

Scientific Trial Report

Soil microbial processes and soil carbon for dairy pastures amended with compost Project No: IN2.1.013 Author: Emeritus Professor Lyn Abbott

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1. Executive Summary

We investigated the impact of application of composted dairy manure on soil microbial communities associated with retention of soil carbon in three dairy soils. The three organic amendments used in this study were those applied in the SWCC Project IN2.1.002 "Measuring the soil health benefits of applying compost and the financial benefits of both on-farm composting of farm waste resources and purchasing compost for the WA dairy industry." This project also builds on the project funded under the Carbon Farming Awareness Project (CA.001) that commenced in April 2015 and finished in May 2016. Soil bacterial communities were characterised using community profiling (semiconductor sequencing of barcoded amplicons generated from the V3-V4 region of bacterial 16S rDNA genes). As in 2015/6, *Proteobacteria* was the dominant bacterial phylum in soil from each of the three farms (soils were collected in September 2017). The overall bacterial community structure in soil differed for each farm but there was relatively little effect of the different compost and manure treatments on the community in soils from each farm. Minor changes in relative abundance of *Bacterioidetes* were observed for Farm 1, *Actinobacteria* for Farm 2, and *Acidobacteria* for Farm 3). In addition to the field experiment, compost was added to soil from each of the three sites used in the field experiment and compost was applied at two levels (5 and 10t/ha equivalent); this was a continuation of the glasshouse experiment established in 2015 but the compost was added at a higher level than in 2015. As observed under field conditions, there was a greater difference in relative abundance of *Proteobacteria* (the dominant bacterial phylum) between farms than within farms with different compost treatments under controlled glasshouse conditions. The same occurred for other bacterial phyla. The ratio of bacteria to fungi was assessed for soils in the glasshouse experiment. Although there was no effect of compost level, the effect of application of compost had different effects on the ratio of bacteria:fungi in each soil. This study further demonstrated that changes in bacterial communities can occur irrespective of measurable changes in soil carbon. It also shows that soil microbial communities differ widely between soils and highlights functional redundancy among bacteria and fungi within soil communities.

2. Introduction

We investigated microbial community responses after 3 year's of application of compost and manure on three dairy farms. We assessed changes in soil bacterial communities between application of compost at two levels and application of manure in comparison with no organic amendment. In each case, a normal rate of application of synthetic fertiliser was applied with the manure and compost.

The organic amendments used in this study were those applied in the SWCC Project IN2.1.002 "Measuring the soil health benefits of applying compost and the financial benefits of both onfarm composting of farm waste resources and purchasing compost for the WA dairy industry." This project also built on the project funded under the Carbon Farming Awareness Project (CA.001) that commenced in April 2015 and finished in May 2016. Soil bacterial communities were characterised using community profiling (semiconductor sequencing of barcoded amplicons generated from the V3-V4 region of bacterial 16S rDNA genes).

In addition to the field experiment, compost was applied at two levels (5 and 10t/ha equivalent) to soil from each of the three sites used in the field experiment. This was a continuation of the glasshouse experiment established in 2015 but the compost was added at a higher level than in 2015. For the glasshouse study, in addition to assessment of the bacterial communities, the abundance of fungi (18S rRNA gene copy number) was assessed and the ratio of bacteria:fungi was assessed based on (based on 16S rRNA and 18S rRNA gene copy number).

Characterisation of changes in microbial communities associated was complementary to the approach used in the previous study. The glasshouse experiment included additional assessments of the relative abundance of fungal abundance and the ratio of bacteria to fungi.

The aims of the project were:

- To quantify microbial dominance associated with soil carbon retention in three diary soils in response to compost application under field conditions.
- To quantify microbial dominance associated with soil carbon retention in three dairy soils in response to compost application under controlled glasshouse conditions (Year 2 of an established glasshouse experiment).

3. Materials and Methods

This project investigated the impact of the application of compost and dairy manure on soil microbial communities associated with retention of soil carbon in three dairy soils. The compost amendments used in this study were those used in the SWCC Project IN2.1.002 "Measuring the soil health benefits of applying compost and the financial benefits of both on-farm composting of farm waste resources and purchasing compost for the WA dairy industry."

