



final report

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Prepared by: Terry Rose/Bronwyn Barkla
Southern Cross University
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Identification of suitable crops for sustainable bio-remediation of wastewater from a bovine service abattoir

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Executive Summary

Background and methods

The continual irrigation of high-nutrient abattoir and/or tannery wastewater relatively levels onto a limited land area can result in accumulation of nutrients such as phosphorus (P and sodium (Na) in soil, and leaching of nitrogen (N) into waterways. Cultivating plant species that have the capacity to grow rapidly and extract relatively high amounts of key nutrients (N, P and possibly Na) may enable the creation of a near-neutral system, where nutrient input in wastewater is matched with nutrient offtake in harvested crop material on an annual basis. This option becomes more economically attractive if these crops can be used for bioenergy as sold as a commodity.

To investigate the feasibility of various annual and perennial 'mop-crop' options at the Northern Cooperative Meat Company (NCCMC), a replicated field trial with five cropping treatments was established on the NCCMC farm (Casino, NSW). The two annual treatments were Rhodes grass/ryegrass (summer/winter) and forage sorghum/ryegrass (summer/winter) while the perennial crops trialled were tea tree (*Melaleuca alternifolia*), Bamboo (*Bambusa oldhamii*) and giant reed (*Arundo donax*). Reports from the literature suggested that these perennial crops have high growth rates and N and P accumulation in subtropical conditions. The Rhodes grass/ryegrass treatment was considered as the control treatment as this is the current practice at the site.

Crop species were established in randomised 3 x 4 m plots in December 2015 either by seed (Rhodes grass and forage sorghum) sown at a relatively high density, or as seedlings planted at 1 m row spacing and 0.5 m between seedlings in a row (tea tree) or 1 m between seedlings in a row (giant reed and bamboo). To assess the value of each crop in removing nutrients, we measured the amount of N and P removed in harvested crop biomass, the amount of nitrate-N that leached below each plot using suction cap lysimeters after each irrigation or large rain event, and measured nutrient concentrations in the topsoil (0-100 mm) after 10 months. The Rhodes grass and ryegrass were harvested on three occasions each, the sorghum was harvested twice, and the three perennial crops were harvested once (September 2016) during the trial period.

Key Results

There was no statistically significant impact of any crop treatment on the levels of key nutrients (N, P, K, Ca, Mg, Na) in the topsoil after 10 months. This is not surprising given that there was inherent variation in soil nutrient levels across the trial site prior to the establishment of the crops, and statistically significant changes in these levels due to difference in crop nutrient removal would not be expected to be seen until after several years of nutrient removal by crops. The total amount of nitrate that leached to 400 mm depth was significantly higher in the tea tree plots (49 mg nitrate per lysimeter) than Rhodes grass/ryegrass plots (6 mg nitrate) or sorghum/ryegrass plots (2 mg nitrate). Bamboo (26 mg nitrate) and giant reed (30 mg nitrate) plots were intermediate but did not leach significantly more nitrate than the Rhodes grass/ryegrass control plots.

The annual crop treatments produced significantly more biomass (39t/ha and 16t/ha for forage sorghum/ryegrass and Rhodes grass/ryegrass, respectively) than the perennial crop treatments which yielded 5-6t/ha each. The removal of N, P and K reflected the biomass production with 537, 63 and 641 kg/ha of N,P,K removed by forage sorghum/ryegrass while removal rates of N, P and K were 4-5 times lower in the perennial crops for all nutrients.

