

Comparative effect of alternative fertilisers on pasture production, soil properties and soil microbial community structure

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Abstract. Different fertiliser products are commonly promoted for use on pastures in order to improve pasture productivity and support a more ‘healthy’ soil microbial environment. However, minimal field research has been conducted to validate such claims. A 6-year study (2009–14) was conducted on phosphorus (P)-deficient soils at three sites near Yass, New South Wales, to investigate the effect of topdressing perennial native-based pastures with a range of alternative fertilisers compared with single superphosphate and an unfertilised control treatment. The alternative fertiliser products included manures, composts, crushed rock, rock-phosphate-derived products, concentrated ash and microbial products. Annual measurements were made of soil chemical properties, botanical composition and pasture yield during spring and/or winter + spring, as well as the relative effectiveness of products per unit of pasture grown. Soil microbial community structure under each fertiliser treatment was also analysed in the sixth year of the study. Fertiliser products with substantial quantities of P increased extractable soil P and resulted in significantly higher pasture growth and clover content compared with the unfertilised control. Superphosphate was found to be the most P-effective fertiliser for increasing pasture growth, along with a range of other products that showed differential responses. However, the cost and P-effectiveness of the products in relation to pasture growth varied considerably and was a function of rate and frequency of application as well as amount and solubility of the P applied. Despite large differences in pasture growth across the various fertiliser treatments, there was no significant effect of the alternative fertiliser products on microbial community structure compared with either the superphosphate or unfertilised control treatments. The observed variation in bacterial, fungal and archaeal community structures across all fertiliser treatments was best explained by soil pH or aluminium (Al) concentration, which was influenced differentially by the fertiliser products. Fungal community structure was also correlated with pasture-productivity parameters (i.e. spring pasture yield, clover content and soil-available P). Our findings reveal a highly resilient soil microbial community that was influenced minimally by use of the alternative fertiliser products, thus highlighting that on-farm management decisions regarding fertiliser product choice should primarily focus on pasture response and cost-effectiveness.

Additional keywords: bio-fertilisers, compost, manure, pasture growth, phosphorus, soil microbiology.

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Introduction

The Southern Tablelands of New South Wales (NSW) are used primarily for wool and meat production. Like many areas used for agriculture in Australia, the Southern Tablelands are noted for their inherently low level of soil phosphorus (P) (Curl 1977). Because P is a key driver of pasture growth, P fertilisers are routinely used to promote pasture growth and ensure high productivity. Historically, farmers have successfully used superphosphate (8.8% P and 11% sulfur, S) to topdress both

native perennial grass based pastures containing introduced legume and improved pastures containing temperate grasses and annual clovers. The system benefits and agronomic efficiency of P for increasing pasture growth and animal production on a Southern Tablelands pasture system have been demonstrated by Simpson *et al.* (2015). That study highlighted the importance of fertilising soil to increase the level of extractable P towards a critical soil-test P (STP) for maximum pasture growth and carrying capacity to achieve

optimised P-balance efficiency and to minimise excessive accumulation of P in soil.

Interest in the use of alternative fertilisers is driven by a range of factors including the fluctuating cost of conventional fertilisers, perceptions of environmental and long-term sustainability benefits from use of organic products compared with 'mineral' fertilisers, and concern that conventional fertilisers are not as effective as they were previously. 'Alternative' fertilisers are marketed for agricultural use on broadacre crops and for the topdressing of pastures (Abbott *et al.* 2018). They include a wide range of manures, composts, sewage products, microbial-based products and compost 'teas', crushed rock products, and reactive phosphate rock, with some of the mineral products also being treated with and/or containing microbial cultures. Broad claims of the benefits of alternative fertilisers include improvements to crop and pasture productivity, increased forage quality, and support for a more 'healthy' soil environment including claims around the enhancement of soil microbial function (Gould 2012; Massy 2017; Jones 2018; www.farmingsecrets.com, accessed 14 January 2019). However, many of these claims are largely anecdotal, and there is a paucity of evidence-based research to support the proposed benefits of most products. Alternative fertiliser products may be highly variable in nutrient and dry matter (DM) content, and the nutrients present in the products are of differing availability to plants (Quilty and Cattle 2011). Any benefits for pasture production of alternative fertiliser products relative to conventional products such as superphosphate have therefore been difficult to ascertain.

This paper reports a 6-year study conducted on three P-deficient, permanent-pasture soils near Yass, NSW, investigating the effect of topdressing pasture with a range of alternative fertilisers of variable nutrient (P and S) content that are available within the Australian agricultural sector. The alternative fertilisers were obtained from locally available sources and were applied to pastures at times and application rates specified by the suppliers. The alternative fertiliser products were compared with an unfertilised control and with pastures fertilised with single superphosphate as the industry standard. The effects of the fertiliser products on key soil properties and pasture growth during spring and/or winter + spring over the study period were measured in ungrazed swards. The impact of the fertilisers on the structure of soil microbial communities was also investigated. The aim of the study was to provide an objective evaluation of the relative effectiveness of different fertiliser products for pasture production.

Materials and methods

Field sites

The project involved three perennial pasture sites in the Binalong and Bookham areas of the Southern Tablelands of NSW: 'Glenroy', Binalong (34°35'14.23"S, 148°38'7.74"E); 'Kia-Ora', Bookham (34°48'6.18"S, 148°34'49.17"E); and 'Te Kooti', Bookham (34°51'16.33"S, 148°35'28.33"E). Sites on loamy-clay soils with low fertility status were selected. Eleven treatments were tested at each site: eight alternative fertiliser treatments, a superphosphate treatment, a treatment with urea only, and an unfertilised control. Treatments were first

applied in May 2009, with three replicates of each treatment at each site arranged in a randomised complete block design. Trials were run at Glenroy and Kia-Ora until 2014 and at Te Kooti until 2013. Individual plots at Glenroy and Kia-Ora were 2 m wide by 10 m long, whereas at Te Kooti, plots were 2 m wide by 8 m long. Soil tests were conducted before the trials were established (November 2008) to ensure site suitability, with levels of extractable P (Colwell P, STP) in the range 6.93–8.71 mg P kg⁻¹, extractable S (KCl-40) 2.90–4.41 mg S kg⁻¹, and potassium (K) 0.16–0.43 cmol(+) kg⁻¹ soil in the 0–10 cm layer. Baseline soil tests for total soil carbon (C), cation exchange capacity (CEC), soil pH and aluminium (Al, % of CEC) content were also performed.

The three sites were located in farmers' paddocks. When the study commenced, they consisted of perennial native-based pasture with small amounts of annual legume present (0–12% legume content), primarily as subterranean clover (*Trifolium subterraneum* L.). Native perennial grass species found across the sites included weeping grass (*Microlaena stipoides* (Labill.) R.Br.), wallaby grass (*Rytidosperma* spp., formerly known as *Austrodanthonia* spp.), common wheatgrass (*Anthosachne scabra* [formerly *Elymus scaber*] (R.Br.) Nevski), red grass (*Bothriochloa macra* (Steud.) S.T.Blake) and spear grass (*Austrostipa scabra* (Lindl.) S.W.L.Jacobs & J.Everett). There were also varying amounts of annual grasses (primarily *Vulpia* spp., *Bromus* spp. and *Lolium* spp.) present across all sites.

Long-term average annual rainfall for the Bookham and Binalong districts is ~650 mm, but rainfall is highly variable between years and locations, with Glenroy generally a drier site than Kia-Ora and Te Kooti. The annual rainfall at each site was recorded in relation to pasture growth.

Pasture and fertiliser treatments

Prior to the study commencing, landholders in the Binalong and Bookham areas were consulted about which alternative fertiliser products to include, and as a result, 10 commercial products (including superphosphate) were selected. Full descriptions of the fertiliser products and key nutrient compositions are shown in Table 1. The timing and rate of application of the fertiliser treatments applied over the trial period are shown in Table 2. Superphosphate was applied each year according to typical district practice (125 kg ha⁻¹, providing ~11 kg P ha⁻¹). Alternative fertiliser products were applied at rates and times according to supplier recommendations based on initial soil data obtained in 2008 and on annual soil measurements and pasture-yield data provided to the suppliers each year. Depending on supplier advice, some products were therefore applied only once in 6 years, whereas other products were applied once in 2 or 3 years or applied annually (Table 2). Granular or powdered fertiliser products were applied to the plots by hand-spreading, and liquid products were applied by using a hand-held 2-m boom spray. All products were applied under conditions specified by the supplier. The fertiliser suppliers did not participate in the physical application of products to trial plots; all field work was carried out by independent, qualified field staff. Comparison of the relative cost-effectiveness of fertiliser products over the course of the trial, determined as cost (AU\$) per tonne increased pasture DM over the unfertilised control, was derived from information and data provided by the suppliers

Table 1. Description of fertiliser products used in the study as provided by the commercial suppliers of each product

Fertiliser product	Description
Single superphosphate	Granulated fertiliser containing 8.8% phosphorus (P) (8.6% as soluble P), 11% sulfur (S) and 20% calcium (Ca). Single superphosphate containing molybdenum (Mo) was used in year 1 (Mo; 0.05%) and year 5 (Mo; 0.025%)
Agri-ash	Burnt human sewage ash product produced at the Lower Molonglo Water Quality Control Centre, the main wastewater treatment plant for the Australian Capital Territory (ACT). The product contains 6.6% P (1.12% as soluble P), 0.85% S and a range of other macro- and micro-nutrients. It has an average neutralising value of 65%. It does contain some heavy metals but all below threshold limits. Product provided by Fertspreed, Gunning, NSW, and commercially marketed as 'Agri-Ash'
Trio-min/Eco-min Balance	Both fertilisers are a crushed rock (semi-granulated) product made from igneous and metamorphic rocks. Trio-min has added phosphate; it contains 4.5% P plus a range of other macro- and micro-nutrients. Eco-min Balance contains 2.4% P plus a range of other nutrients, and contains 10% lime. Products supplied by Munash Natural Fertilizers, Ballarat, Victoria.
SEP Pig Manure	A waste product from pigs. Liquid and solid manure is put into sedimentation evaporation ponds (SEP) to dry and then manure is scooped into piles for further air-drying before being spread on paddocks. Contains a range of macro- and micro-nutrients. Nutrient levels vary from batch to batch. Product sourced from 'Windridge Farms', Young, NSW
Groundswell Compost	A food and garden waste compost, part of a project called 'Groundswell' funded under an Environment Trust Grant by NSW Department of Environment and Heritage. Contains a small amount of macro- and micro-nutrients. Nutrient content varies
YLAD Compost Mineral Blend	A dry product containing humus compost, lime, soft rock phosphate and gypsum. Nutrient content varies. The compost used is produced from local organic waste. This product sourced from YLAD Living Soils, Young, NSW
YLAD Bio TX 500 Compost Tea Extract	A liquid product containing 95% humus compost tea extract, 2% molasses and 3% liquid fish. Product sourced from YLAD Living Soils, Young, NSW
BioAg Blend	A blend of dry and liquid fertilisers. The dry products are a mix of BioAgPhos (reactive phosphate rock treated with a proprietary microbial culture), lime and gypsum. The liquid fertilizers applied in year 1 were a combination of 'Soil and Seed' (product containing nutrients plus microorganisms marketed by BioAg Pty Ltd), milk thistle and vitamin B5. Products supplied by BioAg Pty Ltd, Narrandera, NSW
Ecology Fluid Fertilizer (EFF)/ Dical 64	Ecology Fluid Fertilizer is a liquid foliar fertiliser containing a mix of macro- and micro-nutrients and microorganisms. Dical 64 is a granular fertiliser containing 18% P (~2% of this is soluble P) and 24% Ca. Both products supplied by Ecology Pty Ltd. In years 5 and 6, gypsum was also applied with the Dical 64
Urea	Granulated fertiliser containing 46% nitrogen

based on retail price of the product and associated handling and spreading costs.