The treatments compared were:

- (i) Compost applied at two rates (3t/ha and 6 t/ha) on each farm with standard inorganic synthetic fertiliser
- (ii) Dairy manure applied at one rate (3t/ha) on each farm with standard synthetic fertiliser, and
- (iii) Synthetic fertiliser applied at standard farm application rate for dairy pastures.

The three dairy farms belong to S. Maughan (Harvey, WA) (Farm 1), M. Brett (Dardanup, WA) (Farm 2) and S. Scott (Gelorup, WA) (Farm 3). These farmers are participants in SWCC Project IN2.1.002.

We compared the manure and compost treatments, and synthetic fertiliser treatments on three WA dairy farms in collaboration with a three-year study funded through current SWCC grant IN2.1.002 140422. We sought to add value by evaluating soil biological processes associated with soil carbon storage in these pastures receiving different nutrient inputs including synthetic fertiliser, dairy effluent/manure, and compost. The fertiliser, compost and manures are already being characterized in terms of nutritive value as part of IN2.1.002 140422. The diagram of the location of the trial sites is included in Appendix 1.

Soil microbial community diversity was determined using community profiling techniques (pyrosequencing or ion-tag sequencing of amplicons generated from the V4 region of bacterial 16S rRNA genes).

FIELD TRIAL DESIGN AND SOIL SAMPLING:

The three experimental field sites were the same as those in SWCC Project IN2.1.002.

Soil was collected in collaboration with SWCC Project IN2.1.002 from the three field sites established in SWCC Project IN2.1.002. Three replicate samples were collected from each treatment at each site.

Each replicate soil sample consisted of 5 composite samples. Soil samples for microbial analysis (0-10cm) were frozen and stored prior to analysis.

DATA COLLECTED FROM THE FIFLD EXPERIMENT.

DNA was extracted from 0.5 g of field soil sampled form each soil amendments on each farm using the MoBio Powersoil DNA isolation kit (Geneworks, Australia). For the sequencing, PCR was performed on the 16S rRNA genes and sequenced using the Ion Torrent Personal Genome Machine.

GLASSHOUSE EXPERIMENTAL DESIGN AND SAMPLING:

Soil had been collected adjacent to the field trials on each of the dairy farms for the glasshouse experiment and potted in 2015 (as part of the previous project). The two compost treatments were selected at higher levels than in 2015. There were two levels of compost and a control (no compost).

The compost applied was obtained from C-Wise and applied as (i) Quicken[®] 10 kg ha-1, (ii) Quicken[®] 5 kg ha-1. Fertilisers were mixed in the top 10-20 cm (at the same rates for each pot).

There were 3 replicates (pots) for each treatment for soil collected from each farm. Each pot contained 3 kg soil. Annual ryegrass was grown in the pots. Soil was sampled 11 weeks after sowing the ryegrass seeds.

DATA COLLECTED FROM GLASSHOUSE EXPERIMENT:

The collected bulked soils were sieved (<4mm) prior to soil characterisation and analysis. Microbial community structure, soil C (%) total soil N (%), mineral N (nitrate and ammonium), water holding capacity, electrical conductivity (EC) and pH were assessed at the end of the experiment.

DNA was extracted from 0.5 g of field soil sampled form each soil amendments on each farm using the MoBio Powersoil DNA isolation kit (Geneworks, Australia). For the sequencing, PCR was performed on the 16S rRNA genes and sequenced using the Ion Torrent Personal Genome Machine.

At harvesting of the glasshouse experiment (11 weeks), roots were carefully lifted out of the soil and shaken vigorously to remove loose adhering soil. The tightly adhering rhizosphere soil was collected and used for subsequent soil analyses. Fresh shoot weight was taken and oven-dried at 60°C for 72 h and total shoot dry weights per pot for each treatment was calculated. The roots were washed well with water to remove the remaining adhering soil particles, blotted dry, weighed, cut into 1 cm segments and mixed thoroughly. Known weights of subsamples were taken for DNA extraction and assessing root colonisation by arbuscular mycorrhizal fungi.

Root fragments for DNA extractions were further cut into segments several mm long at the time of harvesting and stored at -80°C for molecular analysis. The remaining roots were oven-dried at 60°C for 72 h and total root dry weights per pot for each treatment was calculated taking to consideration the weight taken for DNA extraction and root staining.

