Wildlife poop too: investigating the role of introduced predators and avian species on microbial water quality assessments

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E. coli = faecal indicator bacteria

- Diverse sources of water pollution

But…

   lack of data on wildlife

- Wildlife shown to harbour "cryptic *Escherichia* clades" in their faeces (*e.g. E. marmotae, E. ruysiae*)

- Routine "*E. coli*" monitoring tests cannot distinguish faecal *E. coli* from those "cryptic clades"

Pet waste

Illicit discharges
Storm water runoff

Agricultural runoff

Wild animal waste

Connected waste and
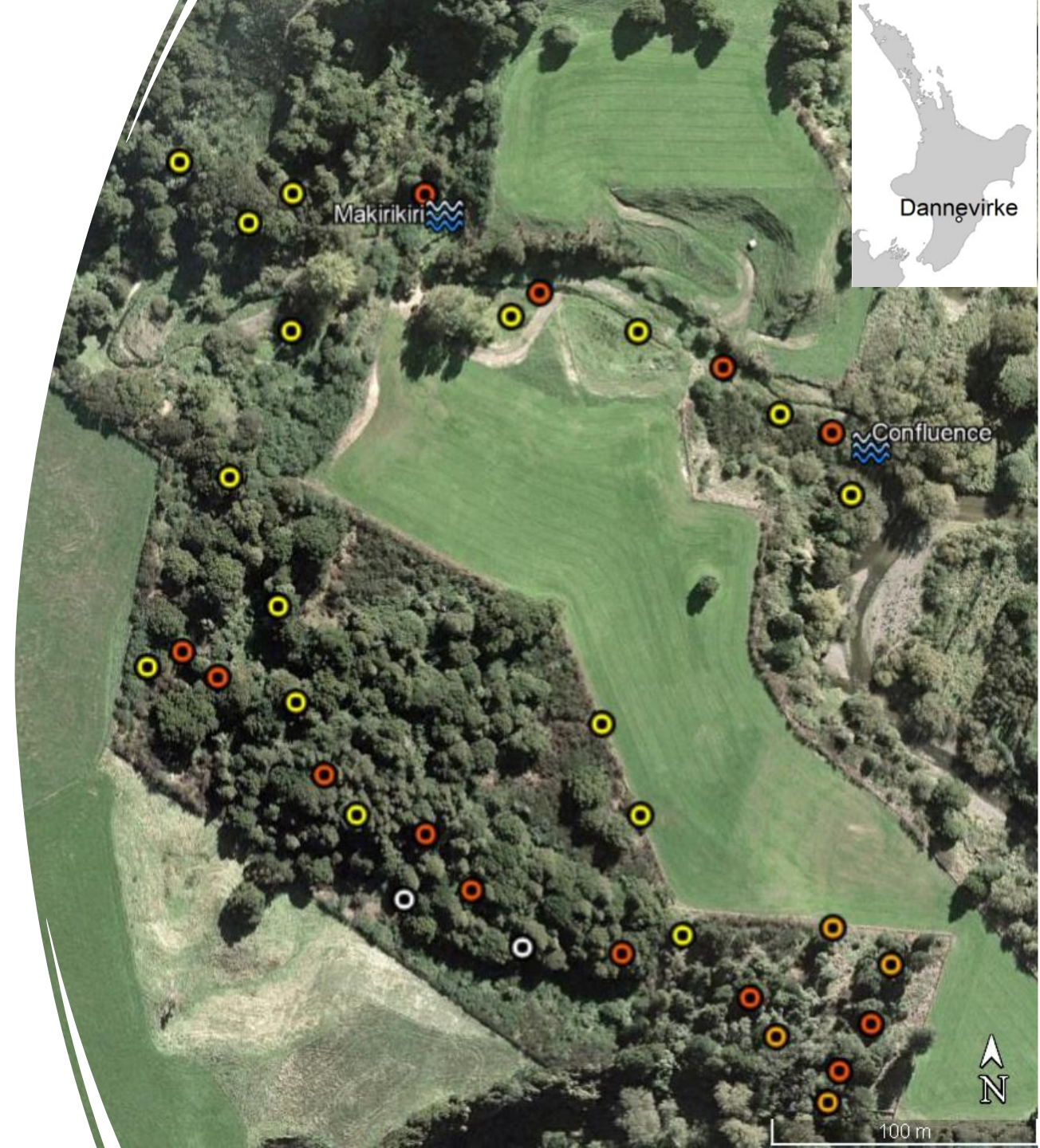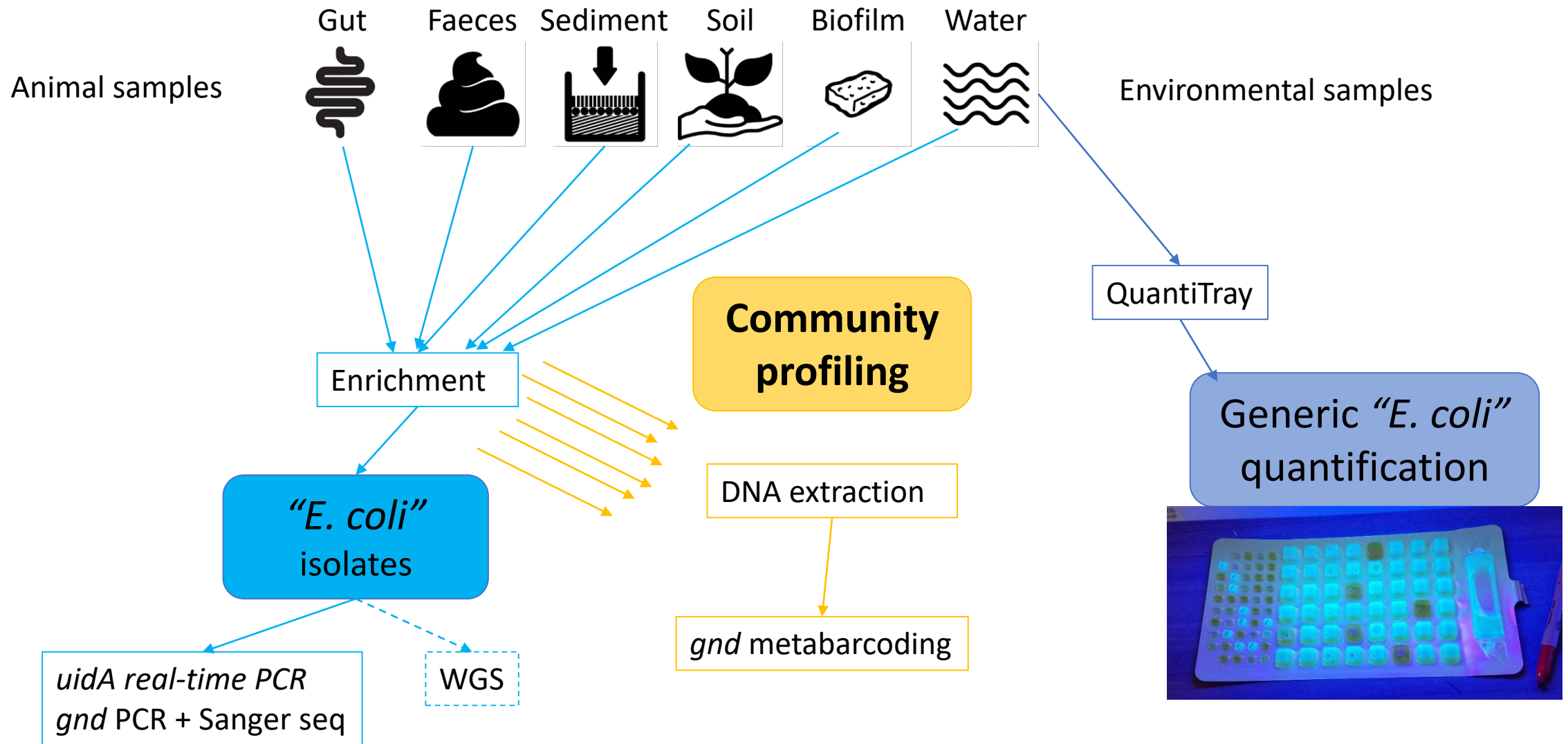storm water systems
Septic systems

