The effect of dung and dung beetle (*Bubas bison*) activity on soil microbial communities.

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Executive Summary

Bioscience received 30 soil samples for microbiological analysis on the 15th of January 2018. The samples were part of a trial that investigated the effect of dung and dung beetle activity on soil microbiology on eight different properties.

The main findings were:

- Dung addition increased diversity of archaea and fast-growing bacteria and fungi but supressed slow-growing Acidobacteria and Actinobacteria.
- Dung beetle activity mitigated the deleterious effects of dung on slow-growing micro-organisms which resulted in treatment A exhibiting the highest microbial diversity.
- Place of origin (i.e. where samples were collected) had a bigger effect on the microbial community structure for bacteria and fungi than treatments. In contrast, the archaeal community was significantly influenced by dung-only addition.

1 Background

Bioscience received 30 soil samples (0-10 cm) from Kathy Dawson (Warren Catchments Council) on the 15th of January 2018. The samples originated from eight properties on which trials with dung beetles were conducted. The dung beetle *Bubas bison* (Linnaeus, 1767) used in the trials is an opportunistic night-flier that utilises the whole dung pad and actively buries dung in tunnels up to 50 cm deep (Kirk, 1983; http://www.dungbeetlesolutions.com.au/about-dung-beetles/). The effect of dung beetle and dung application (treatment A) on soil microbial diversity was compared to a dung-only application (treatment B) and an unamended control (treatment C). Additionally, samples from treatment P were collected from pastures into which dung beetles were released.

2 Methods

DNA was extracted from 10 g soil using the DNeasy PowerSoil Kit (Quiagen, Melbourne, Vic, Australia) after washing soil with phosphate-buffered saline solution containing Tween 20. Polymerase chain reaction (PCR; Mullis and Faloona, 1978) was used to amplify the internal transcribed spacer (ITS) region of the ribosomal RNA operon of nine selected microbial groups (Table 3) for automated ribosomal intergenic spacer analysis (ARISA; Fischer and Triplett, 1999).

3 Results

3.1 Effect of dung and dung beetle activity on soil microbial diversity (alpha diversity)

The Bioscience ARISA assay returns the total number of microbial "operational taxonomic units" (OTUs) or "species" for each sample (alpha diversity). It is generally accepted that a high biodiversity is indicative of a healthy soil, so the more microbial species are present, the healthier the soil.

Total microbial diversity was generally high with an average abundance of 629 OTUs across all 30 samples analysed. When grouping the four different treatments across all properties, the highest biodiversity was detected in samples from treatment A (dung beetle plus dung), followed by treatment B (dung only), C (control), and P (dung beetles released into pasture) (Table 1). Although these results are consistent with expectations of what effect manure and dung beetles would have on the soil microflora, this pattern only occurred consistently at four of the eight properties (Figure 1). This was, however, not statistically significant due to variability between properties. For example, treatment A resulted in both the highest and lowest number of OTUs (781 OTUs at Burton and 527 OTUs Hilwig-Prestridge).

Table 1: Average number of microbial OTUs detected in different treatments.

Treatment	Number of OTUs (±Standard error)
A (dung beetle and dung)	648 ± 28
B (dung only)	640 ± 13
C (control)	624 ± 14
P (dung beetles released into pasture)	598 ± 11

The nine different microbial groups surveyed responded differently to the dung beetle and dung treatments.

Archaea were generally upregulated in manure treatments (21% for A and 36% B) compared to the control ($P = 0.047$). This was especially pronounced for treatment B at properties Burton, Cooper, Fraser, Mottram, and Muir T (Figure 1). This increase in Archaea could reflect an increase in the abundance of methane-producing microorganisms that originate from the dung and continue to proliferate under anaerobic conditions. The latter may be persistent in treatment B due to the lack of bioturbation i.e. no dung beetle activity and thus limited aeration.