Conclusions and recommendations

The lower growth rates and nutrient removal in all perennial crops, and higher nitrate leaching in the tea tree, was not unexpected given that they were planted at a relatively low

plant spacing and take at least 12 months to establish a solid root system before investing in shoot growth. This highlights the need to introduce paddocks of perennial plants into the farm in an incremental manner, should any perennials prove more effective than annual species in subsequent years. **It is recommended that the trial be continued for a further 1-2 years to fully assess any potential benefits of the perennial crops to remove nutrients from the site.**

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1 Background

The slaughtering, and processing activities required for meat production in slaughterhouses, generates large quantities of wastewater and solid waste with predominantly high salt and organic content. The reuse of wastewater and effluents for irrigation can result in excessive nutrient build-up in the soil, in particular significant amounts of N, P and organic carbon, but also sodium, which over time may cause land degradation. Conventional remediation of the effluent is costly and therefore implementation of a more economical wastewater management process is attractive. Phytoremediation, the use of plants to clean up polluted soils and water is not only energy and cost efficient, but can emerge as a more sustainable measure towards wastewater management.

For an effluent irrigation system to be sustainable, the amount of water, nutrients and chemicals that will be applied should be effectively processed by the selected crop or cultivar, and site-specific factors such as climate, topography and soil should be monitored. Plant selection is a crucial factor in phytoremediation since different plant species respond differently to the same wastewater and have different capacities for uptake of nutrients and specific pollutants. By cultivating suitable plant species on the wastewater irrigated land, excess nutrients can be phyto-extracted by the plants for growth. In the process, a large amount of biomass could be produced for energy generation, as a feed source for grazing animals or with other industrial end uses (i.e. hemp fibre or tea tree oil). This approach would be a solution to add value to the existing rural industry processes and effectively close the loop on waste system management.

The selection of plant species for the trial site is based on several key attributes including:

- ability for rapid growth,
- high biomass production,
- high rates of transpiration,
- ability to accumulate sodium,
- nutrient scavenging abilities,
- options for industrial end use.

The aim of this project is to conduct a replicated field trial using small plots to determine the performance of a range of potential phytoremediation crops, namely Giant reed (*Arundo donax*), bamboo (*Bambusa oldhamii*), tea tree (*Melaleuca alternifolia*), sorghum (*Sorghum bicolor*)/ryegrass (*Lolium multiflorum*) compared to the current system of Rhodes grass (*Chloris gayana*)/ryegrass (control treatment).

2 Projective Objectives

The objectives of the project are:

- To characterise the nutrient and salt levels existing in the soil at the trial site;
- To evaluate the performance of a range of phytoremediation crops irrigated using wastewater discharged from the plant;
- To analyse the capacity of the crops to minimise the build-up of N and P in the topsoil and minimise the leaching of nitrate into the subsoil nutrients and salt in the soil;
- Develop tactical recommendations based on the performance and potential yield of the different crops that could be used to inform decisions of other abattoirs.

3 Methodology

3.1 Crop establishment

A randomised plot design was established with 4m x 8m plots with four replicate plots per crop treatment (i.e. 20 plots total) (see Figure 1). *Arundo donax*, Tea tree and Sorghum were planted on December 22nd 2015. This was a delay of approximately 8 weeks from that initially scheduled in the project. This was due to a delay in signing of the initial agreement between MLA and NCMC and the need to obtain permits for the *Arundo donax*.

Arundo donax –

Permit No. OUT15/25960 was obtained from the Department of Primary Industries to have, transport, grow and dispose of *Arundo donax* which is classified as a noxious weed in NSW.

Plants were regenerated from culm cuttings sourced from a mature stand on the banks of Eden Creek at the intersection of Afterlee Road and Brown Knob Road, Eden Creek, outside Kyogle. Young culms were cut into pieces comprising 2 internodes and placed directly in water. Once roots emerged, approximately 2 weeks, culms with emerging plantlets were transferred to soil in 6 cm pots and grown for a further 4 weeks in the greenhouse prior to transplanting in the field. Plants were transferred into plots at a density of 28 plants per plot with an inter row spacing of 1 m (Figure 1).

Tea Tree –

Three different Tea tree ecotypes were selected for the trial; two low land varieties, Devil's pulpit and Barcoongere, and one upland variety, Sundowner. Seedlings were grown from seed obtained from Dr. Mervin Shepherd, SCU, in propagation trays. Two to four month old seedlings were transplanted into 6 cm pots with soil and grown in the green house for a further 4 weeks to ensure they were vigorous enough to survive field transplanting. Seedlings were planted at a density of 56 plants per plot with an inter row spacing of 1m and 0.5m between plants within a row (Figure 1). Ecotypes were planted in each of three rows with the last row being a mix of ecotypes.