Aims

- Co-design and co-innovation of project undertaken with local hapū
- Provide opportunities for local hapū to reconnect with culturally significant Mākirikiri Reserve
- Support hapū with tools to undertake pest management to enhance the mauri of the ngahere during project and after

- Examine faecal material from bird and introduced predator species
  - WGS and phylogenomic analysis of "*E. coli*" isolates
  - "*E. coli*" community profiling (*gnd* metabarcoding)
- Compare with environmental samples

Methods

- Mākirikiri Reserve/Stream, Dannevirke
- Animal trapping (Nov-Dec 2020)
  - ⊙ 13x GoodNature A24
  - ⊙ 4x GoodNature A12
  - ⊙ 16x Timms traps
  - ⊙ 2x Trapinator
- Environmental sampling (4 visits)
  - 〰 Water, soil, sediment, periphyton
    - 2 sites
- Faeces from environment
  - Avian
  - Mammal

Animal samples   Gut   Faeces   Sediment   Soil   Biofilm   Water   Environmental samples

Community profiling

QuantiTray

Enrichment

Generic *"E. coli"* quantification

"E. coli" isolates

DNA extraction

uidA real-time PCR
gnd PCR + Sanger seq

WGS

gnd metabarcoding

OPEN   **Culture independent analysis using *gnd* as a target gene to assess *Escherichia coli* diversity and community structure**

l: 17 November 2016
l: 16 March 2017
d online: 12 April 2017

Adrian L. Cookson[1,2], Patrick J. Biggs[2,3], Jonathan C. Marshall[2,4], Angela Reynolds[1], Rose M. Collis[1], Nigel P. French[2] & Gale Brightwell[1]

gndDb, a Database of Partial *gnd* Sequences To Assist with Analysis of *Escherichia coli* Communities Using High-Throughput Sequencing

