be a good practice to improve resilience in this soil but manure amendment does.

3.2 Contribution to program objectives

The Rural R&D for Profit program aims to realise productivity and profitability improvements for primary producers through:

- generating knowledge, technologies, products or processes that benefit primary producers
- strengthening pathways to extend the results of rural R&D, including understanding the barriers to adoption
- establishing and fostering industry and research collaborations that form the basis for ongoing innovation and growth of Australian agriculture.

3.2.1 Novel wastewater treatment technologies

The project aimed to develop a new treatment technology, using the growth of algae and purple phototrophic bacteria (PPB) biomass that treats the water by capturing and assimilating nutrients and carbon in the wastewater into high-value microbial biomass. The test results demonstrated the proof-of-concept for application of the technology for treatment of various real agricultural industrial wastewaters. The development used broad lab screening study, a larger continuous study to show longer term performance, and finally using a pilot scale installed at a commercial piggery. The robustness of the technology was tested by varying operating conditions and wastewater composition. The project developed simple and effective harvesting and separation methods, growing the microbial biomass directly attached to illuminated surfaces from where it was harvested manually as a concentrated biomass. The high biomass concentration greatly reduces energy inputs and processing cost (e.g. drying). Microalgal treatment was most effective at removing contaminants from wastewater, whereas PPB provided best recovery of carbon and nutrients in the form of microbial biomass. In terms of integration with agricultural wastewater management systems, open algal ponds can be positioned downstream of anaerobic treatment (e.g. covered pond or anaerobic digester) to allow energy recovery as biogas and then subsequently assimilate residual carbon and nutrients into microbial biomass. In contrast, a PPB system can be a standalone (complete treatment) or placed upfront of anaerobic treatment, because it is more resilient to high ammonia than microalgae and it can directly assimilate carbon and nutrients from high-strength wastewater.

3.2.2 Novel feeds

Aquaculture is the fastest growing animal food production industry, now producing over 50% of all food fish, yet aquaculture is dependent on fishmeal derived from capture fisheries, making its continued sustainable growth near impossible. This project for the first time tests the bulk replacement of fishmeal with PPB microbial biomass in diets for barramundi (Asian sea bass, *Lates calcarifer*). The results showed that fishmeal can be bulk substituted with PPB without compromising fish survival rate, feed intake or carcass composition, albeit that PPB inclusion had moderate negative effects on fish growth and feed conversion. This trial was a preliminary yet firm proof-of-concept, demonstrating that bulk replacement of fishmeal with PPB is feasible. The outcomes were considered commercially viable, given that 33% fish meal replacement with PPB had little or no impact. Further research should investigate reasons for increased feed conversion rate at higher PPB replacement extents, to identify opportunities to optimise feed formulations for performance. Future research should also explore other benefits such as reducing high-cost vitamins and micronutrients given their presence in PPB. Supplementing a part of fishmeal in conventional aquaculture feeds could attract a price of >1400-1800 USD tonne^{-1}, provided that quality and consistency could be assured. This may lead to a preference for microbial biomass produced from non-animal derived wastewaters, e.g. sugar mill/stillage to simplify regulatory requirements for use in animal and fish feeds. The estimated PPB production cost were estimated to be up to 1600 USD tonne^{-1} of dry PPB product, but this does not consider savings on wastewater treatment by instead treating the wastewater with PPB. Considering typical discharge costs of organics, nitrogen and phosphorous, up to 1400 USD tonne^{-1} PPB product could be saved. This results in a net production cost of 200 USD tonne^{-1} PPB. We note that this needs to be confirmed in a demonstration plant.

3.2.3 Development of novel fertilisers

Australia's organically grown produce is a growing market with the domestic consumer market predicted to be worth A$2 billion by the end of 2018. This project developed a number of innovative waste treatment technologies that convert different farm by-products into low-cost, sanitised, high quality, balanced soil conditioners (including Frass, microalgae, PPB, high quality composts, pellets, digestate) that have a higher economic and agronomic value and are easy to handle and transport. The application of microalgae and frass in pot trials was shown to have yield gains of up to 80 per cent (equivalent to synthetic fertiliser) on a typical south-eastern Australian horticultural soil. They outperformed current organic products on the market and therefore have a strong competitive advantage in this growing high value, niche market. Also, in their improved sanitized forms, CBH will consider back-loading freight trains thereby greatly reducing the transport cost and further opening the broadacre agriculture market. Aside from producing high quality fertiliser products, these innovative waste treatment technologies have other economic, social and environmental benefits such as bioenergy recapture and waste purification and recovery of water. The recaptured nutrients can be developed as a novel feed.