Bamboo –

Bamboo, *Bambusa oldhamii*, was sourced from Bamboo Land, 87 Old Coach Rd, Torbanlea QLD. Plants obtained were approximately 80 cm in height and growing in 3 L bags. Plants were planted on January 6, 2016 at a density of 28 plants per plot with an inter row spacing of 1m.

Sorghum –

Sorghum seeds were obtained from Landmark (Lismore) and sown by hand at a density of 75 g per plot.

At the time of this report plants are approximately 4 months since transplantation into the field and well established with evidence of good root growth. The progress of plant establishment can be observed in Figures 1 to 3.

3.2 Plant Harvesting and soil measurements

Plant Harvesting

Forage sorghum was harvested on 23rd February and 13th May 2016, Rhodes grass was harvested on 5th April and 1st June 2016 and ryegrass was harvested on 1st August and 29th September 2016 by cutting stalks at a height of approximately 10 cm above ground level. Tea tree, *Arundo donax* and bamboo were harvested on 29th September. Fresh weight of removed biomass was obtained for each plot and a subsample of material was placed in the SCPS drying room (45 °C) to obtain dry weight. The dried subsample was then roughly ground before a further subsample was finely ground and sent to Environmental Analysis Laboratories (at SCU) for nutrient analysis by LECO (for N) and Inductively Coupled Plasma Mass Spectrometry (P and Na).

Soil Sampling and Analysis

Initial soil samples were taken on December 22, 2015 to a depth of 90 cm, using increments of 0-15cm, 15-30cm, 30-60cm and 60-90cm with locations mapped in Figure 4. Based on visual differences in topsoil colour, two areas were chosen for sampling (Eastern side and Western side). Ultimately, the soil chemical properties of the two areas were relatively similar and therefore mean values are presented in Table 1.

Table 1 – Selected chemical properties of the 0-100 mm horizon of the Ferrosol taken from the field site.

Property	Soil depth (cm)			
	0-15	15-30	30-60	60-90
Total carbon (%)	2.3	0.75	0.41	0.25
Total nitrogen (%)	0.17	0.06	0.04	0.03
KCl extractable ammonium-N (mg kg ⁻¹)	25	8.8	7.1	7.3
KCl extractable nitrate-N (mg kg ⁻¹)	141	21	11	5.6
pH (1:5 water)	5.1	5.3	5.3	5.7
EC (dS m ⁻¹)	0.28	0.09	0.08	0.05
Bray 1 P (mg kg ⁻¹)	9.7	1.7	1.6	1.6
Cation exchange capacity (cmol ⁺ kg ⁻¹)	10.8	19.8	23.6	23.9
<i>Base cations (cmol⁺ kg⁻¹)</i>				
Calcium	4.3	1.8	0.48	0.17
Magnesium	3.9	5.1	3.5	2.4
Potassium	0.49	0.26	0.15	0.1
Sodium	1.0	1.2	1.1	0.8
Aluminium				
<i>DTPA extractable metals (mg kg⁻¹)</i>				
Zn	2.0	0.1	0.1	0.4
Mn	74	8.3	3.0	1.8
Fe	20	33	25	67
Cu	0.5	nd	nd	nd

Pore water sampling for nitrate leaching

Following sufficient root establishment, piezometers were installed to 40 cm depth to enable the measurement of nitrate concentrations in pore water. Any nitrate that is detected at or below 40 cm depth is assumed to have leached below the root zone and is therefore a

potential contaminant. Pore water samples were measured on five occasions in each plot following either an irrigation event or large rainfall event. The amount of nitrate leached in each plot was summed to give a value of cumulated nitrate leached. Nitrate in the pore water was measured using colourimetry in the laboratory at SCU.