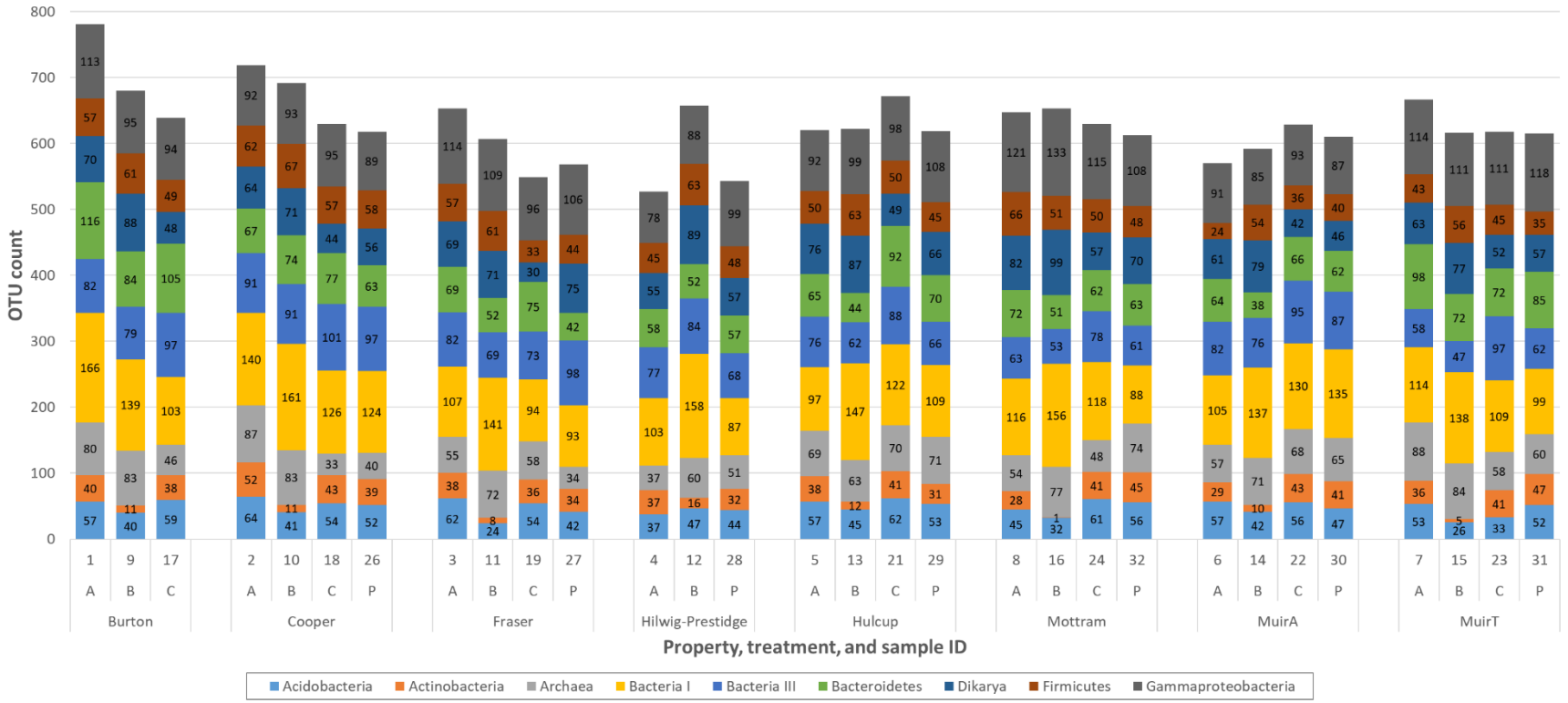


Figure 1: Microbial OTU counts for each sample.
Samples are arranged by property and treatment. Treatment key: A = dung beetles and dung, B = dung only, C = control, P = pasture with dung beetles released. For four of the eight properties the highest microbial diversity was detected in treatment A (Burton, Cooper, Fraser, and Muir T).