Root sub-samples used to determine the extent of root colonisation by arbuscular mycorrhizal fungi were cleared in 10% KOH, acidified and stained with Trypan blue (0.05%) (Abbott and Robson 1981). Total root length colonised and percentage of colonisation by AM fungi were assessed using the gridline intercept method under a microscope at 100x magnification (Abbott and Robson 1981).

Basic soil chemical parameters were measured (EC, pH, available soil P, total carbon and total nitrogen, NH4+ and NO3–). The soil EC was measured in water at 1: 5 (w/v) ratios. Soil pH was measured in CaCl2 at 1:5 (w/v) ratios. Available P within the soil was assessed after extraction with 0.5M aqueous NaHCO-3 (pH 8.5) using colorimetrically (Murphy and Riley 1962). Total C and total N in ground soil were assessed using combustion analysis using an Elementar analyser

(vario Macro CNS; Elementar, Germany). Soil NH4+ and NO3– were measured by extracting 20 g with 80 mL 0.5 M K2SO4. All measurements were completed in triplicate.

DNA was extracted from 0.5 g of soil taken form each amendments using the MoBio Powersoil DNA isolation kit (Geneworks, Australia). For the sequencing, PCR was performed on the 16S rRNA genes and sequenced using the Ion Torrent Personal Genome Machine.

Statistics

The relationship between compost input and the relative abundance of soil biota were assessed using one-way analysis of variance (ANOVA) with a significance level of P<0.05. A data matrix of explanatory variables (pH, TN, TC, EC) was prepared and canonical correspondence analysis (CCA) was performed to explore which parameters had the greatest influence on bacterial and archaeal community structure for each compost amendment (Jenkins et al. 2010). Linear regression analysis was also performed to investigate relationships between colonisation of roots by arbuscular mycorrhizal fungi and carbon. All these approaches were executed using Microsoft Excel 2016 Package.

4. Results

FIELD TRIAL

Soil bacterial community composition on the 3 dairy farms: The most abundant bacterial phyla were *Proteobacteria, Actinobacteria, Acidobacteria*, *Firmicutes* and *Bacteroidetes*. The phyla *Chloroflexi, Verrucomicrobia*, *Gemmatimonadates*, *Planctomycetes* and *Chlorobi* were also present but to a lesser extent. Overall, *Proteobacteria* was the most dominant phyla at all the farms (Figure 1).

Relative abundance soil bacteria on the 3 dairy farms: At the phylum resolution level, treatment had little impact on the relative abundance of most bacterial phyla (Figure 1), however, there were some significant differences in between farms. For Farm 1, there was a significant interaction between soil treatment and the *Actinobacteria*, *Firmicutes* and *Bacteriodetes*. Compost applied at 6t/ha increased the relative abundance of *Firmicutes* and *Actinobacteria* compared to the unamended control soil whilst compost at 3t/ha and manure at 2 t/ha increased the relative abundance of *Bacteriodetes* and *Actinobacteria*, respectively. For Farm 2, all soil amendments lead to an increase the relative abundance of *Acidobacteria* compared to the control whilst the addition of compost at 6 t/ha caused a decrease in the relative abundance of *Actinobacteria*. Finally for Farm 3, the relative abundance of *Bacteriodetes* decreased and the *Actinobacteria*. Increased following amendment of compost or manure relative to the control.

Comparison of soil bacterial community composition on the 3 dairy farms: The bacterial community on each of the three dairy forms was distinct based on a canonical analysis of the data (Figure 2). Farm 2 had the least divergent community, and Farms 1 and 3 had more divergent but distinct bacterial communities. The distribution pattern of some bacterial taxa were more strongly linked to bacterial community structure on each farm (Figure 2).

Figure 1. Relative abundance of bacterial sequences at the phylum resolution for soil on three dairy farms amended with compost (3t/ha, 6t/ha) and manure (2t/ha). Bars represent the mean value and error bars are the standard error of the mean (n=3). Field Experiment sampled in September 2016.