3.2.4 Building subsoil fertility and overcoming soil constraints with organic matter amendment

The benefit of soil amelioration practices is about $124/ha/year (annualised value). The average cost of soil amendment across grain growing regions in WA is about $41/ha. This has a benefit: cost ratio of 3:1 and a **net benefit of about $83/ha/year**. Agricultural semi-arid soils of Western

Australia (WA) are characterised by poor soil structure coupled with low soil fertility, organic matter and carbon content making them susceptible to soil water repellence, soil acidity, soil compaction and herbicide resistance. In particularly, subsoil acidity has been identified as having the most significant economic impact a major yield-limiting constraint, affecting 8.5 million hectares of cropping soils in the south-west with an estimated to cost of at least $1.6 million/year in lost production potential.

We found that the application of high quality novel fertiliser could partially ameliorate soil acidity by improving N cycling, a major soil constraint across Western Australia's cropping regions. Additionally, the application of poultry compost was able to partially replace synthetic fertiliser in both horticulture and broadacre agriculture by addressing soil constraints (particularly acidity). Future research could determine the extent to which novel, low-cost, nutritionally balanced organic amendments can ameliorate soil acidity, address soil constraints and improve root growth by increasing and extending soil nutrient supply in subsoil throughout the season. We hypothesise that if nutrients are applied at depth and in a slow release organic form, there is potential for nutrient availability later in the season and greater yields.

The impact on crop yields is variable and dependent on the crop, soil type and amendment. Majority of the trials showed that crops yields were sustained when receiving the manure and reduced synthetic fertilizer rate (60-80%) inputs compared to conventional synthetic fertiliser at 100%. This means livestock enterprises could potentially increase their productivity, profitability and fertiliser use efficiency without impacting yield. Adding chicken compost had a "liming effect" on the soil when compared to the other amendments. By raising the pH, it could reduce the amount of liming required especially in acidic, semiarid areas of WA. It also overcomes constraints on grain production due to poor nutrient availability and biological activity by increasing the organic matter content, pH and CEC of the soil.

3.2.5 Improving crop resilience to heat and water stress and climate change

Applying compost improved shoot growth and P uptake in pasture soils under water stress much more effective than clay. The benefit of soil amelioration practices (such as liming or adding clay) is about $124/ha/year. The average cost of soil amendment across grain growing regions in WA is about $41/ha. Compost could be a cheaper option if cost transport can be lowered (by back loading CBH freight trucks).

The semi-arid soils of WA were low emitters of nitrous oxide (N2O) and a major sink of methane when amended with manures and composts. Methane uptake could lead to significant reduction is in GHG emissions by negating on-farm GHG emissions. This mitigation methodology could be included as Australian carbon credit units (ACCUs) issued under the Emissions Reduction Fund, currently trading at between $13-15 per tCO2-e of emissions abated

3.2.6 Overcoming barriers to adoption (Stable flies)

The Western Australian Broiler growing industry produces in excess of 225,000m^3 of spent broiler litter per annum. By-products of intensive animal production have long been recognized as an important soil amendment, providing nutritive value for crops and pastures as well as soil health attributes through increased carbon storage and the development of robust soil microbial populations. Unfortunately, poor management practices in the past have led to stringent application restrictions being imposed through Health (Poultry Manure) Regulations 2001 and more recently through the Biosecurity and Agriculture Management Act 2007 (BAM Act). The regulation of manure disposal options have led to loss of important manure marketing options causing significant cost increases (> 4 million) to the WA broiler growing industry.

Currently, the Western Australian compost industry does not have sufficient scale, capacity or end market to process the entire allotment of broiler litter and therefore large quantities of litter are transported to horticulture and broadacre agricultural zones for pasture and crop fertilisation. The broiler industry recognises the importance of responsible manure management and therefore proposes to further develop application framework to ensure that the remaining markets are not closed to manure application, causing significant escalation of manure disposal costs and threatening the viability of the local poultry industry.