Dikarya (fungi), Bacteria I, and Firmicutes were also significantly enriched in soil of treatment B ($P < 0.02$) and to a lesser extent in treatment A compared to the control (Figure 1). This increase is likely related to the initial input of carbon and other nutrients (e.g. P and N) contained in the dung stimulating growth of fast-growing microorganisms (copiotrophs or r-strategists). Although the soil chemical parameters surveyed two years after dung treatment (provided by Kathy Dawson) did not show a consistent increase of soil carbon levels for all eight sites, treatment B resulted in higher carbon stocks for five properties (i.e. Burton, Fraser, Mottram, Muir A, and Muir T) with an average increase of 0.6% (Table 2). Colwell-P was higher in treatment A and B compared to the control for seven out of the eight properties (no control data for Hilwig-Prestidge, Table 2). No clear trend was discernible for N either in form of ammonium or nitrate between treatments and control. However, these forms of N are interconvertible and readily utilised by microorganisms and plants so that historic treatments may have little effect on present N concentrations. Despite this, increased C and P stocks likely caused the significant increase in abundance of copiotrophic soil bacteria and fungi in treatment B, and to a lesser degree in treatment A (Figure 1).

In contrast, dung addition and subsequent higher C and P concentrations led to a significant decrease of Acidobacteria ($P = 0.001$) and Actinobacteria ($P < 0.0001$) in treatment B (Figure 1). These microbial groups are considered oligotrophs or K-strategists meaning they are adapted to low nutrient environments and do not cope well under high nutrient concentrations as evidenced here by their low abundance in the dung-only treatment (B, Figure 1). This deleterious effect was mitigated by the presence and activity of dung beetles and there were no statistically significant differences in the abundance of Acidobacteria and Actinobacteria between treatment A compared to treatment C.

Table 2: Selected soil chemical properties in different treatments

	Treatment A	Treatment B	Treatment C	Treatment P
Colwell-P (mg/kg)	143	137	109	59.9
NO3-N (mg/kg)	26.1	30.9	22.2	24.6
NH4-N (mg/kg)	3.09	3.83	3.86	3.83
Total carbon (%)	6.95	7.51	6.90	4.55

The abundance of Bacteria III, Bacteroidetes and Gammaproteobacteria was seemingly unaffected by the treatments.

In summary, the average alpha diversity was highest in soils treated with dung and dung beetles (treatment A, Table 1) although the abundance of fast-growing copiotrophs was highest for the dung-only treatment (B). However, the concomitant decrease in abundance and diversity of oligotrophs in the dung-only treatment, especially that of Actinobacteria, would be undesirable as they play a vital role in soil ecosystems.

3.2 Effect of dung and dung beetle activity on soil microbial community structure (beta diversity)

*In addition to investigating the number of OTUs present in a sample (alpha diversity), the differences in the community composition can be assessed (beta diversity). In the below principle component analysis plots (PCO, Figure 2*Figure 2*), the microbial community of each sample is represented by a data point. Samples with similar microbial communities cluster closely together. Conversely, samples with different microbial communities are depicted further apart from each other. Data on soil fertility (provided by Kathy Dawson) were integrated with the microbiological data and are represented by vectors (Figure 3). Along each vector the numerical value of the variable increases.*

3.2.1 Community structure of Archaea

The archaeal community structure was significantly affected by both sample location (i.e. property from which the samples were collected, P = 0.001) and treatment (P = 0.012). Interestingly, the archaeal communities in treatment A and C were more similar to each other (34.6%) than to treatment B (32.5% for A vs B and 27.0% for C vs B) as indicated by their close proximity to each other in the PCO plot (Figure 2B). This suggests that the archaeal community in soil from treatment B is likely more dominated by members originating from dung rather than by those from the soil. Conversely, beetle activity seemingly reduced this effect by removing, burying, and thus "diluting" dung in treatment A resulting in an archaeal community more similar to the original soil while generally increasing microbial abundance (Figure 1).

3.2.2 Community structure of Bacteria

The bacterial community structure was also significantly affected by sample location (i.e. property from which the samples were collected, P = 0.001) but not by treatment (P = 0.146). This means that although the abundance of bacteria was increased by dung treatments (Figure 1, Table 1) the community structure was not impacted (Figure 2D) i.e. bacteria contained in the dung were not able to compete with the in situ soil bacteria. This is somewhat unsurprising given the vast environmental differences between conditions inside the animal host and soil.

3.2.3 Community structure of Dikarya

Similar to bacteria, the fungal community structure was significantly affected by sample location (i.e. property from which the samples were collected, P = 0.001) but not by treatment (P = 0.881, Figure 2F). Dikarya present in the soil were able to take advantage of added nutrients without drastic changes to their community composition.