HEMP	ARUNDO	BAMBOO	SORGHUM	TEA TREE
SORGHUM	TEA TREE	HEMP	BAMBOO	ARUNDO
ARUNDO	BAMBOO	SORGHUM	TEA TREE	HEMP
TEA TREE	SORGHUM	ARUNDO	HEMP	BAMBOO

Figure 1: Planting of tea tree and *Arundo donax* and sowing of Sorghum, December 2015 according to the randomized plot design shown in bottom panel.



Arundo donax



Bamboo



Tea tree

Figure 2: Two months of growth.



Arundo donax



Tea tree



Bamboo



Sorghum

Figure 3: Four months of growth.

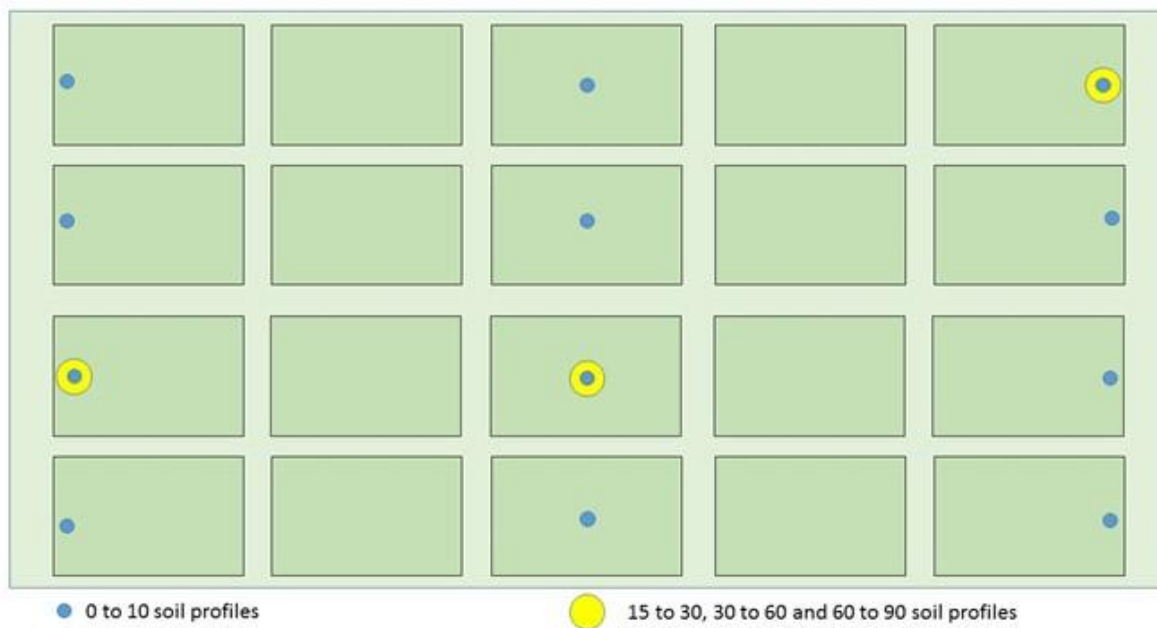


Figure 4: Map of test plot depicting location and depth of soil samples obtained December 22, 2015. Depth values are in cm.

3.3 Statistical analyses

Data for plant biomass, and N, P, K accumulation, were analysed by ANOVA (analysis of variance) to determine variance within and between groups. This was carried out by fitting crop treatment as well as column and block effects using Genstat (VSN International 2014) using a probability level of 0.05. Soil data, including topsoil nutrient concentrations and leached nitrate data, were also analysed in a similar way. Significance of differences between treatment mean values for each trait was tested using Duncan's multiple range test. Data transformation was undertaken where appropriate to satisfy normality assumptions.

4 Results

4.1 Plant growth and nutrient removal

Biomass production across the 10 month growing period was significantly higher in the annual crop treatments, with 39t/ha and 16t/ha for forage sorghum/ryegrass and Rhodes grass/ryegrass, respectively, compared to 5-6t/ha for tea tree, bamboo and giant reed (Figure 5). The biomass production in the forage sorghum/ryegrass treatment was dominated by the forage sorghum crop which accounted for more than 90% of the total biomass for this treatment (data not shown).