Pasture and soil measurements

Nutrient composition of applied fertiliser products

All fertiliser products used in the trials were analysed annually by a commercial laboratory (Environmental Analysis Laboratory, Lismore, NSW) to determine nutrient composition. Fresh samples of each product used were obtained annually from the fertiliser suppliers and analysed for levels of P, S, nitrogen (N), K, molybdenum (Mo) and C (Supplementary Materials table S1, available at the journal's website), with only P and S data considered in detail here. Analyses consisted of a standard fertiliser analysis test (FA-PACK-001 Fertiliser Analysis - Total Acid Extractable Nutrients), and a more detailed test for P speciation (reported as total P, water-soluble P and citrate-soluble P) (FA-PACK-005 Fertiliser Soluble Phosphorus). This nutrient analysis allowed calculation of the total inputs of P and S applied to all plots over the period 2009–14.

Soil chemical analyses

Soil sampling for chemical analysis was conducted annually in late spring. Fifteen individual soil cores (2.5 cm diameter, 10 cm depth) were taken randomly across each plot and pooled into a single soil sample for the plot. Soil samples were stored on ice

when in the field and during transport. Soils were passed through a 5-mm sieve and thoroughly mixed, and subsamples were stored at -20°C . The remaining soil was then air-dried, with ~500 g being sent for laboratory analysis. Soils were analysed for soil pH (in 0.01 M CaCl_2 , 1 : 5 soil : solution), available soil P (Colwell: in 0.5 M NaHCO_3 , 1 : 100 soil : solution, pH 8.5), P buffering index, extractable S (in 0.25 M KCl, 15 : 100 soil : solution), Al content, total CEC and % soil base saturation, and total soil C content (Dumas elemental analysis; LECO analyser, LECO Corp., St. Joseph, MI, USA) according to methods outlined by Rayment and Lyons (2011). Analytical services were provided by the NSW Department of Primary Industries Diagnostic and Analytical Services Laboratory (Wollongbar, NSW). Results for soil pH, Al, P and S only are reported here, because other soil parameters showed little variation over the course of the trial.

Pasture yield

Comparative pasture yield measurements (in kg DM ha^{-1}) were determined by sampling in spring over the period 2009–12 on all trial sites. A winter + spring pasture yield measurement was taken over 2013 and 2014 for the Glenroy and Kia-Ora sites, and only in 2013 for the Te Kooti site. Spring was initially chosen as the key time for measurement because it is usually the time when moisture and temperature are not limiting production and pasture growth is at its greatest, thus allowing for a comparative measure of growth response to fertiliser applications. A winter + spring

Table 2. Treatments and spreading rates (per ha) of fertiliser products applied in autumn at the Glenroy and Kia-Ora trial sites over the 6-year period 2009–14 and at Te Kooti over the period 2009–13

	Year 1 (2009)	Year 2 (2010)	Year 3 (2011)	Year 4 (2012)	Year 5 (2013)	Year 6 (2014)
Rates of application of each fertiliser product in each year were determined according to product supplier recommendations based on their assessment of initial and annual soil-test results						
Control (nil fertiliser)	Nil	Nil	Nil	Nil	Nil	Nil
Single superphosphate	125 kg (molybdenum (Mo) 0.05%)	125 kg	125 kg	125 kg	125 kg (Mo 0.025%)	125 kg
Agri-ash	2.5 t	Nil	Nil	Nil	Nil	Nil
Trio-min/Eco-min Balance	300 kg Trio-min	300 kg Eco-min Balance ^A	300 kg Eco-min Balance ^B	300 kg Eco-min Balance ^A	300 kg Eco-min Balance ^{A,C}	300 kg Eco-min Balance ^{A,C}
SEP Pig Manure ^D	4.88 t	Nil	Nil	4.0 t	Nil	Nil
Groundswell Compost ^E	3 t	Nil	3 t	Nil	3 t	Nil
YLAD Compost Mineral Blend	1.1 t ^F	1.235 t ^G	1.58 t (Kia-Ora) ^H 1.23 t (Glenroy) ^I 1.23 t (Te Kooti) ^J	0.81 t (Kia-Ora) ^J 0.41 t (Glenroy) ^K 0.7 t (Te Kooti) ^L	0.5 t Compost ^E	0.40 t (Kia-Ora) ^M 0.29 t (Glenroy) ^N
YLAD Bio TX 500 Compost Tea Extract	100 L	100 L	100 L	100 L	100 L	100 L
BioAg Blend	130 kg BioAgPhos + 400 kg lime +3 L Soil and Seed + 30 g milk thistle + 0.45 g vitamin B5	200 kg BioAgPhos + 100 kg gypsum	Nil	200 kg BioAgPhos + 100 kg gypsum	Nil	200 kg BioAgPhos + 100 kg gypsum + 50 g Mo
Ecology Fluid Fertilizer (EFF)/Dical 64	50 L EFF	50 L EFF	80 kg Dical 64	80 kg Dical 64	80 kg Dical 64 + 75 kg gypsum	80 kg Dical 64 + 75 kg gypsum (+ Mo coated on product to deliver 50 g ha ⁻¹)
Urea	100 kg	22 kg	100 kg	100 kg	100 kg	100 kg

^ATreatment contains 10% lime. ^BTreatment contains 20% lime. ^CTreatment contains 33.3% biosolids and 66% Eco-min Balance. ^DProduct contained on average 50% dry matter (DM). ^EProduct contained on average 64% DM. ^FTreatment contains 45.5% compost (average DM content 64%), 45.5% lime, 9% soft rock phosphate (SRP). ^GTreatment contains 42.8% compost, 38.9% lime, 6.2% SRP, 11.7% gypsum, 0.39% zinc hepta. ^HTreatment contains 31.6% compost, 47.5% lime, 5.1% SRP, 15.8% gypsum. ^ITreatment contains 40.7% compost, 40.7% lime, 6.5% SRP, 12.1% gypsum. ^JTreatment contains 31% compost, 31% lime, 7% SRP, 31% gypsum. ^KTreatment contains 61% compost, 15% SRP, 24% gypsum. ^LTreatment contains 36% compost, 36% lime, 7% SRP, 21% gypsum. ^MTreatment contains 57% compost, 9% SRP, 34% gypsum. ^NTreatment contains 86% compost, 14% SRP.

measurement was taken in the later years to provide both a more accurate measure of total biomass per annum, and a comparative measure of pasture growth rate ($\text{kg DM ha}^{-1} \text{ day}^{-1}$). Prior to the measurements of pasture yield, all plots were mown to an equal height (~2 cm above ground level) in early to mid-August (or mid-May) in each year and then allowed to grow until harvest. Material from the initial mowing was captured and discarded. Following pasture growth periods, a strip (0.48 m wide) was mown through the full length of each plot and fresh biomass was recovered and weighed. Three representative subsamples of the freshly harvested pasture from each plot (~300 g) were taken to determine DM percentage for the plot. The area of the mown strip was measured to allow calculation of pasture yield. The material from the mown strip was discarded. The remaining standing pasture on all plots was mown during the late spring–summer period each year and the cut material evenly distributed and left on the plots. The position of the mown strip used to measure pasture growth on each plot varied across years to minimise possible effects on pasture composition.

Botanical composition and pasture quality

Botanical composition of all pasture treatments was monitored annually, in winter. A fixed-transect procedure was used down the centre of each plot with four evenly distributed sampling points. At each sampling point, a wire mesh grid 0.25 m by 0.25 m containing 25 internal squares was positioned centrally and pasture species present were recorded under 25 crosswise points, providing 100 data points per plot at each monitoring. Plants were identified as clover, grass and broadleaf weed or points were designated as bare ground and/or as dead litter. Pasture quality was based on percentage clover content along with predicted measures of metabolisable energy and crude protein of entire herbage as determined on representative composite subsamples from each plot in 2012 and 2014, using the Standard Forage near-infrared (NIR) analysis package offered by NSW DPI Feed Quality Service (Wagga Wagga, NSW).

Soil microbial diversity

Soil samples were collected in spring 2014 from selected treatment plots at the Kia-Ora and Glenroy sites, and DNA was extracted from the samples by using a Bio101 Power Soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) according to manufacturer's recommendations. Concentrations of extracted DNA were determined by NanoDrop spectrophotometry (Thermo Fisher, Waltham, MA, USA). The archaeal 16S rRNA gene, bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS) regions were amplified from each sample by polymerase chain reaction (PCR), using procedures and primers as outlined by Banerjee *et al.* (2018). The amplified samples were then analysed by terminal restriction fragment length polymorphism (T-RFLP) (see Supplementary Materials text: *Community structure assessed by T-RFLP*) and, for the Kia-Ora site (except in the urea and Trio-min/Eco-min Balance treatments), by Illumina (San Diego, CA, USA) 300-bp paired-end DNA sequencing performed at the Australian Genome Research Facility (Brisbane, Queensland). The DNA sequence dataset was processed according to procedures outlined in Banerjee *et al.* (2018) with operational taxonomic units (OTUs) being defined by clustering at 97% similarity followed

by removal of singleton OTUs. Sequences were then mapped to these OTUs to produce abundance tables and classified to species and higher order taxonomic levels according to SILVA Release 102 using the naïve Bayesian classifier (Wang *et al.* 2007) as implemented in mothur (https://www.mothur.org/wiki/Main_Page).

Data analyses and statistics

Soil chemical properties and pasture yield and quality data were assessed by linear mixed models, fitted using ASReml 2.0 (VSN International, Hemel Hempstead, UK), with treatment, year (for repeated-measures) and the treatment \times year interaction fitted as fixed effects, and blocking structures used for replicate, plot and laboratory processing fitted as random effects. Spatial correlations were included in the model using an autoregressive AR1 structure (i.e. a correlation structure for plot residuals). The significance of the fixed effects (at $P = 0.05$) in all models was determined by using approximate *F*-tests (Kenward and Roger 1997). Botanical composition data were assessed by cubic smoothing splines fitted as linear mixed models according to the methods of Verbyla *et al.* (1999), where trends in percentage composition were compared across treatments and over time. Fixed effects included the factor 'treatment', the variate 'year', and the interaction of treatment and year (treatment \times year). The significance of fixed effects was assessed using *F*-tests (at $P = 0.05$), and random terms by using residual maximum likelihood ratio tests (Kenward and Roger 1997; Verbyla *et al.* 1999). In cases where an experiment consisted of comparison between only two treatments (i.e. control vs a specific fertiliser treatment), a *t*-test ($P = 0.05$) was performed to compare treatment means when all other model assumptions were met. When multiple comparisons were made by *t*-test, a Bonferroni adjustment for multiple pair-wise comparisons at a significance level of $P = 0.05/55 = 0.0009$ was used to minimise the false discovery rate.