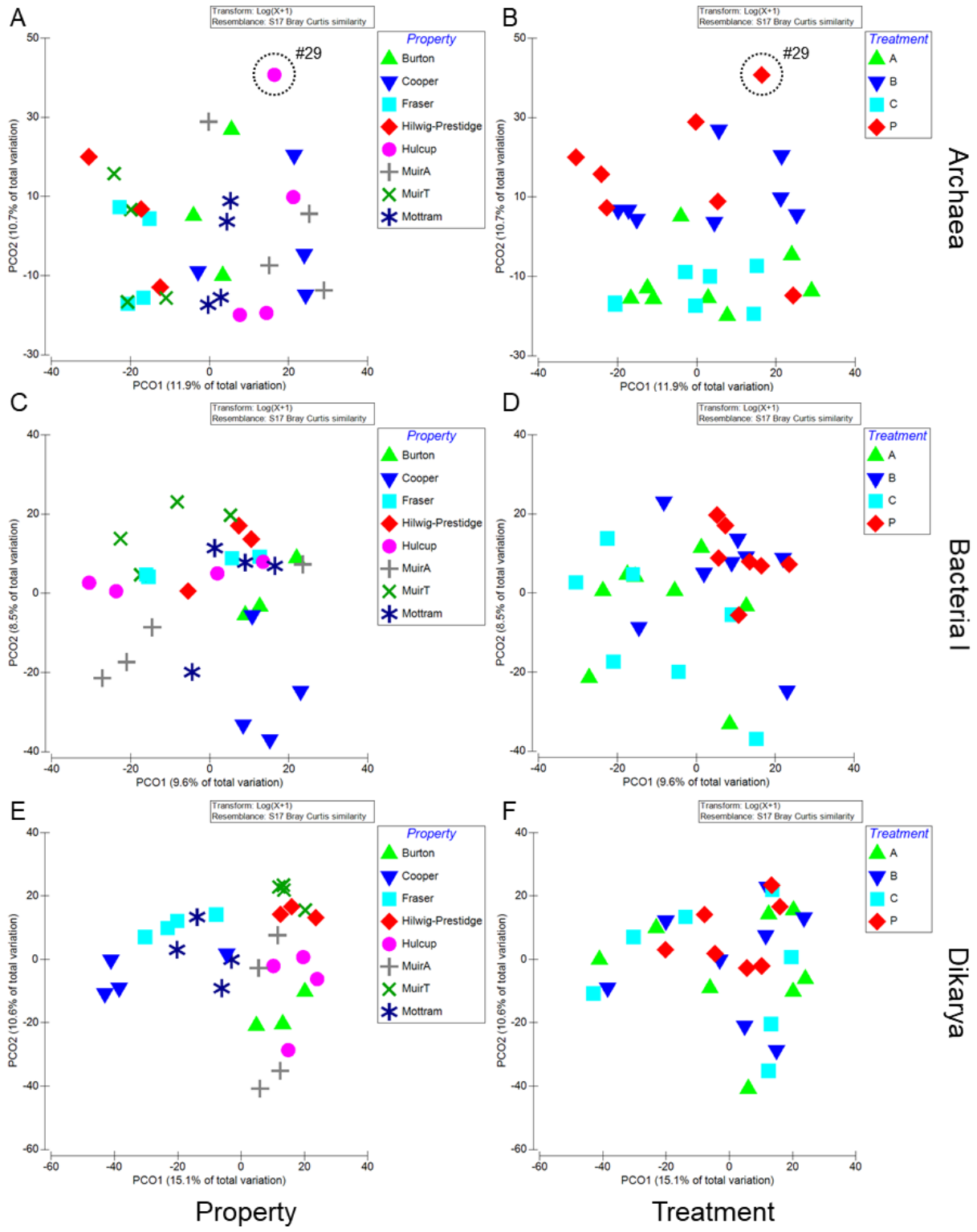


Figure 2: PCO plots for dung beetle treatments

PCO plots for Archaea (A and B), Bacteria I (C and D), and Dikarya (E and F) are presented. Samples are separated by property in figures on the left-hand side (A, C, and E), and by treatment on the right-hand side (B, D, and F). Data points in the same location in the two plots for each microbial group are identical, for example, the two circled dots in A and B correspond to sample number 29 (Hulcup: Pasture).