Recently, opportunities have arisen that could open up new markets for the use of spent litter in broadacre. To exploit these opportunities, guidelines on the best management practices for its land application are needed to avoid stable-fly outbreaks whilst improving crop productivity and soil quality.

Overall, the field trials showed that the emergence of stable fly and other nuisance was very low from broadacre soils amended with raw chicken manure. The application rate (5 or 10t/ha), application method (broadcast on the surface or incorporated into to soil) application timing (winter versus summer) and application type (raw manure versus composted manure) had no significant influence on stable fly development and emergence. Moreover, stable fly emergence from soils amended with chicken manure or compost were comparable to soils amended with pig manure and compost.

Research result based on other horticulture and broadacre field trials at Brookton, Pingelly and Gingin in Western Australia funded through Agrifutures chicken meat program (PRJ 009946) on stable flies has led to an amendment to the Biosecurity & Agricultural Management Act 2007 permitting poultry litter/compost to be applied to broadacre agriculture in previously banned Shires.

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4 Collaboration

The interdisciplinary approach between the research institutions (UQ and UWA) has ensured that new technologies, products and knowledge has been developed and delivered in accordance with the project's schedule. The research teams have developed an extensive network of scientists, industry representatives and government officials across Australia to disseminate the project outcomes to producers and growers. The project was monitored and evaluated throughout by the project advisory group, consisting of members from each of the project partner industries.

We relied heavily on the advice and support of the project advisory group who provided essential contacts at industry sites from where real wastewater was collected for testing of the novel treatment technologies. The project work would not have been possible without this invaluable input and the willingness of industry sites to collect and analyse wastewater samples.

We developed an internal collaboration with The Aquatic Animal Health Laboratories (UQ) which further led to an external alliance with Ridley Aqua feeds. The Aquatic Animal Health Laboratories (UQ) executed the fish feed trials and Ridley assisted with the formulation and provided base materials for the barramundi (*Lates calcarifer*) feed trial. The project team is exploring other funding options with Ridley to expand the testing and proof of concept with other commercially relevant aquatic species with particular focus on Tilapia and prawns. The wastewater products that have been produced and characterized by UQ appear to be suitable as a partial fish feed substitute. The information obtained as part of this program was used to initiate an aquaculture feed trial and link up with a potential future supplier. There is potential for future investment from an Australian Research Council (ARC) Linkage or other funding sources to extend the project, conduct in depth analysis with multiple species including barramundi, Atlantic salmon, Tilapia and tiger prawns. An ARC Linkage application has been drafted and funding is currently negotiated with Ridley.

We collaborated with Prof. Stephen Rushton at Newcastle University, UK who specialises in the development and application of modelling techniques in biological systems and assisted in our data analysis and modelling of the fertilizer laboratory, pot and field trials.

We have formed new collaborations with Mr Luke Wheat at Future Green Solutions, Dr Bede Mickan at Richgro Ltd (fertiliser company) and Dr Navid Moheimani at the algae centre, Murdoch University. These collaborations have led to the development of innovative, high quality, concentrated, balanced fertilisers (algae and frass) and soil improvers (composts, pellets) generated from low-cost cleaner composting technologies. Dr Moheimani and his team assisted UWA with the pot trial assessing the suitability of microalgae biomass produced from piggery waste as a fertilizer by providing the algae biomass and contributing to the experimental design and writing of the manuscript. Dr Bede Mickan and his team at Richgro assisted in the development of high quality, balanced composts and contributed to the experimental designs, undertaking of the experiments, data analysis and co-authored the paper. Additionally, Richgro and Murdoch University provided in-kind contributions of salaries, materials and analysis tools.

The collaboration members are committed to developing high quality products from a variety of waste streams. During the final year of this grant UWA conducted further experiments with Dr Mark Farrell at CSIRO, Dr Bede Mickan and Dr Navid Moheimani to examine the specific mode of action of the new fertiliser products so that they can be refined and tailored to different end-users. This has aided the development of new markets for the products and that will hopefully maximize the generation income and profit for the livestock industries.