High-throughput-sequencing data tables consisting of OTUs for archaea, bacteria and fungi were analysed using PRIMER version 6.1.15 and PERMANOVA+ version 1.0.5 software from PRIMER-E (Quest Research, Auckland, New Zealand). A square-root transformation was conducted on the data before principal component analysis (PCA). Differences between treatments were assessed with PERMANOVA analyses on the transformed data, using Bray–Curtis resemblance matrices with 999 permutations to assess fertiliser treatment effects. Alpha diversity indices for Margalef richness, Pielou evenness and Shannon–Weaver diversity were determined on OTU sequence data in PRIMER. OTU community data were also examined by correlation with other soil and pasture variables by using a Pearson correlation coefficient of 0.43 (representing probability $P = 0.01$; $n = 36$). Statistical analysis of the relative abundance of each microbial grouping (species, genus, class or phylum) based on percentage contribution to the total community was performed with both raw data and arcsine-transformed data. One-way ANOVA with Duncan post hoc tests were conducted (SPSS Statistics version 20; IBM, Armonk, NY, USA) to assess the effect of treatment on the various microbial groups ($P < 0.05$ to $P < 0.001$).

Results

Composition of fertiliser products and amounts of P and S applied

Total quantities of P and S applied for each treatment over the duration of the study for the three sites are shown in Table 3. This analysis is based on both the nutrient composition of the products (table S1) and the applied rates of each fertiliser across years (Table 2). There were substantial differences in the amounts of total P applied across treatments, with the largest amounts (165 and 177 kg P ha⁻¹) being applied in the Agri-ash (165 kg P ha⁻¹) and SEP Pig Manure (177 kg P ha⁻¹) treatments (compared with 66 kg P ha⁻¹ for superphosphate), and the smallest amount (apart from the unfertilised and urea treatments) in the YLAD Compost Tea treatment (<1 kg P ha⁻¹). Total amounts of S applied over the trial period also differed across treatments, ranging from 11 to 34 kg S ha⁻¹ for most treatments, except for the superphosphate (69–83 kg S ha⁻¹) and YLAD Compost Mineral Blend (71 kg S ha⁻¹) treatments. Some treatments also received S through added gypsum, whereas the control, urea and YLAD Compost Tea treatments received <1 kg S ha⁻¹ (Table 3).

The quantities of water-soluble and citrate-soluble P, which would be expected to be more readily available to plants, also varied across fertiliser products (Table 3). The amount of water-soluble P provided over the trial period was relatively small for each of the fertilisers (representing 0–6% of total P) other than superphosphate (with 77% of P water-soluble). By comparison, citrate-soluble P content was more varied and proportionally lower for several of the fertiliser products, including BioAg Blend, YLAD Compost Mineral Blend and EFF/Dical 64 (range 3–11% of total P), and at the rates applied over the trial period, these products provided an average of 2, 3 and 7 kg citrate-soluble P ha⁻¹, respectively. Agri-ash and superphosphate contained proportionally higher levels of citrate-soluble P (17% and 20%), and these two products also delivered large quantities of total P (165 and 55–66 kg P ha⁻¹, respectively). Both

SEP Pig Manure and Groundswell Compost contained a relatively high proportion of citrate-soluble P in relation to total P applied (47% and 45%, respectively) and they were applied at similar biomass rates (total 8.9 and 9.0 t ha⁻¹) over the trial period. However, SEP Pig Manure contained approximately eight times more citrate-soluble P than Groundswell Compost, and hence provided larger quantities of citrate-soluble P (83 v. 9 kg P ha⁻¹). Trio-min/Eco-min Balance also contained a relatively high proportion of citrate-soluble P in relation to total P (average 36%), but had only small amounts of total P overall, and at annual application rates of 300 kg ha⁻¹ delivered ~6 kg citrate-soluble P ha⁻¹ in total (Table 3). Several fertiliser products had high content of insoluble P (94–98%), including YLAD Compost Mineral Blend, BioAg Blend and EFF/Dical 64.

Soil chemical status

Extractable soil phosphorus

Average P buffering index before commencement of treatments was 53 mg P kg⁻¹ (range 50–57 mg P kg⁻¹) at Glenroy, 71 mg P kg⁻¹ (range 65–77 mg P kg⁻¹) at Kia-Ora, and 58 mg P kg⁻¹ (range 53–61 mg P kg⁻¹) at Te Kooti. Based on these values, critical STP levels that would be expected to support 95% of maximum pasture growth were similar across the three sites, being 29, 31 and 30 mg P kg⁻¹ soil at Glenroy, Kia-Ora and Te Kooti, respectively (Gourley *et al.* 2007; Moody 2007).

Initial STP levels across all sites ranged from 6.93 to 8.71 mg P kg⁻¹ soil, confirming the low P fertility of the soils. Measurements of soil extractable P were subsequently taken annually on all treatments and are presented for selected years (Table 4) representing the initial, mid and final periods of the study (full dataset provided in table S2d). In each year, significant differences ($P < 0.05$) due to fertiliser treatment were observed relative to the unfertilised treatment. Fertiliser products that

Table 3. Amounts (kg ha⁻¹) of water-soluble, citrate-soluble and insoluble phosphorus (P) and total quantities of P and sulfur (S) applied in each treatment at Glenroy, Kia-Ora and Te Kooti trial sites over 6 years (2009–14)

Treatment	Site	Water-soluble P	Citrate-soluble P	Insoluble P	Total P	Total S
Control	All	Nil	Nil	Nil	Nil	Nil
Single superphosphate	Glenroy and Kia-Ora	51	13	2	66	83
	Te Kooti	43	11	1	55	69
Agri-ash	All	Nil	28	137	165	21
Trio-min/Eco-min Balance	Glenroy and Kia-Ora	1	6	10	17	13
	Te Kooti	1	6	9	16	12
SEP Pig Manure	All	6	83	88	177	34
Groundswell Compost	All	1	9	11	20	15
YLAD Compost Mineral Blend	All ^A	<1	3	46	49	71
YLAD Compost Tea	All	<1	Nil	<1	<1	<1
BioAg Blend	Glenroy and Kia-Ora	<1	2	70	72	29
	Te Kooti	<1	1	51	52	19
EFF/Dical 64	Glenroy and Kia-Ora	<1	8	64	72	22
	Te Kooti	<1	6	49	55	11
Urea	All	Nil	Nil	Nil	Nil	Nil

^ABased on nutrient analysis of fertiliser product applied to the Kia-Ora site; small variation to this input occurred at the Glenroy and Te Kooti sites as indicated in Table 2.

Table 4. Soil pH, aluminium (Al) content (% of cation exchange capacity), and available P and extractable S (mg kg⁻¹) in soil (0–10 cm) at the Glenroy, Kia-Ora and Te Kooti sites over the trial period

SSP, Single superphosphate. Results from selected years are presented as initial (2008), mid-trial (year 3, 2011) and final (year 6, 2014, for Glenroy and Kia-Ora, and year 5, 2013, for Te Kooti). Within a row, values (means, $n = 3$) followed by the same letter are not significantly different ($P > 0.05$); treatments differing from the unfertilised control are shown in bold

Site	Soil parameter	Year	Control	SSP	Agri-ash	SEP Pig Manure	BioAg Blend	EFF/Dical 64	YLAD Compost Mineral Blend	Groundswell Compost	Trio-min/Eco-min Balance	YLAD Compost Tea	Urea
Glenroy	pH	2008	4.15a	4.15a	4.15a	4.15a	4.15a	4.15a	4.15a	4.15a	4.15a	4.15a	4.15a
		2011	4.40a	4.40a	5.18b	4.45a	4.59a	4.51a	4.94b	4.53a	4.50a	4.47a	4.37a
		2014	4.47a	4.35a	4.84b	4.47a	4.50a	4.34a	4.76b	4.46a	4.44a	4.36a	4.36a
Kia-Ora	pH	2008	4.02a	4.02a	4.02a	4.02a	4.02a	4.02a	4.02a	4.02a	4.02a	4.02a	4.02a
		2011	4.29a	4.35a	4.76b	4.33a	4.41a	4.30a	4.79b	4.40a	4.36a	4.38a	4.33a
		2014	4.38a	4.49ab	4.69bc	4.48ab	4.47ab	4.42ab	4.84c	4.51ab	4.50ab	4.58abc	4.35a
Te Kooti	pH	2008	4.03a	4.10a	4.10a	4.03a	4.07a	4.10a	4.13a	4.20a	4.13a	4.20a	4.00a
		2011	4.27a	4.28a	4.63ab	4.27a	4.42ab	4.35a	4.74b	4.51ab	4.38ab	4.53ab	4.27a
		2013	4.31a	4.27a	4.67c	4.33a	4.43ab	4.34a	4.63bc	4.46abc	4.42ab	4.34a	4.30a
Glenroy	Al	2008	28.03a	27.43a	24.61a	22.61a	25.81a	23.61a	27.54a	24.21a	19.37a	22.30a	25.39a
		2011	23.66bc	22.68d	2.75a	19.23cd	13.21d	18.57cd	5.12ab	19.22cd	19.34cd	22.33d	22.10d
		2014	21.86d	21.81d	4.88a	13.35abcd	12.45abc	17.07cd	5.31ab	17.44cd	14.22bcd	18.76cd	21.28cd
Kia-Ora	Al	2008	31.24a	31.24a	31.24a	31.24a	31.24a	31.24a	31.24a	31.24a	31.24a	31.24a	31.24a
		2011	29.28b	23.33b	6.15a	23.82b	20.89b	28.48b	6.71a	21.72b	22.02b	23.22b	25.91b
		2014	19.32c	15.27c	5.77ab	12.12abc	13.73bc	18.47c	4.28a	13.20bc	13.55bc	16.08c	19.99c
Te Kooti	Al	2008	20.33a	20.33a	20.33a	20.33a	20.33a	20.33a	20.33a	20.33a	20.33a	20.33a	20.33a
		2011	23.46b	24.35b	9.20a	23.79b	17.31ab	21.84b	8.69a	14.18ab	21.84b	15.87ab	22.80b
		2013	15.79bc	18.01c	5.80a	13.46bc	10.31ab	15.84bc	5.89a	11.95abc	12.24abc	16.04bc	13.80bc
Glenroy	P	2008	7.47a	7.47a	7.47a	7.47a	7.47a	7.47a	7.47a	7.47a	7.47a	7.47a	7.47a
		2011	7.98a	12.00bcd	13.31cd	16.10d	8.18a	10.62abc	8.07a	8.16a	9.13ab	7.85a	7.58a
		2014	6.53a	14.45b	13.02b	22.52c	6.95a	11.78b	6.74a	6.87a	6.31a	6.26a	5.82a
Kia-Ora	P	2008	6.93a	6.93a	6.93a	6.93a	6.93a	6.93a	6.93a	6.93a	6.93a	6.93a	6.93a
		2011	7.07a	8.18ab	18.17c	12.30bc	10.42ab	8.88ab	7.83ab	7.73ab	9.15ab	6.75a	6.87a
		2014	5.47a	8.57bc	15.93d	15.22d	7.90bc	9.93c	6.51ab	6.53ab	5.80a	5.12a	5.37a
Te Kooti	P	2008	8.71a	8.71a	8.71a	8.71a	8.71a	8.71a	8.71a	8.71a	8.71a	8.71a	8.71a
		2011	9.90ab	12.61b	17.15c	17.05c	10.36ab	10.80ab	10.46ab	10.82ab	10.32ab	9.40a	8.78a
		2013	15.23ab	20.44bc	29.04cd	30.35d	15.41ab	19.14ab	15.13ab	16.71ab	15.38ab	13.83a	14.76ab
Glenroy	S	2008	3.61a	3.61a	3.61a	3.61a	3.61a	3.61a	3.61a	3.61a	3.61a	3.61a	3.61a
		2011	3.04a	5.57b	4.72ab	3.54ab	3.23a	3.69ab	5.56b	2.84a	4.06ab	3.25a	2.91a
		2014	2.03a	8.98b	2.26a	2.22a	3.87a	3.29a	2.97a	2.38a	2.17a	2.65a	1.92a
Kia-Ora	S	2008	2.90a	2.90a	2.90a	2.90a	2.90a	2.90a	2.90a	2.90a	2.90a	2.90a	2.90a
		2011	2.10a	3.17a	2.50a	2.15a	2.18a	2.70a	3.43a	2.08a	2.55a	2.19a	2.15a
		2014	2.00a	4.01b	2.04a	2.06a	2.12a	2.20a	3.00ab	2.00a	2.04a	1.87a	2.01a
Te Kooti	S	2008	4.41a	4.41a	4.41a	4.41a	4.41a	4.41a	4.41a	4.41a	4.41a	4.41a	4.41a
		2011	3.55ab	4.78b	3.49ab	3.13a	3.16a	3.37a	4.11ab	3.65ab	3.39ab	4.00ab	2.77a
		2013	3.02ab	5.31b	2.69a	2.80a	3.09ab	3.23ab	2.91a	2.78a	2.83a	2.97ab	2.54a