4 Discussion

Dung beetle and dung addition generally had a positive effect on the abundance of soil microbial taxa (Table 1, Figure 1). This is in line with the expectation that added dung would deliver organic carbon and nutrients to the soil and consequently stimulate microbial activity and plant growth locally. Only at two properties, Hulcup and Muir A (Figure 1), did dung addition not increase microbial abundance compared to the control. Reasons for this are not apparent.

Dung beetle activity seemingly resulted of a redistribution of added carbon along the soil profile given that the carbon content of treatment A was slightly higher than the control but markedly lower than treatment B in the uppermost soil layer (0-10 cm, Table 2) with presumably very similar initial inputs. This is supported by Menendez et al. (2016) who reported that a tunneler species (*Typhaeus typhoeus*) increased C transfer down the soil profile.

Unlike carbon, phosphate appears to have been retained in the top soil either by fixation onto soil minerals or by biological uptake (both plant and microbial). This increase in surficial phosphate would be of agronomic importance given that phosphate limitation to plant growth is common in Western Australian sandy soils and could potentially reduce expensive fertiliser application to grazed pastures.

The functional tunnelling behaviour of *Bubas bison* (Kirk, 1983) incorporated dung into the soil and resulted in similar archaeal communities in treatments A and C. The lack of beetle activity (treatment B) caused a shift in the archaeal community which likely resembles the one in the original dung. Without bioturbation and aeration, methanogenic archaea were allowed to persist and retain their influence on the soil microbiota even after two years (Figure 2) which is somewhat astonishing. In contrast, the bacterial and fungal community composition were unaffected by the presence of dung beetles and / or dung. Similar results were obtained by Slade et al. (2016) who reported that initially distinct microbial communities in dung and soil converged during a 60 day experiment. The analysis of soil microbiota two years after dung application suggests that microbial abundance remains positively influenced even if any effects on the community composition have dissipated.

Sample provenance and therefore local edaphic factors such as soil chemical and physical properties govern the microbial community structure. As such it is not surprising that sample origin (i.e. property) had a bigger influence on the bacterial and fungal community than added dung beetles and dung. This is supported by the distance-based redundancy analysis plots (dbRDA, Figure 3) that take edaphic factor into account when computing similarities between microbial communities. It is evident that samples from the same property group are closely together (Figure 3). Nonetheless, dung beetle and dung addition stimulated the local microbial communities (treatments A and B, Figure 1, Table 1).With treatment A likely being most beneficial as treatment B which had a negative effect on oligotrophic Acidobacteria and Actinobacteria.

In the context of this study, a publication by Menendez et al. 2016 (attached) raises interesting points:

- On pasture stocked at rates of 700 cow days ha-1 y-1, dung deposition adds around 22 t ha-1 of C (Bol et al., 2000), providing a significant input of C to soil. This C input is thought to contribute to soil C stocks in temperate grasslands with 10-16% of cow-dung C incorporated into the soil in only two months (Bol et al., 2000; Dungait et al., 2005).
- a significant proportion of dung-C is lost through microbial respiration (Lovell and Jarvis, 1996; Chen et al., 2011; Grilo et al., 2011).
- In addition, dung can stimulate microbial activity in the soil underneath the dung, resulting in the loss of pre-existing soil C (Bol et al., 2003).
- Soil macro-invertebrates, including dung beetles, have also been reported to strongly influence greenhouse gas emissions (e.g. CO2, N2O and CH4) from dung (Lubbers et al., 2013; Penttila et al., 2013), suggesting that these organisms influence microbial activity and dung decomposition rates.
- Interestingly, the presence of the dweller and tunneler beetle species together had a synergistic, positive effect on soil microbial respiration
- Aphodius beetles (dwellers) have been reported to increase bacterial density through substrate mixing (Lussenhop et al., 1980), while tunnelers have been shown to enhance fungal growth (Yokoyama et al.,1991).
- This discrepancy between activity and biomass results is consistent with the idea that, whereas microbial activity is influenced rapidly by the input of labile C, soil microbial biomass is determined by the long term input of stable organic C (Bardgett et al., 1998).