The collaboration (UWA, Murdoch University, Richgro and CBH) will actively seek further funding to ensure the high-quality fertilizer products developed during this project are fully commercialized. UWA have won an AusIndustry grant to allow Dr Bede Mickan to do a placement at UWA and Murdoch University and students to work at Richgro. Further work will focus on the development of a simple, low-cost algae waste treatment facility to produce algae biomass commercially.

By creating opportunities to generate new revenue from their waste streams this project has gained support and collaboration across the primary sector organisations including; Western Australian Lot Feeders Association (WALFA); Western Australia Broiler Grower Association (WABGA); West Australian Pork Producers' Association (WAPPA); Altona hatcheries; Western Dairies, South West Catchments Council (SWCC); Department of Agricultural and Food Western Australia (DAFWA), West Australian Pork Producers' Association (WAPPA), Grower Group Alliance, Soil Alliance and composting companies (e.g. C-WISE). The establishment of these networks has aided the dissemination of information arising from the project that has been utilised by early adopters. Consultation with these industry partners identified a number of barriers to adoption including cost of product, cost effective transportation, legislation, regulatory and licencing requirements, variability of product (both physical and nutrient status), pathogens, GHG emissions, application methods and research to substantiate benefits to soil and plant health.

To overcome barriers relating to cost of transportation, UWA has engaged with CBH (freight distribution network), farmers and composting company about the distribution of high quality compost via freight trains. Also, the CBH group, a grain growers' cooperative that handles, markets and processes grain from the wheatbelt of WA, has expressed an interest in converting manures and other agricultural wastes into high-value agronomic products for their grower client base. In this improved form (high quality soil improver), CBH will consider back-loading freight trains (thereby greatly reducing the transport cost) if the agronomic and economic value in broadacre agriculture as well as the consistency and safety of the product can be demonstrated. This provides an opportunity to reduce the cost associated with transportation, making the re-use of alternative fertiliser in broadacre an economically viable option for both the producer and grower.

The project partners have also worked extensively collaborated with DPIRD, Department of Environment, Regulation and Water, regulatory relevance group (based in WA, that reports and makes recommendations to the Stable Fly Ban Act), Agrifutures and other policy makers and regulatory bodies to demonstrate that applying poultry litter to broadacre agriculture didn’t significantly increase the emergence of stable fly. By providing the scientific evidence for economic value of poultry litter, sustainable practices for their application to avoid stable fly emergence, environmental management to aid planning approval the research was able to change regulation.

This project has also developed collaboration between Future Green Solutions (FGS) and The University of Western Australia (UWA) to progress a novel concept into a new industry to support the long-term sustainability of the fish and animal feed market.

With support for the Director General of the Department of Fisheries (WA), Western Australian Fishing Industry Council (WAFIC) and Aquaculture Council of Western Australia (ACWA), the UWA team have applied for funding with FGS. During the development of this FRDC application, new relationships with Ridley Corporation, Richgro, Department of Fisheries (WA), Department of Food and Agriculture (WA), ChemCentre(WA) and GAE were have been developed.

5 Extension and adoption activities

Over the life of the project, the project leaders disseminated the knowledge to producers, growers, consultants, policy makers, stakeholders, policy makers and scientists by presenting at field days, conferences, meetings, workshops and forums. In addition, the project leaders have disseminated information by writing plain English review, factsheets, YouTube videos, articles, technical notes and a practical guide on using organic amendments to address soil constraints and economic scenarios in which biological inputs may improve farm profitability. The project leaders have also sought opportunities to reach a wider audience through media outlets (print and radio) including rural specific media (e.g. farm weekly and Countrymen and appearing on Landline) was also utilised to facilitate communication of the project outcomes. To ensure a high degree of visibility within the scientific community, data arising from this project was published in high impact journals and the project leaders have presented papers and lectures at international and national conferences and meetings.

In addition, this project disseminated the knowledge to growers, stakeholders, policy makers and scientists by presenting at field days, conferences, workshops and preparing plain English review, factsheets, YouTube videos, articles, technical notes and a practical guide on deep placement organic amendments for addressing soil constraints and economic scenarios in which biological inputs may improve farm profitability.