resulted in the most consistent increase in STP levels were Agri-ash and SEP Pig Manure (Table 4). A significant increase in STP occurred with the SEP Pig Manure treatment in all years and at all sites; with Agri-ash, an increase occurred in all years at Te Kooti and only in the later years at Glenroy and Kia-Ora. Both Agri-ash and SEP Pig Manure provided large amounts of total P input (Table 3), which resulted in STP being nearer to the critical value, in the range 15.03–29.04 mg P kg⁻¹ for Agri-ash and 25.33–33.20 mg P kg⁻¹ for SEP Pig Manure. At the Glenroy site, superphosphate also resulted in a significantly higher STP in the last four years of the trial, with the highest level of 14.45 mg P kg⁻¹ observed in the final year (2014). At both the Kia-Ora and Te Kooti sites, superphosphate resulted in a significant increase in STP in only one of the six years: the final year at Kia-Ora (8.57 mg P kg⁻¹ v. 5.47 mg P kg⁻¹ in the unfertilised control), and the

fourth year at Te Kooti (15.38 mg P kg⁻¹ v. 10.62 mg P kg⁻¹ in the control). At Kia-Ora, both BioAg Blend and EFF/Dical 64 also showed some effect on STP, albeit in the sixth year only (7.90 and 9.93 mg P kg⁻¹, respectively), whereas at Glenroy, EFF/Dical 64 (but not BioAg Blend) resulted in a significant increase in STP in the final year (11.78 mg P kg⁻¹ v. 6.53 mg P kg⁻¹ in the control). The fertiliser products YLAD Compost Mineral Blend, Groundswell Compost, Trio-min/Eco-min Balance and YLAD Compost Tea, as well as urea, had no effect on levels of STP as measured by the Colwell test.

Extractable soil sulfur

Extractable S was measured annually at all three sites, with mean values for the initial, mid and final stages of the trial

presented in Table 4 (full dataset provided in table S2e). Initial levels of extractable S ranged from 2.90 to 4.41 mg S kg⁻¹ soil across all sites. The greatest difference for soil extractable S in response to the fertiliser treatments occurred at the Glenroy site. A significant increase in soil S occurred with the superphosphate treatment over five years of the trial (2010–14), with the highest level of 8.98 mg S kg⁻¹ soil observed in 2014. Alternative fertiliser treatments Agri-ash and YLAD Compost Mineral Blend also resulted in increased soil S at Glenroy in two of the six years. Soil S increased with Agri-ash treatment to 5.06 and 5.52 mg S kg⁻¹ in 2009 and 2010, and with YLAD Compost Mineral Blend to 5.56 and 4.40 mg S kg⁻¹ in 2011 and 2012 (table S2e). For both products, no further change was observed thereafter. By comparison, a limited effect on soil extractable S was observed at the Kia-Ora and Te Kooti sites (Table 4). At Kia-Ora, superphosphate resulted in a small increase to 3.61 mg S kg⁻¹ in 2009 and 4.01 mg S kg⁻¹ in 2014, with no change in the intervening years, and at Te Kooti, to 6.70 mg S kg⁻¹ in 2009 only. At both Kia-Ora and Te Kooti, Agri-ash resulted in higher soil S (7.77 and 7.32 mg S kg⁻¹, respectively) in the first year of the trial only (2009). Fertiliser products applied with added gypsum (i.e. BioAg Blend and EFF/Dical64) had no significant effect on levels of extractable S (Table 4).

Soil pH and aluminium content

Initial soil pH in 2008 varied between 4.02 and 4.20 across all sites, and several of the fertiliser products resulted in increased soil pH throughout the trial (Table 4, table S2a). Increases in soil pH were in all cases associated with corresponding decreases in Al (% of CEC) content. Products that contained substantial amounts of lime (e.g. Agri-ash and YLAD Compost Mineral Blend) significantly ($P < 0.05$) raised soil pH at all sites compared with the control (Table 4). Agri-ash provided 1.63 t ha⁻¹ of lime in total, all of which was applied in the initial year of the trial (Table 2). This treatment significantly raised soil pH (and decreased %Al) across all six years at Glenroy, in five of six years at Kia-Ora, and in four of five years at Te Kooti. At Glenroy, soil pH was highest (5.28) in the second year after application, whereas at Kia-Ora it was highest (pH 4.81) in each of the first three years, and at Te Kooti it was highest (pH 4.83) immediately after application. YLAD Compost Mineral Blend provided 1.48, 1.93 t ha⁻¹ and 1.73 t ha⁻¹ of lime in total at the Glenroy, Kia-Ora and Te Kooti sites, respectively, and was applied in varying quantities over the different years (Table 2). Nonetheless, YLAD Compost Mineral Blend consistently raised soil pH (from 4.65 to 4.94 at Glenroy, from 4.43 to 4.96 at Kia-Ora and from 4.47 to 4.74 at Te Kooti) from the second year (2010) and thereafter through to the end of the trial (Table 4). The BioAg Blend treatment provided 0.4 t ha⁻¹ of lime in the first year of the trial only (Table 2), and it increased soil pH in the second year at the Glenroy site (pH 4.57). The annually applied treatment Trio-min/Eco-min Balance, which contained small quantities of lime (180 kg ha⁻¹ of lime in total delivered at each site; Table 2), did not result in any change in soil pH, as was evident for all other fertiliser products, including single superphosphate (Table 4).

Total soil carbon

Total C levels at the commencement of the trial in 2008 were similar across all three sites. Mean soil C (0–10 cm) at the Glenroy site was 1.79% (\pm s.e. 0.07%), at Kia-Ora 1.84% (\pm 0.04%), and at Te Kooti 1.98% (\pm 0.09%). Soil C was measured annually in the top 10 cm in all treatments, and there was no difference due to fertiliser treatment throughout the trial period (Glenroy $P = 0.419$, Kia-Ora $P = 0.471$, Te Kooti $P = 0.373$). This included treatments such as YLAD Compost Mineral Blend, SEP Pig Manure and Groundswell Compost, all of which would be expected to have significant inputs of C throughout the trial, with combined applications of ~0.22–0.76 t C ha⁻¹ across the different treatments.

Pasture yield response

Mean spring (2009–12) and winter + spring (2013 and 2014) pasture yields from the control plots at each site over the trial period are shown in Table 5. Large differences in pasture growth rates were observed across all sites and years (being highest at Te Kooti), reflecting clear site and seasonal differences in pasture growth. Consequently, relative growth responses to fertiliser products were compared with the control within each year at each site (determined as percentage growth increase over the control; Fig. 1a–c). Significantly higher pasture yield was evident for several of the fertiliser products across all three sites. Most notable was the response to applications of superphosphate, Agri-ash and SEP Pig Manure, which generally showed significantly higher ($P < 0.05$) pasture yields in most years of the trial (Fig. 1). In the later years of the study, pasture yield was significantly higher for the BioAg Blend and EFF/Dical 64 treatments at all sites, indicating a delayed response time for these products. YLAD Compost Mineral Blend also showed significantly higher pasture production in some years across the sites, with responses at Te Kooti evident in the first year, whereas at Glenroy, a significant pasture growth response occurred in the third year. Other fertiliser products such as Groundswell Compost, Trio-min/Eco-min Balance and YLAD Compost Tea had no effect at Glenroy or Kia-Ora and only a small effect in some years at Te Kooti (Fig. 1).

The DM response for the three sites was averaged across all years (Fig. 2). This combined analysis indicates that the highest pasture yield response across all fertiliser treatments consistently occurred at the Kia-Ora site and the lowest response at Te Kooti. Across all years and sites, highest pasture yield response was evident for superphosphate (average increase of 178% at Kia-Ora, 130% at Glenroy and 55% at Te Kooti; Fig. 2). Importantly, this response occurred despite superphosphate providing only a modest amount of P (i.e. 55–66 kg P ha⁻¹) relative to other treatments, especially Agri-ash and SEP Pig Manure (165 and 177 kg P ha⁻¹, respectively). By comparison, average pasture yield responses for SEP Pig Manure and Agri-ash across the three sites were generally lower (98–143% at Kia-Ora, 58–107% at Glenroy, and 20–49% at Te Kooti). Average pasture yield responses were similarly lower for BioAg Blend, EFF/Dical 64 and YLAD Compost Mineral Blend treatments despite them providing levels of P (49–72 kg P ha⁻¹) similar to superphosphate. Average pasture yield responses for all other fertiliser products were not significantly different from the

Table 5. Annual rainfall, periods of pasture growth measurement and pasture yield in control plots at the Glenroy, Kia-Ora and Te Kooti trial sites
n.d., Not determined

Year	Site	Annual rainfall (mm)	Period of pasture yield measurement (days)	Mean pasture yield in growth period (kg DM ha ⁻¹)	Av. daily pasture growth (kg DM ha ⁻¹ day ⁻¹)	Time of year when measurement taken
2009	Glenroy	535	64	801	12.5	Spring, mid-Aug.–late Oct.
	Kia-Ora	643	76	2503	32.9	
	Te Kooti	660	82	3452	42.1	
2010	Glenroy	975	77	2132	27.7	Spring, mid-Aug.–late Oct.
	Kia-Ora	1280	82	2390	29.1	
	Te Kooti	1235	82	3513	42.8	
2011	Glenroy	619	78	1278	16.4	Spring, mid-Aug.–late Oct.
	Kia-Ora	798	88	2165	24.6	
	Te Kooti	775	85	2831	33.3	
2012	Glenroy	694	76	1446	19.0	Spring, early Aug.–late Oct.
	Kia-Ora	718	80	1314	16.4	
	Te Kooti	803	83	2238	27.0	
2013	Glenroy	480	156	1012	6.5	Winter + spring, mid-May–mid-Oct.
	Kia-Ora	444	145	811	5.6	
	Te Kooti	459	154	1354	8.8	
2014	Glenroy	659	141	791	5.6	Winter + spring, early May–late Sept.
	Kia-Ora	824	145	707	4.5	
	Te Kooti	n.d.	n.d.	n.d.	n.d.	

unfertilised control (range 0–31% at Kia-Ora, 0–21% at Glenroy and 4–19% at Te Kooti).