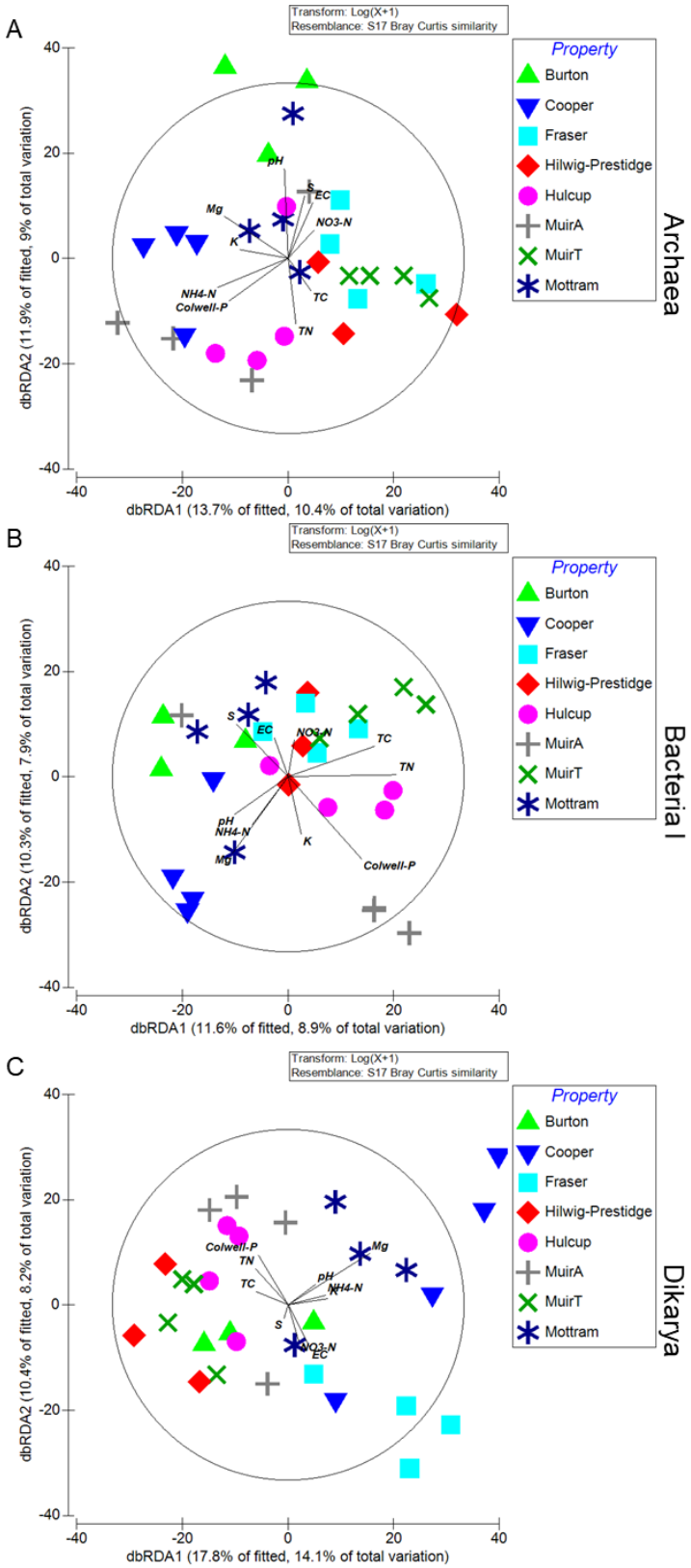


Figure 3: distance-based redundancy analysis of microbial communities and edaphic factors. dbRDA plots for Archaea (A), Bacteria I (B), and Dikarya (C) are presented.