Comparison with previous assessment of soil bacterial community composition on the 3 dairy farms: Sampling of soil in 2016 compared with previous samples at the same site demonstrated that the bacterial community is largely influenced by the prevailing environmental condition including soil and climatic factors at each farm rather than individual soil treatments. In the 2015 samples overall, Farm 1 had a higher relative abundance of *Proteobacteria* whilst Farms 2 and 3 had a higher abundance of *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Bacteriodetes*. In contrast, only Farm 3 had a higher abundance of *Actinobacteria* and all farms had a similar abundance of *Proteobacteria* in 2015 (see previous report). Also, there was a marked increase in the relative abundance of *Chloroflexi* in Farm 1 during 2015 but this was not observed in samples taken in 2016.

Figure 2. Soil bacterial community composition for soil on three dairy farms amended in the field experiment with compost (3t/ha, 6t/ha) and manure (2t/ha) on each of three dairy farms. Symbols represent loading scores for experimental units of the total bacterial community for soil; numbers represent bacterial taxa (groups). Each point represents an individual sample (n=3).

Glasshouse experiment (sampled at 11 weeks)

There was relatively little difference in soil C and soil N with application of the two levels of compost in the glasshouse experiment after 11 weeks (Table 1). There were minor effects on plant growth (at 11 weeks) with application of the two levels of compost in the glasshouse experiment (Figure 3). No increased plant growth was recorded with application of compost.

There were inconsistent effects of compost addition on colonisation of ryegrass roots by arbuscular mycorrhizal fungi for plants when grown in soil from each farm in the glasshouse experiment (Table 2).

The estimate of bacterial abundance in the glasshouse experiment using 16S rRNA gene copy number showed inconsistent effects with addition of compost in each soil (Figure 4). The highest bacterial abundance using this estimate in soil from Farm 1 occurred for compost application, but the opposite was observed for soil from Farm 3. There was no effect on bacterial abundance for soil with application of compost to soil from Farm 2. For fungal abundance using 18S rRNA gene copy number, there was no effect of application of either 5 or 10 t/ha compost in any of the three soils (Figure 5).

Table 1. Soil characteristics for Farm 1, Farm 2 and Farm 3 after application of either 5 or 10 t/ha compost or no compost in the glasshouse experiment. Values are means of all three replicates with standard errors (assessed at the 11 week harvest)

Treatment					
Farm 1	pH	EC	TN (%)	TC (%)	Available P (µg/g soil)
10 t/ha	6.37	381.13	0.32	4.42	6.93
	(± 0.05)	(± 51.38)	(± 0.00)	(± 0.10)	(± 4.25)
5 t/ha	6.26	499.67	0.29	4.18	2.68
	(± 0.03)	(± 98.62)	(± 0.00)	(± 0.09)	(± 1.46)
Control	6.12	306.4	0.25	3.63	0.219
	(± 0.06)	(± 47.08)	(± 0.02)	(± 0.25)	(± 0.56)
Treatment					
Farm 2	рH	EC	TN (%)	TC (%)	Available P (µg/g soil)
10 t/ha	5.74	79.27	0.14	1.72	0.90
	(± 0.03)	(± 2.37)	(± 0.00)	(± 0.06)	(± 0.90)
5 t/ha	5.78	68.27	0.13	1.62	11.81
	(± 0.08)	(± 4.45)	(± 0.01)	(± 0.05)	(± 8.38)
Control	5.87	39	0.134	1.66	0
	(± 0.12)	(± 2.35)	(± 0.00)	(± 0.02)	(± 0.42)
Treatment					
Farm 3	рH	EC	TN (%)	TC (%)	Available P (µg/g soil)
10 t/ha	5.76	130.87	0.46	5.20	10.62
	(± 0.20)	(± 7.66)	(± 0.01)	(± 0.16)	(± 2.87)
5 t/ha	5.58	109.87	0.43	4.82	14.37
	(± 0.06)	(± 1.82)	(± 0.01)	(± 0.08)	(± 6.51)
Control	5.41	87.13	0.44	4.97	5.24
	(± 0.07)	(± 2.78)	(± 0.01)	(± 0.14)	(± 3.11)

Figure 3. Shoot dry weight (g per pot) for ryegrass grown in soil amended with either 5 or 10 t/ha compost for each of the three dairy farms in the glasshouse experiment (11 weeks). Bars represent standard errors.

Table 3. Mycorrhizal colonisation (% of root length colonised) for ryegrass grown with each of the compost amendments for each of the three farms in the glasshouse experiment (11 weeks). Values represent mean of all three replicates and standard errors.