Botanical composition and pasture quality

There were significant differences in subterranean clover content of pastures across years and in response to fertiliser treatments compared with the control treatment at all three sites (Table 6). For Glenroy and Kia-Ora in particular, clover content was higher over the duration of the trial. This was evident as early as the second year (e.g. for superphosphate) or third year after application of various fertiliser products. Significant ($P < 0.05$) effects occurred for all fertiliser products except Trio-min/Eco-min Balance and YLAD Compost Tea at all sites and Groundswell Compost at Kia-Ora and Te Kooti. There was also no significant change in the clover content of the control and urea treatments over time at any site. The Te Kooti site was notable for having lower percentage clover content and lesser responses to fertiliser than the other sites (Table 6). Nonetheless, significant increases in clover content were observed at this site in several years for pastures that received superphosphate, Agri-ash, SEP Pig Manure, BioAg Blend, YLAD Compost Mineral Blend and EFF/Dical 64. In all cases, higher clover content of the pastures was associated with increases in the NIR-predicted metabolisable energy content and crude protein content of representative pasture samples from each fertiliser treatment taken for all sites in 2012 and 2014 (fig. S1).

Soil microbial community analysis

The DNA sequence dataset for archaea consisted of ~130 000 filtered reads in total, representing 319 OTUs. These OTUs were predominantly affiliated with two phyla and one

unclassified group at phylum level. The bacterial dataset consisted of ~800 000 filtered reads represented by 3971 OTUs that were affiliated with 29 phyla. The bacterial dataset was assessed primarily at the phylum taxonomic level, with the Proteobacteria also being classified down to class level, because this was the dominant phylum. The fungal community was made up of ~2.7 million filtered reads represented by 4142 OTUs that were affiliated with six phyla and one unclassified group.

Principal component analysis of the OTU sequence data was conducted separately for archaea, bacteria and fungi (Fig. 3). In all cases, the first two ordines plotted for each kingdom explained a reasonable percentage of the total cumulative variation (45.1% for archaea, 40.7% for bacteria and 30.1% for fungi). PERMANOVA analysis indicated no effect of fertiliser treatment on the community structure for either archaea or bacteria ($P = 0.264$ and $P = 0.226$, respectively; Fig. 3a, b). For fungi, there was an overall significant effect ($P = 0.011$) on the community structure across fertiliser treatments; however, post hoc pairwise testing showed no significant differences for any of the fertiliser product treatments compared with either the superphosphate or unfertilised control (Fig. 3c). The overall community structure for archaea was correlated with soil pH, Al (% of CEC) and CEC; the structure of the bacterial community was correlated with soil pH, Al, CEC and clover content in the pasture treatments (Fig. 3). Fungal community structure was similarly correlated with pH and Al, as well as with parameters associated with pasture productivity, including Colwell P, clover content and spring yield (Fig. 3).

In terms of α diversity indices, archaeal richness was significantly higher in all fertiliser treatments than in the control, and the archaeal Shannon–Weaver index was increased in the Agri-ash and YLAD Compost Mineral Blend

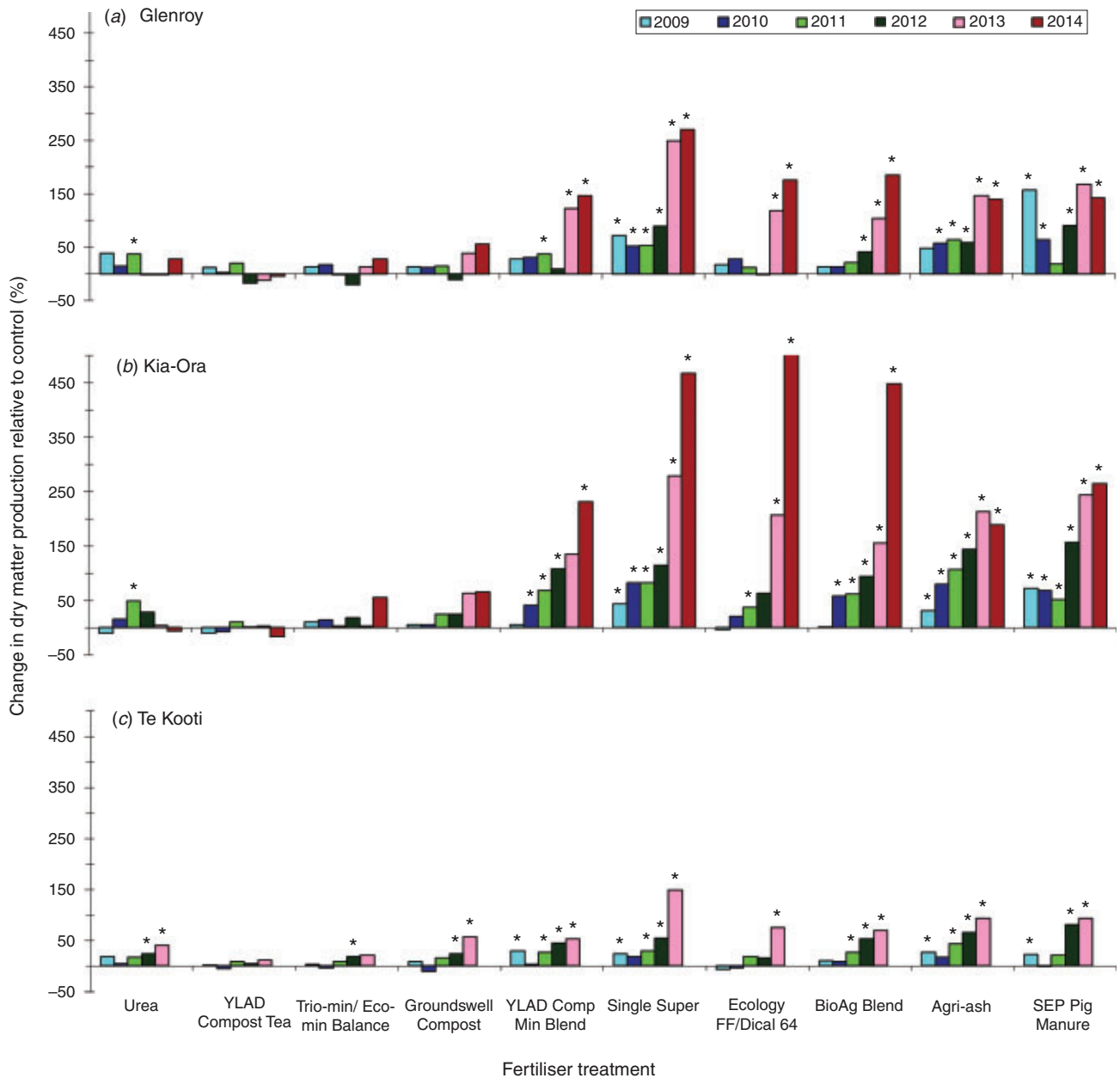


Fig. 1. Pasture yield in response to fertiliser products, presented for each year as percentage change in dry matter production relative to the control treatment (as indicated in Table 5). Spring pasture yield is shown for 2009–12 and winter + spring yield for 2013 and 2014 at (a) Glenroy, (b) Kia-Ora, and (c) Te Kooti (not determined in 2014). Fertiliser product treatments are presented in ascending order according to total P applied. For each site and for each year, bars marked with an asterisk (*) indicate a significant difference ($P < 0.05$) of the mean ($n = 3$) compared with the unfertilised control.

treatments (Table 7); these are the two products with significant liming effect. Interestingly, these two products also influenced bacterial diversity compared with the superphosphate treatment. For fungi, a significant increase in evenness and diversity relative to the unfertilised control was found only in response to the SEP Pig Manure treatment. No differences were found when the alternative fertiliser treatments were compared with the superphosphate treatment (Table 7). These results for sequence analysis at the Kia-Ora site are consistent with

results of T-RFLP analysis of microbial communities conducted at both the Kia-Ora and Glenroy sites (fig. S2).

Relative abundance based on percentage composition for the communities of archaea (major class level), bacteria (phylum and major class level) and fungi (genus level) are shown in Fig. 4. Archaea were predominantly represented by seven classes as well as an unclassified group. ANOVA indicated that DSEG and MCG were the only two archaeal groups for which fertiliser treatment affected relative abundance ($P < 0.01$), with lower

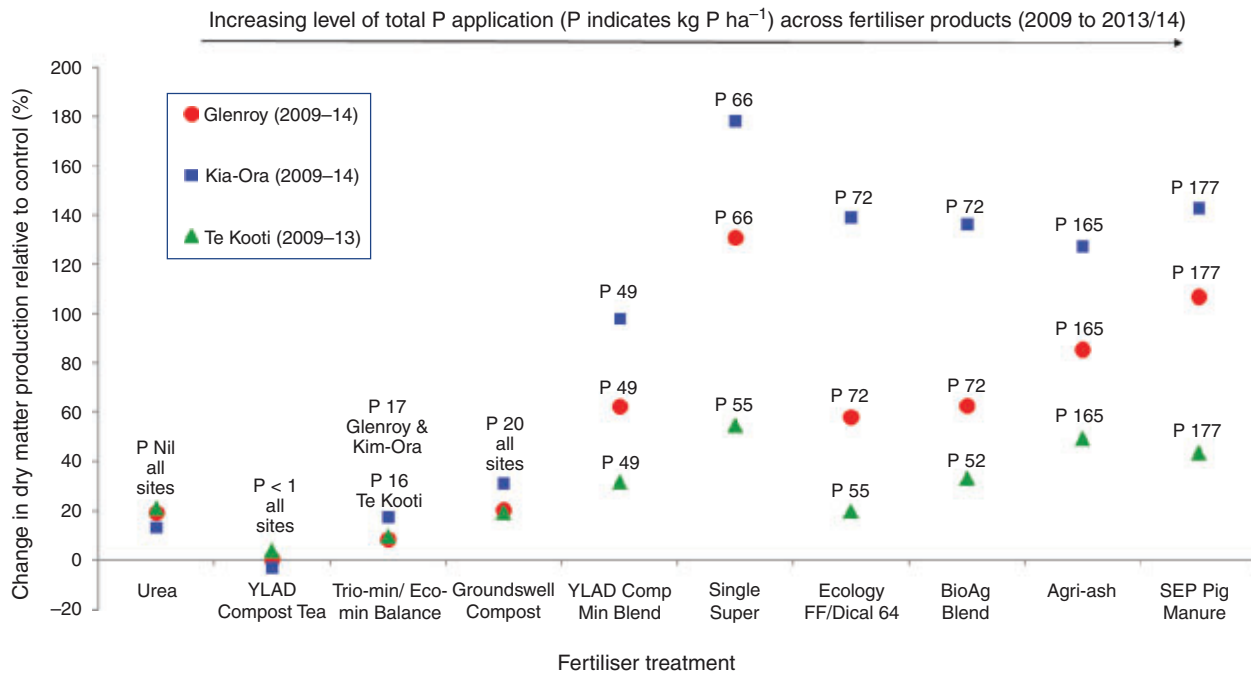


Fig. 2. Average annual spring and winter + spring combined pasture yields in response to fertiliser products measured as percentage change in dry matter production relative to the control treatment over a 6-year period (2009–14) for Glenroy and Kia-Ora and 5-year period (2009–13) for Te Kooti. Fertiliser product treatments are presented in ascending order according to total P applied. Total P applied (kg ha⁻¹) in each treatment at each site over the trial period is presented above the data points.