The removal of N, P and K reflected the biomass production with 537, 63 and 641 kg/ha of N,P,K removed by forage sorghum/ryegrass while removal rates of N, P and K were 4-5 times lower in the perennial crops for all nutrients. Notably, the forage sorghum/ryegrass treatment produced significantly more biomass and removed more N, P and K than the control treatment (Rhodes grass/ryegrass).

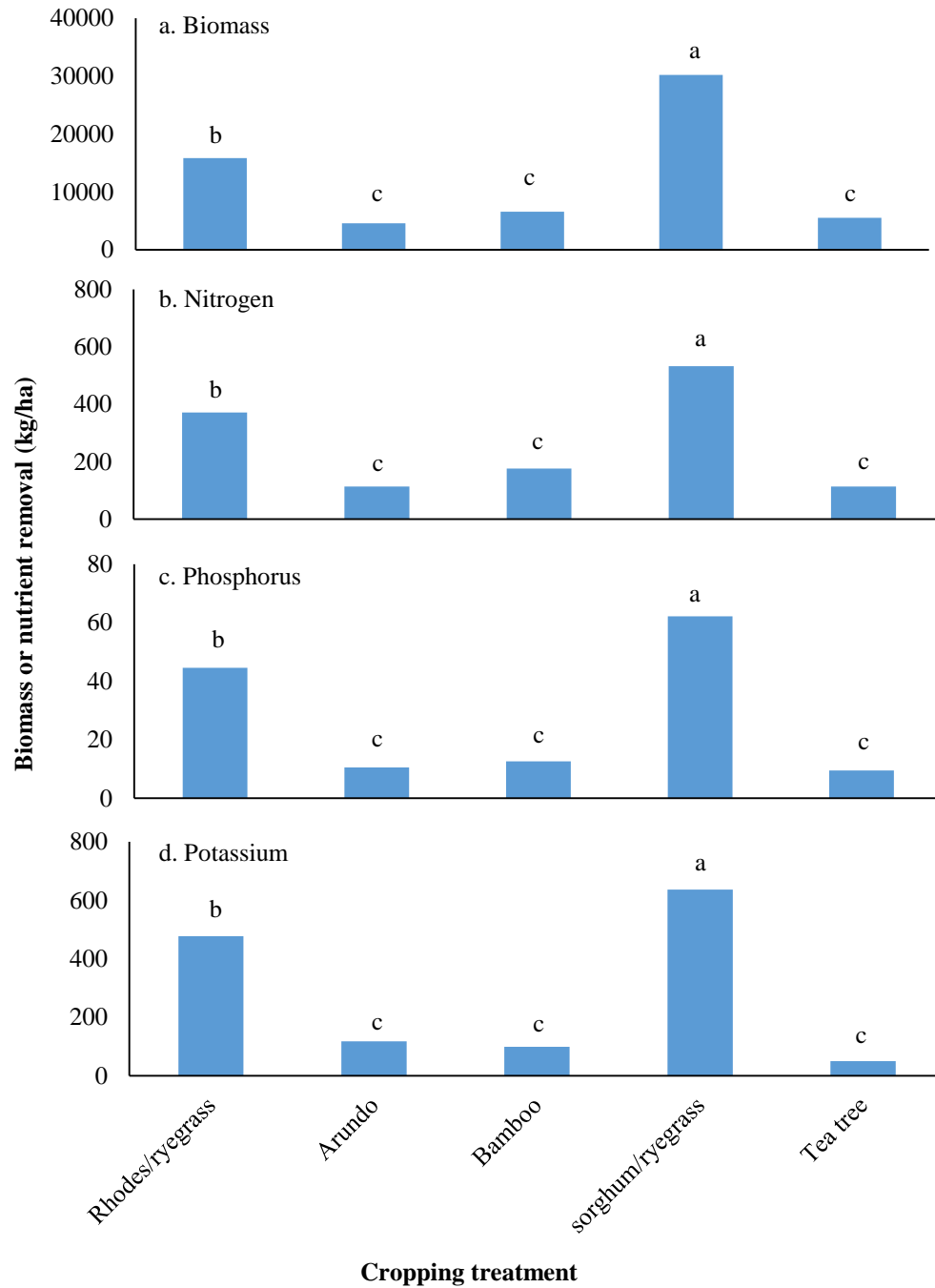


Figure 5. Biomass and nutrient removal by five crop treatments from December 2015 to September 2017. Bars not followed by a common letter are significantly different at $P < 0.05$.

4.2 Topsoil nutrient accumulation and nitrate leaching

The concentration of nutrients in the topsoil (0-100 mm horizon) did not differ among cropping treatments for any nutrient examined. The mean nutrient levels in the topsoil are shown in Table 2.

Table 2 – Mean nitrogen and phosphorus concentrations and key soil characteristics in the 0-100 mm horizon.