Figure 4. Mean abundance of bacteria (16S rRNA gene copy number) for each compost treatment and control for each of the three farms in the glasshouse experiment sampled after 11 weeks of growth of ryegrass. Error bars are standard errors.

Figure 5. Mean abundance of fungi (18S rRNA gene copy number) within each compost treatment and control for each of the three farms sampled after 11 weeks of growth of ryegrass in the glasshouse experiment. Error bars are standard errors.

The bacterial to fungal ratios based on these assessments showed a lower level in soil from Farm 1 and a higher level in soil from Farm 3 with application of either level of compost. There was little effect of application of compost on bacterial to fungal ratio in soil from Farm 2.

Figure 6 The bacteria:fungi ratio (based on 16S rRNA and 18S rRNA gene copy number for bacteria and fungi respectively) following application of compost at either 5 or 10 t/ha in comparison to non compost, for each of the three farms in the glasshouse experiment sampled after 11 weeks of growth of ryegrass.

Relative abundance soil bacteria (Glasshouse Experiment)

As for the field samples, the phylum *Proteobacteria* was dominant in soils from each farm and the relative abundance of the main taxa comprising the *Proteobacteria* was little influenced by compost addition (Figure 7). Although the relative abundance of *Proteobacteria* was unaffected by compost addition at all three farms, there was a marked increase in relative abundance of *Bacteriodetes* observed in soils at all three farms amended with compost at both 5 t/ha and 10 t/ha equivalent (Figure 8).

Figure 7. Relative abundance of each of the major groups within *Proteobacteria* **found within each farm and compost treatments in the glasshouse experiment sampled after 11 weeks of growth of ryegrass. Error bars are standard errors.**

Figure 8. Relative abundance of dominant bacterial groups within different compost amendments applied within each farm in the glasshouse experiment sampled after 11 weeks of growth of ryegrass. Error bars are standard errors

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In contrast to the Proteobacteria, the relative abundance of *Acidobacteria* decreased with compost applications for all farms soils. The relative abundance Cyanobacteria also decreased within compost-amended soils from Farms 1 and 2. There was an increase in the relative abundance of *Actinobacteria* in soil from Farms 1 and 3 but not soil from Farm 2. The relative abundance of both *Firmicutes* and Cyanobacteria increased in the soil at Farm 3 amended with 5 t/ha of compost but not 10 t/ha.

To further explore treatment effects and farm effects on the community composition of soil bacteria, a CCA was performed to determine which environmental variables (pH, EC, TC and TN) best explained changes in bacterial community composition (Figure 9).

While there was no effect of compost on the bacterial community structure, the bacterial community composition within the soil from Farm 1 was strongly influenced by high pH and EC levels and moderate levels of TC and TN. The bacterial community composition at Farm 2 appeared was influenced by low TC and TN and medium to low levels of pH (acidic) and EC, whilst the bacterial community composition at Farm 3 appeared to be influenced by high soil TC and TN and lower soil pH and EC levels (Figure 9).

Figure 9. CCA plot showing the relationship between measured environmental variables (soil pH, EC, TC and TN), bacterial community composition, farm and treatment. Farm 1 is represented by circles, Farm 2 is represented by squares and Farm 3 is represented by triangles. The control treatment is represented by red, and the 5 t/ha and 10 t/ha applications of compost are represented by blue and red respectively (Glasshouse experiment).

5. Discussion

This study demonstrated that the most abundant bacterial phyla present in the dairy soils sampled in this field experiment 3 years after establishment of the treatments of compost and manure application were *Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes* and *Bacteroidetes*. Bacteria in these phyla play a major role in soil processes and services and are commonly reported in agricultural systems across the world (Janssen, 2006; Zeng et al., 2016). The phyla *Chloroflexi, Verrucomicrobia, Gemmatimonadates, Planctomycetes* and *Chlorobi* were also present but to a lesser extent. Overall, Proteobacteria was the most dominant phyla at all the farms. This phylum represent the largest and most metabolically and ecologically diverse phylum which are known to be particularly adept at responding to a variety of C and N compounds entering soils and include metabolic specialists such as nitrogen fixers, nitrifiers, methanotrophs (Fierer et al., 2007). Also, members of this phylum have been identified as plant growth promoting and disease-suppressing bacteria that facilitate nutrient acquisition and provides protection against soil-borne fungal plant pathogens such as *Rhizoctonia* root rot on wheat (Barnett et al., 2017; El-Tarabily and Sivasithamparam, 2006). Therefore, *Proteobacteria* are a key component of healthy soils.