Table 6. Percentage composition of subterranean clover in pastures in response to fertiliser treatments at the Glenroy, Kia-Ora and Te Kooti sites. Within a column, values (means, *n* = 3) followed by the same letter are not significantly different (*P* > 0.05); treatments differing from the unfertilised control are shown in bold

Treatment	Glenroy						Kia-Ora						Te Kooti				
	2009	2010	2011	2012	2013	2014	2009	2010	2011	2012	2013	2014	2009	2010	2011	2012	2013
Control	4ab	1a	6a	5ab	9a	21abc	2a	11ab	1a	1a	4a	12a	0ab	3a	2a	5ab	0a
Single superphosphate	6ab	9bc	23bc	23de	35cde	20ab	3a	13ab	5ab	14bc	14abc	48cd	1ab	10ab	4ab	18cd	12d
Agri-ash	4ab	4ab	36c	29e	49e	57g	3a	20b	6b	16c	24bcd	63d	2b	8ab	10c	23d	9cd
Trio-min/Eco-min Balance	5ab	2a	6a	3a	12ab	28bcd	4a	12ab	1a	0a	6a	18ab	0a	5a	2a	8ab	2a
SEP Pig Manure	6ab	10c	23bc	15bcd	38de	31cde	7a	11ab	5ab	17c	34d	68d	0a	8ab	4ab	22d	4ab
Groundswell Compost	11b	4a	7ab	7ab	20abc	34de	7a	12ab	0a	2a	11abc	35abc	2b	5a	5 ab	7ab	4ab
YLAD Comp Min Blend	6ab	3a	12ab	9abc	25bcd	48fg	3a	14ab	1ab	4a	18abcd	39bc	1ab	11ab	8bc	17cd	2a
YLAD Compost Tea	4ab	2a	5a	2a	9a	20ab	2a	6a	0a	0a	4a	18ab	0ab	6a	1a	4ab	0a
BioAg Blend	1a	4ab	17ab	18cd	30cd	42ef	5a	16ab	3ab	7ab	26cd	63d	0ab	17b	3ab	20d	6bc
EFF/Dical 64	6ab	3a	9ab	8abc	25bcd	33de	5a	15ab	1ab	6ab	16abcd	50cd	1ab	4a	2a	12bc	2ab
Urea	1a	1a	2a	4ab	5a	11a	9a	9ab	1a	1a	7ab	21ab	2 b	7a	2a	2a	2ab
<i>l.s.d.</i> (<i>P</i> = 0.05)	7.6	4.6	16.5	10.5	15.8	10.8	7.0	11.9	5.1	8.6	17.8	23.3	1.6	9.9	4.8	8.3	3.9

abundance for the YLAD Compost Mineral Blend than for the superphosphate treatment, but no difference from the unfertilised control (Fig. 4a). Bacteria were primarily represented by 10 major phyla, with the remaining 19 phyla being grouped as ‘others’. The phylum Proteobacteria was also represented at class level with five categories identified. Acidobacteria (*P* < 0.001), Actinobacteria (*P* < 0.05), Planctomycetes (*P* < 0.05), WPS2 (*P* < 0.01) and class Betaproteobacteria (*P* < 0.05) differed significantly across fertiliser treatments (Fig. 4b). Of these, only the abundances of Acidobacteria and WPS2 were different in the fertiliser treatments compared with either the superphosphate or unfertilised control treatments. For

Acidobacteria, the control and superphosphate treatments provided significantly greater abundance than YLAD Compost Mineral Blend and Agri-ash, the two treatments that had a liming effect. The abundance of phylum WPS2 was also higher in the control than in SEP Pig Manure, BioAg Blend, Agri-ash or YLAD Compost Mineral Blend treatments, whereas there was no difference compared with superphosphate. The fungal community was predominantly represented by eight major genera, with 371 other genera showing lesser dominance and being grouped as ‘others’. One unclassified group at the genus level represented 20–43% of fungal microorganisms across treatments. The fungal genera *Fusarium* (*P* < 0.0001) and

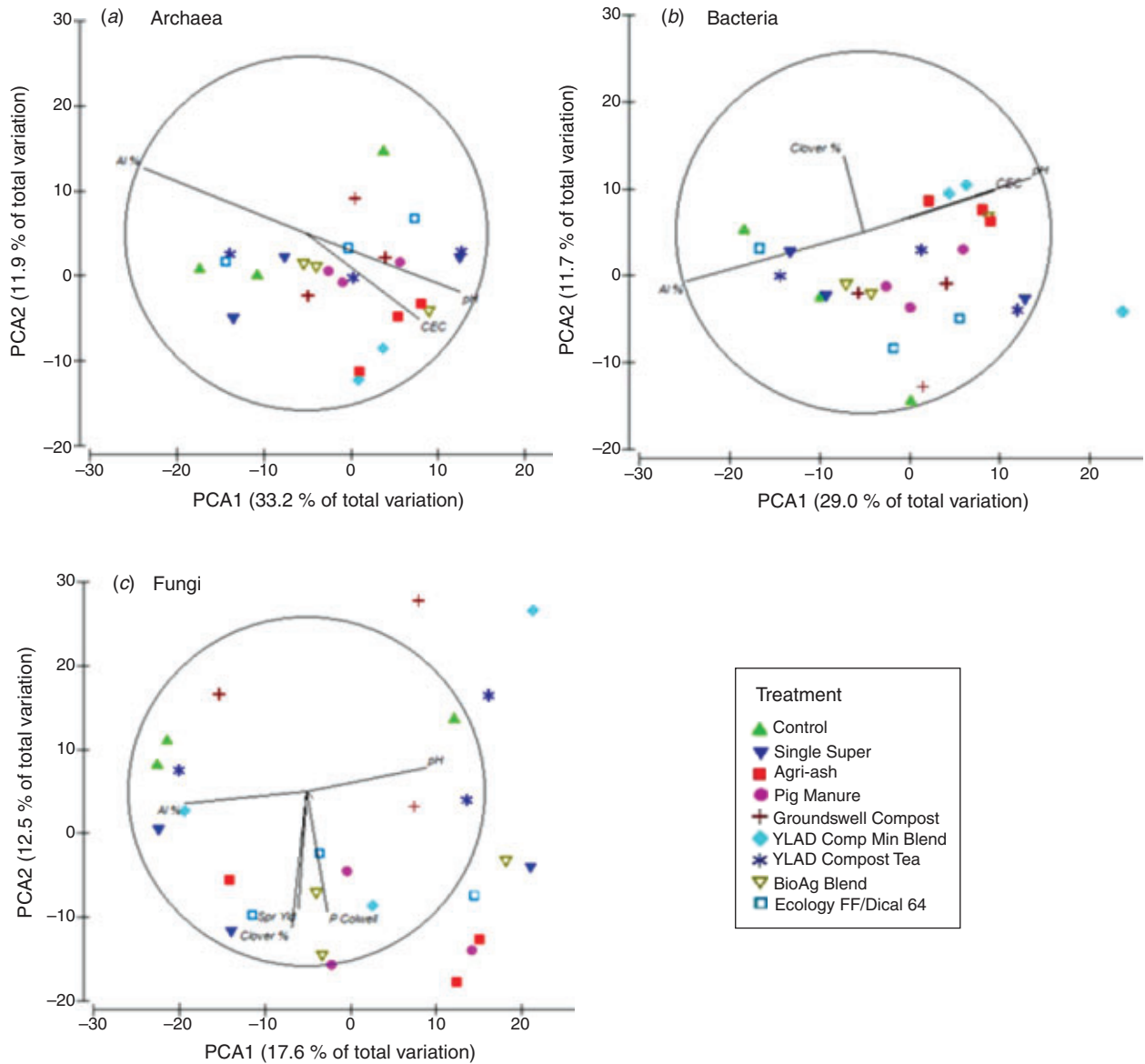


Fig. 3. Structure of (a) archaeal, (b) bacterial and (c) fungal communities in response to fertiliser treatments and unfertilised control at the Kia-Ora field trial site in 2014 ($n = 3$). Principal component analysis (PCA) is shown, performed on Bray–Curtis similarity matrices of archaeal and bacterial 16S rRNA and fungal ITS sequencing data (PERMANOVA; archaea $F = 1.16$, $P < 0.226$; bacteria $F = 1.125$, $P < 0.264$; fungi $F = 1.322$, $P < 0.011$). Vectors show Pearson correlations ($R > 0.43$) with soil parameters pH, CEC (cation exchange capacity), AI (% of CEC) and Colwell P, and with pasture parameters clover content (%) and Spr Yld (spring yield), based on significance at $P = 0.01$. Ellipses indicate 95% confidence interval.