5 Appendix

5.1 Bioscience ARISA Assay Report Details

5.1.1 General Information for Clients

The Bioscience ARISA (Automated Ribosomal Intergenic Spacer Analysis) Assay quantifies the microbial diversity of environmental samples using the latest genetics technologies. Soils with high microbial diversity are generally healthier than soils with low microbial diversity. Soils with high microbial diversity can: suppress plant diseases spread in soil; drive higher rates of soil nutrient cycling; cause increased rates of decomposition of organic compounds in soil; drive higher plant productivity.

Our assay has been used by a wide range of clients including land managers, water scientists, and research organisations. The Bioscience ARISA assay uses a 10 g aliquot of each sample submitted, from which total DNA was extracted including microbes. We then use genetics technologies to estimate the number of microbial species in each of nine groups of microbes, and to determine how similar the different microbial communities are in the submitted samples.

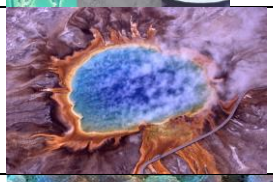
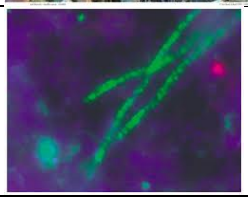
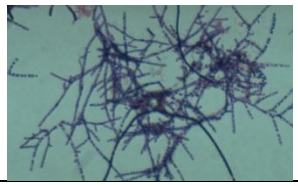
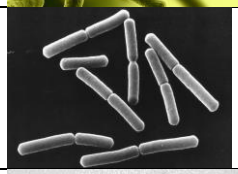
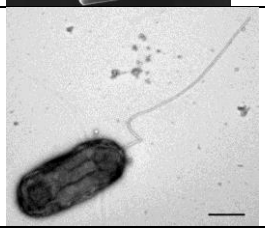
5.1.2 What Do My Bioscience ARISA Results Show?

The table overleaf gives background information on the nine different groups of microbes that the Bioscience ARISA assay measures. The Bioscience ARISA assay produces a count of the number of “operational taxonomic units” (OTUs) for each of the nine groups of microbes assayed. The OTU count is equivalent to the species richness or species diversity of the sample (number of microbial species per group per 10 g sample).

The Bioscience ARISA assay also produces a measure of community similarity for each of the nine groups of microbes assayed. We expect that samples taken from a similar location, at a similar time, or that received a similar treatment, will be similar in the microbial species that they contain. We measure similarity of community composition and plot this visually in two dimensions – the more similar the species present in the sample, the closer together those samples will be plotted.

If your samples were from a trial where treatments were applied, our results will produce estimates on how significantly the treatments applied affected the microbial OTU counts and community composition compared to non-treated samples.

Table 3: Information about different microbial groups detected with ARISA.

Microbial Group	Illustration	Description	Function
Dikaryotic Fungi		Include all mushrooms, and smuts, rusts, mildews, and penicillin.	Many species form mycorrhizal interactions with plants. Some fungal species cause plant disease. Decompose organic matter.
Archaea		Kingdom of microbes growing especially in extreme environments e.g. hot springs. Common in soil.	Many species in healthy soil, these break down ammonia to nitrate. Some species are symbiotic.
Bacteria I Bacteria III		Target bacteria generally across a wide range of different bacterial groups.	Greater numbers of bacteria occur in the plant rhizosphere compared to bulk soil, where they increase the supply of nutrients to plants.
Acidobacteria		Very common bacterial group in soil and water but difficult to culture so poorly studied. Increase in diversity in acid soils.	Function unclear. Species diversity decreases in soils with abundant organic carbon.
Actinobacteria		Bacterial group most commonly found in soil and water, with many species. They make soil smell “earthy”.	Break down soil organic compounds, very important in humus formation. May break down waxes that cause soil water repellence. A few species can cause plant disease.
Bacteroidetes		Bacterial group common in soil. Also common in faecal material.	Break down soil carbon-based organic compounds, very important in humus formation. Adding different carbon sources to soil increases species diversity.
Firmicutes		Fairly common in soil. Produce resistant endospores and can survive environmental extremes.	Common in the soil rhizosphere, assist in the supply of nutrients to plants. Common in soils high in metals.
Gamma Proteobacteria		Very species-rich bacterial group.	Functionally very diverse, living on a variety of soil compounds. Related to nitrogen-fixing bacteria found in pea root nodules.

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