At the phylum resolution level, application of compost and manure had little impact on the relative abundance of most bacterial phyla. However, there were some significant differences in between farms as follows:

- (i) For Farm 1, there was a significant interaction between soil treatment and the *Actinobacteria, Firmicutes* and *Bacteriodetes*. Compost applied at 6t/ha increased the relative abundance of *Firmicutes* and *Actinobacteria* compared to the unamended control soil whilst compost at 3t/ha and manure increased the relative abundance of *Bacteriodetes* and *Actinobacteria*, respectively.
- (ii) For Farm 2, all soil amendments lead to an increase the relative abundance of *Acidobacteria* compared to the control whilst the addition of compost at 6 t/ha caused a decrease in the relative abundance of *Actinobacteria*.
- (iii) For Farm 3 the relative abundance of *Bacteriodetes* decreased and the *Actinobacteria* Increased following amendment of compost or manure relative to the control.

Overall, the bacterial community on each of the three dairy forms was distinct and while application of compost /and or manure influenced the bacterial community to some extent, it is likely that the background farm community structures were greater between farms than the influence of the soil amendments within the farms. This applied to both the field and the glasshouse study.

For fungi, the effects of compost on arbuscular mycorrhial fungi (assessed as the proportion of roots colonised) and total fungal abundance (assessed as 18S rRNA gene copy number) were not predictably influenced by additions of compost (as determined using the glasshouse experiment) for the three soils investigated. Soil factors influencing arbuscular mycorrhizal fungi include the level of available soil P, but there was no relationship between this parameter and the proportion of roots colonised by arbuscular mycorrhizal fungi. Other soil factors or combinations of soil factors may influence fungal abundance, but these effects were apparently less marked for fungi than for bacteria.

DNA sequencing can be used to match DNA extracted from soil samples with sequences known to belong to bacteria and fungi. Soil bacteria, fungi and fauna work together to break down organic matter and release nutrients into soil for use by microbes and plants. However, characterising these communities is complex because they are very diverse. It is not easy to identify bacteria and fungi in soil samples and even within a singly group (phylum) there are species with quite different functions. While the DNA technologies can be used to characterise bacteria and fungi in soil samples, it is difficult to identify organisms to species level. Therefore,

identification at phylum or even family level is only a broad characterisation. Identification of specific organisms (such as species of bacteria and fungi that can cause plant disease) can be done using highly specific DNA primers (sequences of DNA). Molecular diversity indices can be estimated for bacteria and fungi (separately) using DNA extracted from the same soil sample.

6. Conclusion

Although soil bacterial and fungal communities can be influenced by soil amendments such as manure and compost, the magnitude of such changes may not be as great as the differences in the community structure or abundance of bacterial and fungal taxa between soils. The heterogeneity of soil bacterial and fungal communities is extensive and redundancy in function within these communities occurs. Soil microbial communities influence higher-level soil organisms, including soil microfauna and soil mesofauna, with impacts on processes such as nutrient cycling and soil aggregation, However, despite divergences in microbial community structure among soils from farms at different locations, there can be similar outcomes in terms of beneficial soil processes. Therefore, it is likely to be more important to monitor changes within a farm than between farms in order to predict benefits of soil organic amendments such as compost and manure.

7. Acknowledgements

We gratefully acknowledge the Western Dairy collaboration from project SWCC Project IN2.1.002 "Measuring the soil health benefits of applying compost and the financial benefits of both on-farm composting of farm waste resources and purchasing compost for the WA dairy industry." We are appreciative of the collaboration of S. Maughan (Harvey, WA), M. Brett (Dardanup, WA) and S. Scott (Gelorup, WA), the owners of the three dairy farms on which this experiment was established. We are also most appreciative of the contribution of Elana Christou (Masters student, UWA) for her significant contributions to the analysis of the glasshouse experiment.

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9. Appendix 1

Diagram of trial site