An adoption program was developed that targets both the intensive animal industries (generators of waste by-products and feed substitution users) and the end-users of the products such as composting facilities, pasture, horticulture, tuft, grains and cropping industries. Producers were directly engaged in field trials and pilot sites, including integrated production, and generation of fertilizer products. Cost-benefit analysis was done throughout the project with feasibility analyses and case studies published. Broader stakeholders were engaged through position papers (including global scale impacts). Technical feed and fertiliser products were developed through existing and innovative technology providers (particularly Richgro, CBH, C-WISE, Future Green Solutions, KBR and Trojan technologies)

An extension program was also developed to support new and emerging industries (e.g. developing new waste management technology that uses black soldier fly larvae to convert animal waste into fish feed, biodiesel and composts) that offer alternatives commercial pathways for growers to sell their products. The extension program worked closely with the industries partners to ensure the outcomes were tailored towards the needs of the end users in terms of product production and how they would like to receive any information or technology transfer. Key champions were identified from each industry, field trials occurred where possible on 'real farms' to encourage ownership of the technologies and products.

New resources, tools and knowledge bases (factsheets, user guides) have been developed that could facilitate profitability in new emerging industries (e.g. Truffles) as well as industries that traditionally haven’t utilised waste by products.

This project identified novel advanced wastewater treatment technologies, and novel biological products tailored to different end-users and applications. Benefits have included improved sustainability (wastewater treatment energy inputs, feed displacement, soil quality, low GHG potential) and profitability (wastewater treatment efficiency, feed displacement, crop productivity, reduced input costs).

It is envisaged that further work (2-4 years) will be needed in the future to show the feed benefits, and to demonstrate the agronomic and economic benefits. These could include aquaculture trials on different species, and in terms of fertilizer applications could be carried out via regional field trials.

Beyond this (3-6 years) it is envisaged to demonstrate the long-term benefits of new fertilisers/soil improvers needed along with an extension program to raise general awareness and deliver clear, consistent and evidence-based recommendations (and tools) emerging from the research. This is needed to engage a wider farmer and adviser audience and provide support for the second wave of adopters. In terms of feed supplements, it is worth optimising and confirming commercial performance, and then to carefully explore markets to maintain a reasonable price. This would be to some extent buffered by production capacity of microbially derived protein, because it is unlikely possible to produce enough microbial protein for full market displacement; however, the supply-demand of microbial biomass still needs to be carefully managed.

Future funding will be sought to develop the novel fertilisers as certified organic products and biostimulants in order to take full advantage of the emerging niche markets such as hydroponics. The biostimulant program, which take advantage of novel by-products, could be used to replace conventional fertilisers to reduce cost of production and provide alternative income streams for agricultural enterprises.

The scale up and deployment of the current cartridge system seems feasible. This would require a commercialisation partner and a major primary industry partner. From the testing in the present project, it seems most feasible to deploy the technology in the pork, poultry or red meat processing sectors, because the baseline data from this project provides some certainty about the treatability of wastewaters from these sectors. The use of sunlight instead of active illumination would ensure cost-feasibility, and the use of wastewater derived nutrients will also be important to circumvent nutrient costs.

6 Lessons learnt

One of the highlights of this project has been engaging with policy makers and producing research that has effectively changed regulation under the Stable Fly Ban Act, to permit the application of poultry litter to non-irrigated broadacre agriculture. This experience demonstrated the importance of involving government and regulatory bodies during the initial stages of the research process. As a result, we were able to identify barriers to adoption and other concerns with regards to the application of novel fertilisers to both horticulture and broadacre agriculture. Consequently, we were able to adjust our research programme to ensure that the scientific data generated supported any policy, licensing and regulatory changes needed to overcome restrictions on their use. It is hoped that the data will continue to aid in the reclassification of the fertiliser products from environmental hazardous waste to soil improvers and lead to further amendments to the Stable Fly Ban Act. This would then permit the use of poultry litter in irrigated horticultural systems, thereby opening up new markets for chicken meat producers. We highly recommend that scientists engage with government, policy makers and regulatory bodies at the beginning and throughout their research program.

7 Appendix - additional project information

7.1 Project, media and communications material and intellectual property

Journal papers (Activity 5, Output 5(b); Activity 6, Output 6(a))

Abbott, L., Macdonald, L., Wong T.F., Wong, M; Webb, M., Jenkins, S., Farrell, M. (2018) 'Potential roles of biological amendments for profitable grain production – a review'. Agriculture Ecosystems & Environment. 256, pp. 34-50.