Table 7. Indices for species richness (Margalef), evenness (Pielou) and diversity (Shannon–Weaver) for archaeal, bacterial and fungal soil communities from fertiliser treatments sampled in 2014 at the Kia-Ora trial site

SSP, Single superphosphate. Within a row, values (means, $n = 3$) followed by the same letter are not significantly different ($P > 0.05$); treatments differing from the unfertilised control are shown in bold

Kingdom	Index	Control	SSP	Agri-ash	SEP Pig Manure	Groundswell Compost	YLAD Compost Mineral Blend	YLAD Tea	BioAg Blend	EFF/Dical 64
Archaea	Richness	17.3a	18.9bc	20.2c	19.2bc	19.3bc	20.0c	18.5b	19.5bc	19.0bc
	Evenness	0.66a	0.69ab	0.71ab	0.68ab	0.68ab	0.73b	0.70ab	0.69ab	0.68ab
	Diversity	3.29a	3.50ab	3.66b	3.46ab	3.48ab	3.70b	3.52ab	3.50ab	3.45ab
Bacteria	Richness	149.7a	154.9ab	163.8b	157.6ab	157.1ab	158.3ab	155.7ab	157.3ab	153.1ab
	Evenness	0.81ab	0.78a	0.84ab	0.83ab	0.82ab	0.84b	0.83ab	0.83ab	0.82ab
	Diversity	5.95ab	5.77a	6.23b	6.12ab	6.08ab	6.22b	6.12ab	6.11ab	6.07ab
Fungi	Richness	49.6a	49.0a	47.2a	49.4a	53.3a	52.9a	50.7a	50.6a	45.5a
	Evenness	0.55a	0.62ab	0.61ab	0.66b	0.56a	0.58a	0.60ab	0.60ab	0.57a
	Diversity	3.51a	3.90ab	3.87ab	4.18b	3.57a	3.68a	3.86ab	3.86ab	3.55a

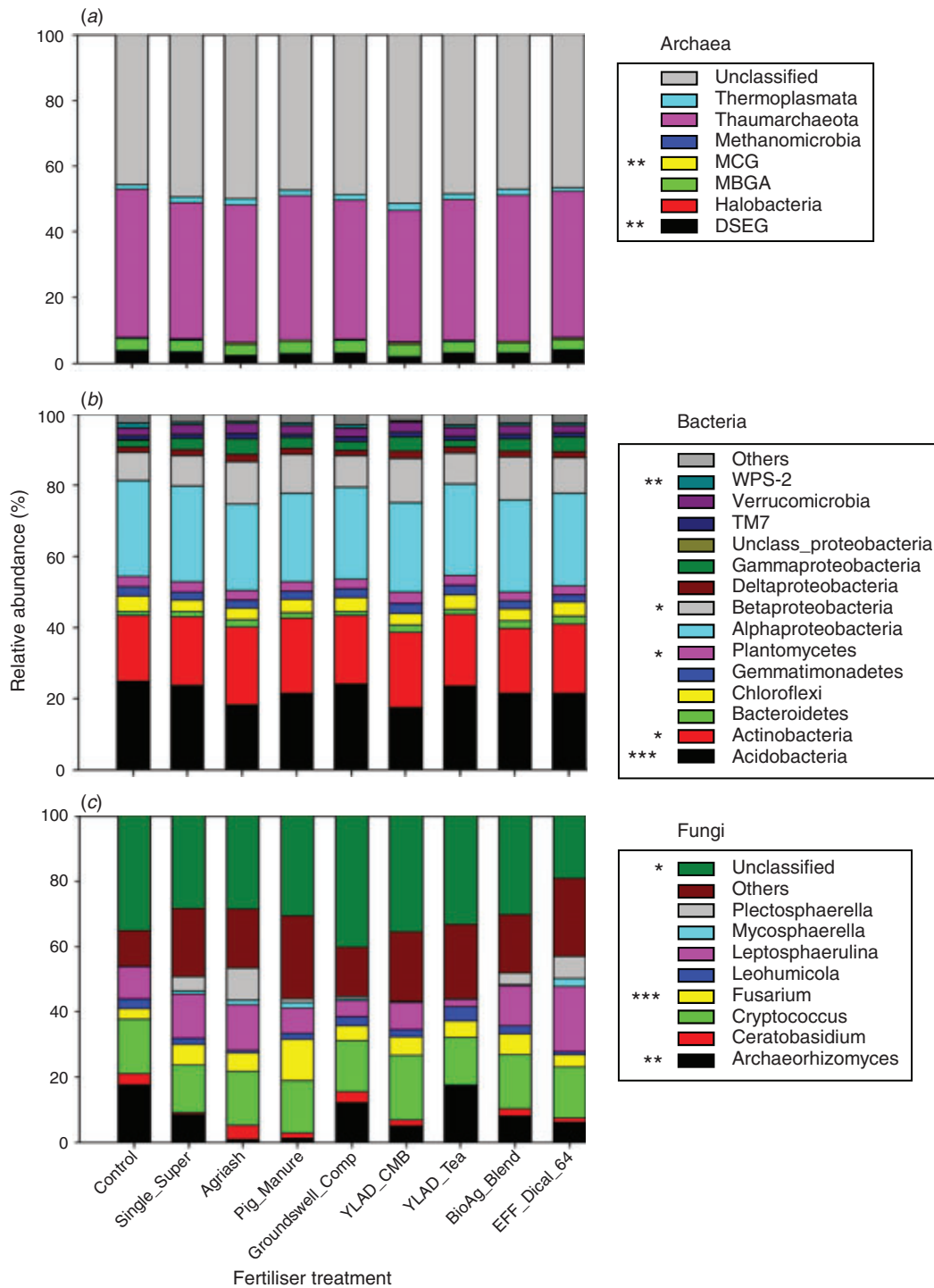


Fig. 4. Relative community abundance for major (a) archaeal class, (b) bacterial phylum and class, and (c) fungal genus present in soil of fertiliser treatments and unfertilised control at the Kia-Ora site in 2014. Unclassified operational taxonomic units (OTUs) are indicated, and minor abundances are grouped as ‘Others’. Asterisks (*) denote significant difference of means ($n = 3$) between treatments (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Archaeorhizomyces ($P < 0.01$) were significantly different across fertiliser treatments. Relative abundance of *Fusarium* was higher in the SEP Pig Manure treatment than in the control and all other fertiliser treatments. Relative abundance of *Archaeorhizomyces* was significantly higher in the unfertilised control than Agri-ash and SEP Pig Manure treatments, whereas superphosphate was not significantly different from any other treatment. Interestingly, the YLAD Compost Tea treatment containing minimal nutrients (similar to the control) also had a higher relative abundance of *Archaeorhizomyces*.

Discussion

This 6-year field study investigated the use and impact of a range of fertiliser products available in the Australian agricultural sector for use on pastures. Differential effects of the products on pasture production and composition were shown, along with their influence on key soil chemical and microbial properties. The fertiliser products used contained differing amounts of key nutrients required for pasture growth, were applied at varied application rates and frequencies based on supplier recommendations, and had differing effects on soil pH. As such, the products would be expected to vary in their ability to address different nutrient limitations simultaneously across the three soils. Unless all nutrients are adequately supplied and growth is not restricted by other soil constraints (e.g. low pH, disease, moisture), production will be restricted by the most limiting factor. In this study, we recognised that the fertiliser products varied considerably with respect to total nutrient supply; nonetheless, assessment was primarily in regard to P (and S), notwithstanding that other factors may have influenced the responses observed. For example, soil K varied across the sites (being lowest at Kia-Ora) and correction of any possible K limitation across the products and sites was not considered. Likewise, the fertiliser products differed markedly in the supply of other nutrients (e.g. Mo), and differential effects of Mo supply on observed pasture responses cannot be discounted.

Pasture growth response

Analysis of the pasture growth response revealed large differences between products. Differences in pasture growth were generally associated with significant increases in pasture quality, as indicated by increased clover content and higher nutritive value, shown by predicted protein content and metabolisable energy content. Consistent with other studies, our results indicated that superphosphate was most effective for increasing pasture productivity and clover content (Curl 1977; Graham and Hazell 1999; Alcock *et al.* 2012). The results also showed that some alternative fertiliser products (e.g. Agri-ash, SEP Pig Manure) were effective for increasing pasture growth, to varying extents, as reported in other studies (Kahn 2014; Nicholson 2014; Farrell *et al.* 2017). However, a range of fertiliser products did not increase pasture yield or alter composition, and treatments with these products were found to be no different from the unfertilised control. It is well recognised that nutrient content and availability vary widely for different alternative fertiliser products, and that the insoluble or organic forms of nutrient present in some fertilisers must first undergo solubilisation and/or mineralisation to be available to plants. The effectiveness of different fertiliser products for

increasing pasture growth therefore varies widely (Quilty and Cattle 2011; Abbott *et al.* 2018).

The degree of response in pasture growth to alternative fertilisers should be interpreted with some caution, in relation to both the frequency of application of the products and the quantities of nutrients (in particular P) applied over time. Consideration of further variables such as provision of other nutrients and differential liming effects is also required. In addition, direct comparison of fertiliser products across different years, as occurred in our study, requires care because of seasonal variability across years and because the period of herbage collection differed across the years (especially in 2013 and 2014 when pasture growth was assessed over winter + spring). Irrespective of this, across all fertiliser treatments the highest relative pasture yield response was consistently observed at the Kia-Ora site, and the lowest at Te Kooti (Fig. 2). Across all years and sites, highest pasture growth was found with superphosphate. Average pasture yield responses with SEP Pig Manure, Agri-ash, BioAg Blend, EFF/Dical 64 and YLAD Compost Mineral Blend treatments across the three sites were generally lower, and average pasture yield with all other fertiliser products was not significantly different from the unfertilised control. The general lack of response of pasture to the urea treatment at all three sites (13–21%) supports the underlying assertion that P was the most limiting nutrient for pasture production.

Direct comparison of the products used in the present study requires consideration of both the total amount of P applied and the potential availability of the applied P. Analysis of the fertiliser products revealed considerable variation in P content and form (water-soluble, citrate-soluble or insoluble). Water-soluble P is considered more highly available to plants than insoluble P, whereas citrate-soluble P has been suggested to become available to plants over a longer period (weeks to months or years; Sale *et al.* 1997). The pasture growth response we observed was associated with available P content as a function of total P supplied across the different products. Growth response of pasture was clearly greatest with treatments that received both 'high' amounts of total P, and the P in an 'available' form, such as superphosphate. Pasture growth was also positively stimulated by products containing a significant proportion of citrate-soluble P provided either through large initial application of total P or by regular applications (e.g. Agri-ash and SEP Pig Manure, respectively). Pastures fertilised with products containing a high proportion of P in 'unavailable' (or insoluble) form (i.e. YLAD Compost Mineral Blend, BioAg Blend, EFF/Dical 64) showed little immediate response to application, with any increase in pasture growth occurring only in later years of the trial. Products that consist almost entirely of insoluble P forms (i.e. rock phosphate fertilisers) take considerable time to be transformed into 'plant-available' forms of P, hence delaying their effectiveness for pasture growth (Johnson *et al.* 1997; Garden *et al.* 1997; Sale *et al.* 1997). The effectiveness of rock phosphate is highly dependent on average annual rainfall and soil type, with acidic soils with high-rainfall environments being more effective (Sale *et al.* 1997).

Two fertiliser products, Agri-ash and SEP Pig Manure, showed comparatively good response relative to superphosphate. SEP Pig Manure contains a large component

of organic plant and faecal material, with ~47% citrate-soluble P and 3% water-soluble P, indicating that at the rates applied (Table 2) it would be capable of providing a response in the short term (Fig. 1), as observed at all sites. SEP Pig Manure was applied every third year and pasture yield showed a substantial increase in the year of application, followed by a lesser response in following years. This is not atypical with manure-based fertilisers, where the longevity of the response will depend on frequency and rates of application (Nicholson 2014). Agri-ash was provided as a one-off application at the start of the study. Although Agri-ash contains a high proportion of insoluble P, 17% of total P is present as citrate-soluble P, suggesting that it could supply adequate P when applied at the very high rate (2.5 t ha^{-1}) in the first year of the study. Agri-ash then yielded consistently higher DM than the unfertilised control treatment through to the fifth year (Te Kooti) and sixth year (Glenroy and Kia-Ora) of the study. This suggests that Agri-ash provided an adequate supply of 'plant-available' P over the course of the trial. By contrast, several of the fertiliser products had low P content (e.g. Groundswell Compost, Trio-min/Eco-min Balance and YLAD Compost Tea; Table 3) and were applied at rates that would not be expected to address any P nutrient deficiencies directly, and hence did not influence STP or provide a pasture growth response. Apart from the control, YLAD Compost Tea delivered the smallest amount of P ($<1 \text{ kg ha}^{-1}$) and consistently resulted in no increased growth over the control. This finding is consistent with other studies that have reported small effects on crop and pasture production of liquid-based biological fertilisers derived from natural products, especially when applied at low rates (Edmeades 2002; Kahn 2014). Kahn (2014) highlighted that these products commonly contain very low levels of nutrient, similar to YLAD Compost Tea trialled in this study. Farrell *et al.* (2017) and Abbott *et al.* (2018) similarly found little evidence for effects on either aboveground biomass or associated root biomass of wheat following application of a wide range of different liquid and microbially based bio-stimulants.