Property	
Total carbon (%)	2.4
Total nitrogen (%)	0.16
KCl extractable ammonium-N (mg kg ⁻¹)	5.0
KCl extractable nitrate-N (mg kg ⁻¹)	46.4
pH (1:5 water)	5.3
EC (dS m ⁻¹)	0.17
Bray 1 P (mg kg ⁻¹)	20.1
Total P (mg kg ⁻¹)	320

The total amount of nitrate that leached to 400 mm depth was significantly higher in the tea tree plots (49 mg nitrate per lysimeter) than Rhodes grass/ryegrass plots (6 mg nitrate) or sorghum/ryegrass plots (2 mg nitrate) (Fig. 6). Bamboo (26 mg nitrate) and giant reed (30 mg nitrate) plots were intermediate but did not leach significantly more nitrate than the Rhodes grass/ryegrass control plots.

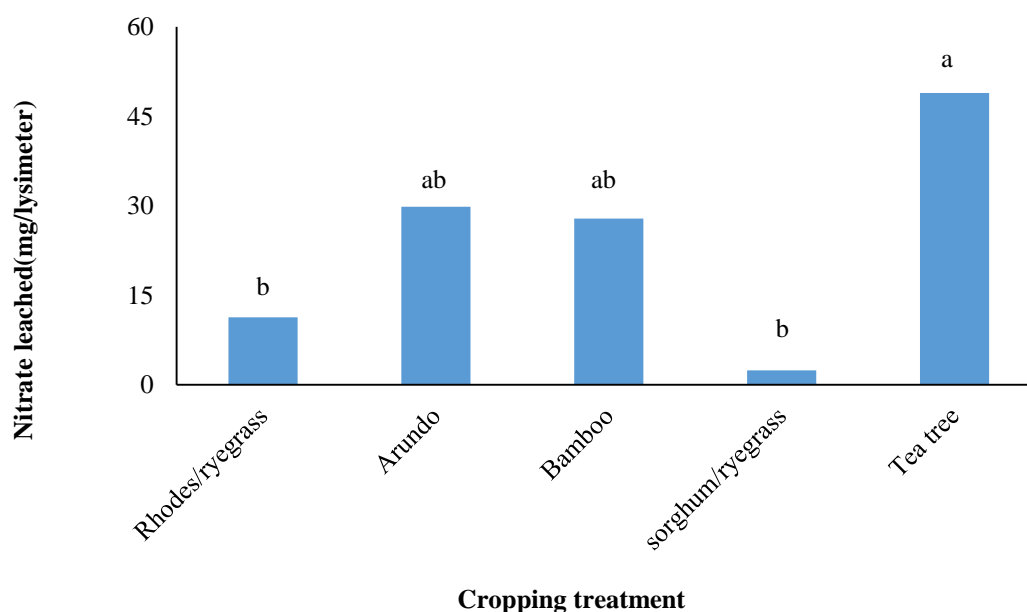


Figure 6. Cumulative nitrate leached to 40 cm depth under five crop treatments from December 2015 to September 2017. Bars not followed by a common letter are significantly different at $p < 0.05$.