Cook, D.F., Jenkins, S. N., Abbott, L.K., Voss, S. C., Telfer, D.V., Deyl, R.A., Lindsey, J.B. Amending poultry broiler litter to prevent the development of stable fly, Stomoxys calcitrans (Diptera: Muscidae) and other nuisance flies. Journal of Economic Entomology (accepted)

Delamare-Deboutteville, J., Batstone, D.J., Kawasaki, M., Stegman, S., Salini, M., Tabrett, S., Smullen, R., Barnes, A.C., Hülsen, T. 2018. Mixed culture purple phototrophic bacterial biomass is effective as a partial replacement for fishmeal in feeds for barramundi aquaculture. Environmental Science & Technology*. **In Submission.***

Hülsen, T., Hsieh, K., Lu, Y., Tait, S., Batstone, D.J. 2018a. Simultaneous treatment and single cell protein production from agri-industrial wastewaters using purple phototrophic bacteria or microalgae – A comparison. *Bioresource Technology*, **254**, 214-223.

Hülsen, T., Hsieh, K., Tait, S., Barry, E.M., Puyol, D., Batstone, D.J. 2018b. White and infrared light continuous photobioreactors for resource recovery from poultry processing wastewater – a comparison. *Water Research. **Accepted.***

Jenkins, S.N., Mickan, B., Weerasekara, A., Schwab, S., Waite, I.S., Solaiman, Z., Abbott, L.K. Enhanced bacterial processes associated with soil C following application of compost and manure to a dairy pasture. Animal Production Science (second review).

Jenkins, S.N., Rushton, S.P., Waite, I.S., Mathes, F., Abbott, L.K., Murphy, D.V. Do soils receiving organic amendment have a greater resilience to heat stress? Soil Biology & Biochemistry (in submission)

Mickan, B., Abbott, L., Fan, J., Hart, M., Siddique, K., Solaiman, Z., Jenkins, S. (2018), 'Application of compost and clay under water-stressed conditions influences functional diversity of rhizosphere bacteria', Biology and Fertility of Soils. 54, pp. 55–70

Conference presentations

Single cell protein production from agricultural and industrial wastewater with simultaneous organics and nutrient removal by purple phototrophic bacteria; 15th Anaerobic World Congress, 17-20th October 2017, Beijing, China.

Resource recovery from wastewater by purple phototrophic bacteria; Nutrient stewardship & Next-Generation Fertilizers, 10-13th Sep 2016, Heron Island, Australia

Phototrophic bacteria for complete nutrient recovery from domestic wastewater; 14th Anaerobic World Congress, 15-18th Nov 2015, Vina del Mar, Chile

Purple Phototrophic Bacteria for Resource Recovery from Domestic and Industrial Streams; Changing Paradigms of Wastewater Treatment; From Waste to Resource, 27-29th March 2017, KAUST, Saudi Arabia (invited speaker).

Methane uptake is significant in semi-arid soils amended with manures and composts. New Zealand and Australian Soil Societies joint conference, 12-16th November 2016, Queenstown, Australia.

Bacterial processes associated with soil carbon after application of compost and manure to dairy pastures. Australasian Dairy Science Symposium (ADSS2016). 16 – 18th November 2016, Sydney, Australia

How can organic farming practices contribute to sustainability and profitability of agriculture? National Trade Fair 2017, Organics and Millets, 28 – 30th April 2017, Bengaluru, India (invited speaker).

Assessing the suitability of microalgae biomass produced from piggery waste as a fertilizer. The Australasian Pig Science Association (APSA), 19-22nd November 2017, Melbourne, Australia

Amending poultry broiler litter to prevent the development of stable fly. National Soils Conference 18-23 November 2018, Canberra, Australia

7.2 Equipment and assets

Not applicable

7.3 Monitoring and evaluation

Final Monitoring and Evaluation Report included with Appendices folder to this document. This report details the project's outcomes against the program objective and include quantitative and qualitative information on outcomes achieved and expected.

7.4 Budget

The final financial report will be submitted within 60 days of the final milestone report. APL will provide a statement of funds and contributions received and spent as per the grant agreement