The total P content and relative availability of P in each of the fertiliser products are consistent with the measured pasture growth response and the change in STP observed across treatments and sites. Both Agri-ash and SEP Pig Manure consistently increased STP over the control and supported near-maximum pasture growth. Higher STP and associated increased pasture growth was similarly observed with the superphosphate treatment. Despite similar quantities of P being applied for these products, a differential effect on STP and associated pasture growth was evident across the three sites, illustrating the influence of soil type and specific site effects on pasture growth and response to fertiliser. Importantly, superphosphate was applied in all cases at the same rate ($11 \text{ kg P ha}^{-1} \text{ year}^{-1}$) across sites and years and was not differentially applied to obtain maximum pasture growth according to STP values. Based on the observed variability in STP, it is evident that the rate of superphosphate used was below that required to build P for optimal production at all three sites. This also needs to be considered with respect to the level of P supplied and relative response with the alternative fertiliser products. Insufficient amounts of other key nutrients (e.g. S or K) required to achieve maximum production may further limit the ability of these alternative fertilisers to achieve optimal

response given the differences in nutrient compositions and rates applied. The importance of understanding critical P requirements to support pasture growth based on STP has been demonstrated on a comparable permanent pasture system with differential P inputs (Simpson *et al.* 2015).

The fertiliser products used in this study also had differential effects on soil pH, soil Al and, in some cases, the level of extractable soil S. The two products that contained significant quantities of lime (e.g. Agri-ash and YLAD Compost Mineral Blend) significantly raised soil pH and lowered Al. The direct impact of these changes on pasture growth or on soil processes that influence nutrient availability was not possible to discern over this 6-year study; however, longer term experiments investigating the effects of lime applied to pastures have been reported elsewhere (White *et al.* 2000; Leech 2006; Li *et al.* 2006; Norton *et al.* 2018). Similarly, the various fertiliser products supplied different amounts of S and, in some cases, also had gypsum added. Despite this, only the superphosphate treatment had a consistent influence on soil S levels across the sites. This effect was most evident at Glenroy, which was generally drier than Kia-Ora and Te Kooti and may have had less potential for leaching of S. Irrespective of this, the possible influence on pasture growth of differing S input provided by superphosphate relative to the alternative fertiliser products across the three sites cannot be discounted, because S deficiency may also represent a potential limitation to pasture productivity on some soils.

Assessment of relative cost effectiveness

The present study provides a useful platform for a comparative assessment of the cost-effectiveness of the fertiliser products for pasture production. An annualised cost was calculated for each product for each year of the trial (2009–14). This analysis was based on AU\$ values in 2014 (excluding GST; table S3), using product price information provided by the fertiliser suppliers, along with an annual estimation of handling and spreading costs (noting that these will vary depending on many factors including market access, quantities purchased and proximity of farms to the fertiliser sources). The annualised cost per hectare was adjusted for application frequency and was determined for each product in relation to the additional pasture grown relative to the unfertilised control within each year. Values are presented only for products that grew significantly ($P < 0.05$) more pasture than the control (Table 8); products that did not yield more than the control were regarded as not being cost-effective.

Based on this analysis, superphosphate was generally the most cost-effective product. Within each site, the cost-effectiveness of the alternative fertiliser products relative to superphosphate varied, and differed over time. This variation was associated with both the rate and frequency of their application and supply and availability of P (and S) in the products. Fertiliser products containing predominantly insoluble P were more cost-effective in later years; these included BioAg Blend, EFF/Dical 64 and YLAD Compost Mineral Blend. Despite producing significantly more pasture than the control, several products (e.g. YLAD Compost Mineral Blend) were less cost-effective across all sites. The

Table 8. Comparison of cost effectiveness of fertiliser products (AUS t⁻¹ DM) used at the Glenroy, Kia-Ora and Te Kooti sites

Analysis of fertiliser products over the trial period (6 years for Glenroy and Kia-Ora, and 5 years for Te Kooti) was determined from information provided by the suppliers based on annual purchase of the product and associated handling and spreading costs. SSP, Single superphosphate. Values represent the cost of additional pasture grown above the unfertilised control (as shown in Fig. 1) for the period of measurement within each of the years 2009–14. Only products that grew significantly more pasture than the control have a recorded value; products that did not differ to the control were regarded as not being cost-effective and are represented with a dash (–). Bolded values within each year indicate the product that was most cost-effective for the particular site and year

Site	Year	SSP	Agri-ash	SEP Pig Manure	BioAg Blend	EFF/Dical 64	YLAD Compost Mineral Blend	Groundswell Compost	Trio-min/Eco-min Balance	YLAD Compost Tea	Urea
Glenroy	2009	82	–	59	–	–	–	–	–	–	–
	2010	41	38	58	–	–	–	–	–	–	–
	2011	81	56	–	–	–	372	–	–	–	128
	2012	31	41	42	89	–	–	–	–	–	–
	2013	19	34	42	88	63	155	–	–	–	–
	2014	22	42	65	53	51	138	–	–	–	–
Kia-Ora	2009	45	69	41	–	–	–	–	–	–	–
	2010	25	24	47	46	–	150	–	–	–	–
	2011	27	20	66	51	86	111	–	–	–	60
	2012	28	21	34	51	68	108	–	–	–	–
	2013	20	26	37	56	42	–	–	–	–	–
	2014	14	34	40	21	19	100	–	–	–	–
Te Kooti	2009	53	45	86	–	–	151	–	–	–	–
	2010 ^A	–	–	–	–	–	–	–	–	–	–
	2011	45	41	–	86	–	185	–	–	–	–
	2012	39	30	41	57	–	164	218	336	–	117
	2013	24	46	80	153	107	247	223	–	–	184

^AWinter and spring period 2010 experienced extensive waterlogging at the Te Kooti site, resulting in no significant differences in pasture yield between treatments.

frequency of application of each fertiliser product also had bearing on relative cost-effectiveness; for example, SEP Pig Manure was applied every third year (2009 and 2012) and showed a cost-effectiveness similar to superphosphate in the year of application (i.e. Kia-Ora site in 2009) but declining thereafter. On the other hand, Agri-ash was applied only once, at the start of the study, and was shown to be cost-effective compared with superphosphate, especially in the earlier years following application (Table 8).

Impacts on soil microbial communities

A key requirement to the use of alternative fertiliser products by growers is an understanding of their wider impacts on soil microbial communities, biological function and 'perceived soil health' over the longer term. In the present study, no major effects on the soil microbial community were found at the Kia-Ora site despite the large differences found in pasture production and cost-effectiveness of fertiliser products. Importantly, at the level of OTUs (as an indicator of species diversity) there was extensive microbial diversity with no direct and significant effects of fertiliser products on the structure of archaeal, bacterial or fungal soil communities compared with either the unfertilised control or superphosphate treatments (Fig. 3, fig. S2). However, there was correlation of the soil archaeal, bacterial and fungal overall community structures with some associated soil and pasture parameters, with soil pH (and Al as % of CEC) being the most apparent. The importance of soil pH as a key driver of community structure has been well documented (Fierer and Jackson 2006; Lauber *et al.* 2009; Rousk *et al.* 2010; Banerjee *et al.* 2016a). In addition,

for the bacterial and fungal communities, correlation was also found with the clover content of the pasture. Although soil N was not specifically measured in the study, this observation suggests that differences in N cycling within the pastures, as a result of the promotion of legume growth through P fertilisation, may have subsequent and longer term influence on community structures. The C:N ratio in soil is known to have a major influence on fungi:bacteria ratios (Six *et al.* 2006; Cleveland and Liptzin 2007; Lauber *et al.* 2008) and as such would be expected to have some effect on community structure for both bacteria and fungi. Interestingly, the OTU-based community structure for fungi across all fertiliser treatments was also correlated with Colwell P. This is consistent with a previous study on a similar soil type (Wakelin *et al.* 2009) where fungal communities were specifically influenced by long-term fertilisation with superphosphate, with differences being associated with particular groups of mycorrhizal fungi. Given the effect of the alternative fertiliser products in the present study on soil P availability and associated response of pastures to P supply, further research would be of value to evaluate specifically the impact of fertiliser treatments on mycorrhizal community structure.

Analysis of the microbial communities at a higher taxonomic level based on phylogenetic assignment to a major phylum, class or genus similarly revealed only small, but in some cases significant, changes in community structure. Most notable was the response of the bacterial phylum Acidobacteria to the Agri-ash and YLAD Compost Mineral Blend fertiliser treatments. These products increased soil pH, and both were associated with significantly lower relative abundance of Acidobacteria. Effects of soil pH on various indices of archaeal and bacterial richness

and diversity were also evident for the Agri-ash and YLAD Compost Mineral Blend treatments, with higher diversity observed in both cases compared with the unfertilised control. For fungi, abundance of the genera *Archaeorhizomyces* and *Fusarium* was significantly affected by fertiliser treatment. In particular, *Archaeorhizomyces* was found to be more abundant in the control, YLAD Compost Tea and Groundswell Compost treatments, which were the most P-limited. This suggests that members of this genus are more strongly associated with lower levels of soil fertility and supports the general correlation found between fungal community structure at the species level and associated soil and pasture productivity parameters. *Archaeorhizomyces* is a relatively newly recognised genus (first reported in 2011) and, although considered non-symbiotic, appears to have a strong association with plant roots (Rosling *et al.* 2011). Relative abundance was similarly higher for the fungal genus *Fusarium* in the SEP Pig Manure treatment than in all other treatments including the superphosphate and unfertilised controls. This difference was consistent with significantly higher fungal diversity in this fertiliser treatment alone. SEP Pig Manure is a complex form of organic fertiliser with high DM input when applied to pastures. However, whether differences in genera such as *Fusarium* have functional implication for soil processes remains to be established, because this genus has wide diversity, including some species that can negatively affect pasture growth and quality. In general, linking functional significance to changes in community structure remains a major future challenge for soil community analysis.

The soil community analysis undertaken in this study highlights that on-farm management decisions regarding fertiliser product choice are unlikely to have a major impact on soil microbial communities. Soil communities are known to retain high levels of functional diversity and structural resilience irrespective of management (Bissett *et al.* 2011, 2013; Banerjee *et al.* 2016b), and would be expected to continue to support a 'healthy' soil regardless of fertiliser product choice. Importantly, our results therefore support the view that decisions on fertiliser choice for pasture and livestock production systems should be based primarily on economic rationale associated with productivity responses.

Conflicts of interest

The authors declare no conflicts of interest with their involvement in this study and in the production of the manuscript.

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