5 Discussion

1) Characterisation of the nutrient and salt levels existing in the soil at the trial site

Nutrient levels were characterised in the top 100 mm of the soil (see Table 1) and indicated that some existing variation in key nutrients was already present prior to the establishment of the crop treatments.

2) Evaluation of the performance of a range of phytoremediation crops irrigated using wastewater discharged from the plant

That the annual crop treatments produced significantly more biomass (39t/ha and 16t/ha for forage sorghum/ryegrass and Rhodes grass/ryegrass, respectively) than the perennial crop treatments which yielded 5-6t/ha each, was not surprising given that the perennial crops produced very little biomass during the first 6 months of the trial. During this establishment phase it appeared that the perennial species invested energy into establishing root systems and rhizomes in the case of *Arundo donax*. It is expected that the benefits of the perennial crops would be seen in subsequent years. The removal of N, P and K reflected the biomass production with 537, 63 and 641 kg/ha of N,P,K removed by forage sorghum/ryegrass while removal rates of N, P and K were 4-5 times lower in the perennial crops for all nutrients. It may be possible to increase biomass production and nutrient removal in the perennial treatments if the seedlings had been planted at higher density. However for the purpose of this study we elected to use a density that is more likely to be commercially viable, which inevitably led to lower biomass production during the 9 month period of the trial.

3) Capacity of the crops to minimise the build-up of N and P in the topsoil and minimise the leaching of nitrate into the subsoil nutrients and salt in the soil

The lack of statistically significant differences in topsoil nutrient levels among crop treatments was not surprising given the variation that existed prior to the establishment of the cropping treatments. To achieve significant changes in soil nutrient levels as a result of cropping treatments, it is expected that at least 3-4 seasons of nutrient removal would be needed. Nitrate leaching data agreed well with crop growth, in that the slower growing perennial plants allowed more nitrate to leach beyond the root zone in the first 9 months after crop establishment. Once again, this is not overly surprising given that the perennial crops used the first 6 months as an establishment phase and did not produce much biomass during this period.

4) Tactical recommendations based on the performance and potential yield of the different crops that could be used to inform decisions of other abattoirs.

The crop treatment that removed the most N and P from the site after 10 months was the forage sorghum/ryegrass treatment, which produced significantly more biomass and removed more N, P and K than the control treatment (Rhodes grass/ryegrass). This suggests that a summer sorghum crop may be an attractive option to remove nutrients if suitable paddocks are available. Ultimately, it was not possible to determine if perennial cropping options may be viable in the longer term because their low biomass production in the first year simply reflects their perennial growing habit. It is expected that they would perform to a level equal to, or greater than, the annual cropping treatments in subsequent years. However, the slow establishment of the perennial crops does highlight the need to introduce paddocks of perennial plants into the farm in an incremental manner in future, should they prove to be a viable longer term option.

6 Conclusions/Recommendations

The lower growth rates and nutrient removal in all perennial crops, and higher nitrate leaching in the tea tree, was not unexpected given that they were planted at a relatively low plant spacing and take at least 12 months to establish a solid root system before investing in shoot growth. This highlights the need to introduce paddocks of perennial plants into the farm in an incremental manner, should any perennials prove more effective than annual species in subsequent years. It is recommended that the trial be continued for a further 1-2 years to fully assess any potential benefits of the perennial crops to remove nutrients from the site.

7 Key Messages

1. Forage sorghum may be a viable option for summer cropping to replace Rhodes grass in suitable paddocks, but the extra time and monetary costs of establishing the crop need to be considered given that Rhodes grass pasture already exists;
2. It is not possible to judge the performance of the perennial crops after one season given the time it takes these crops to establish: assessment over subsequent years is needed;
3. Any possible introduction of perennials into the overall farming operation would need to be done on a gradual basis given the apparent lag time for these crops to fully establish and achieve rapid biomass production.