Adrian L. Cookson,[a,b] David W. Lacher,[c] Flemming Scheutz,[d] David A. Wilkinson,[b,f] Patrick J. Biggs,[b,e,f] Jonathan C. Marshall,[b,e] Gale Brightwell[a,f]

Results

- 1523.5 corrected trap-nights
    - 49
    - 3
    - 5
    - 1

- 58 Gut samples

    + 16 faecal samples

    (7 mammal/ 9 avian)

- 32 environmental samples

- 420 isolates
    - 207 underwent *gnd* sequence typing (gST)
    - 102 sent for WGS
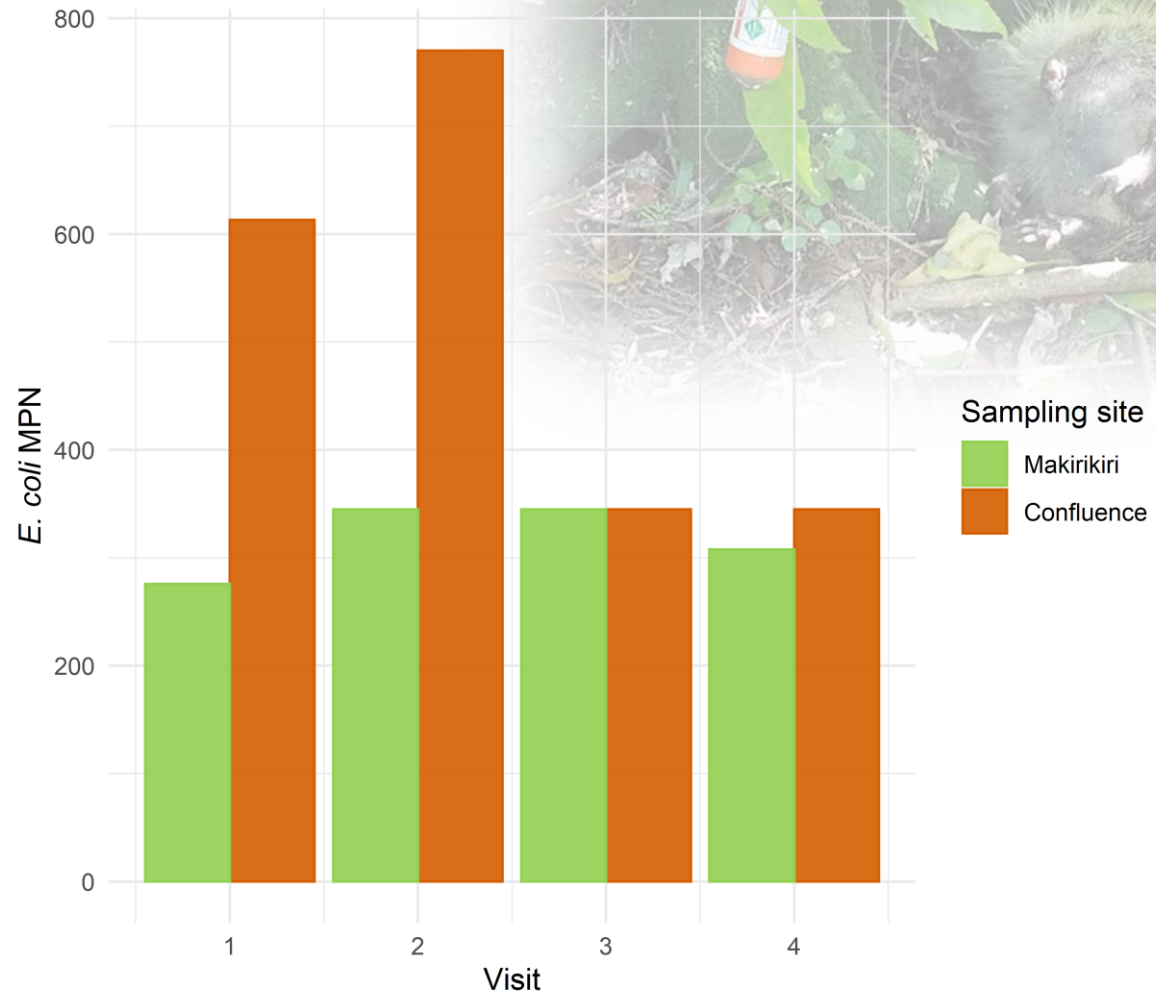
Diversity of isolates

- *uidA* gene not amplified in 32/420 isolates (12 samples)

  4   5   2   1 avian

- Animal (gut + faeces):
  36 gSTs/143 isolates

- Environment:
  41gSTs/64 isolates

- gST258 and gST535 most frequent gSTs (almost ½ of animal samples)

- gST258
  - 1 sediment
  - 3 avian
  - 22   +1

  +1   +1
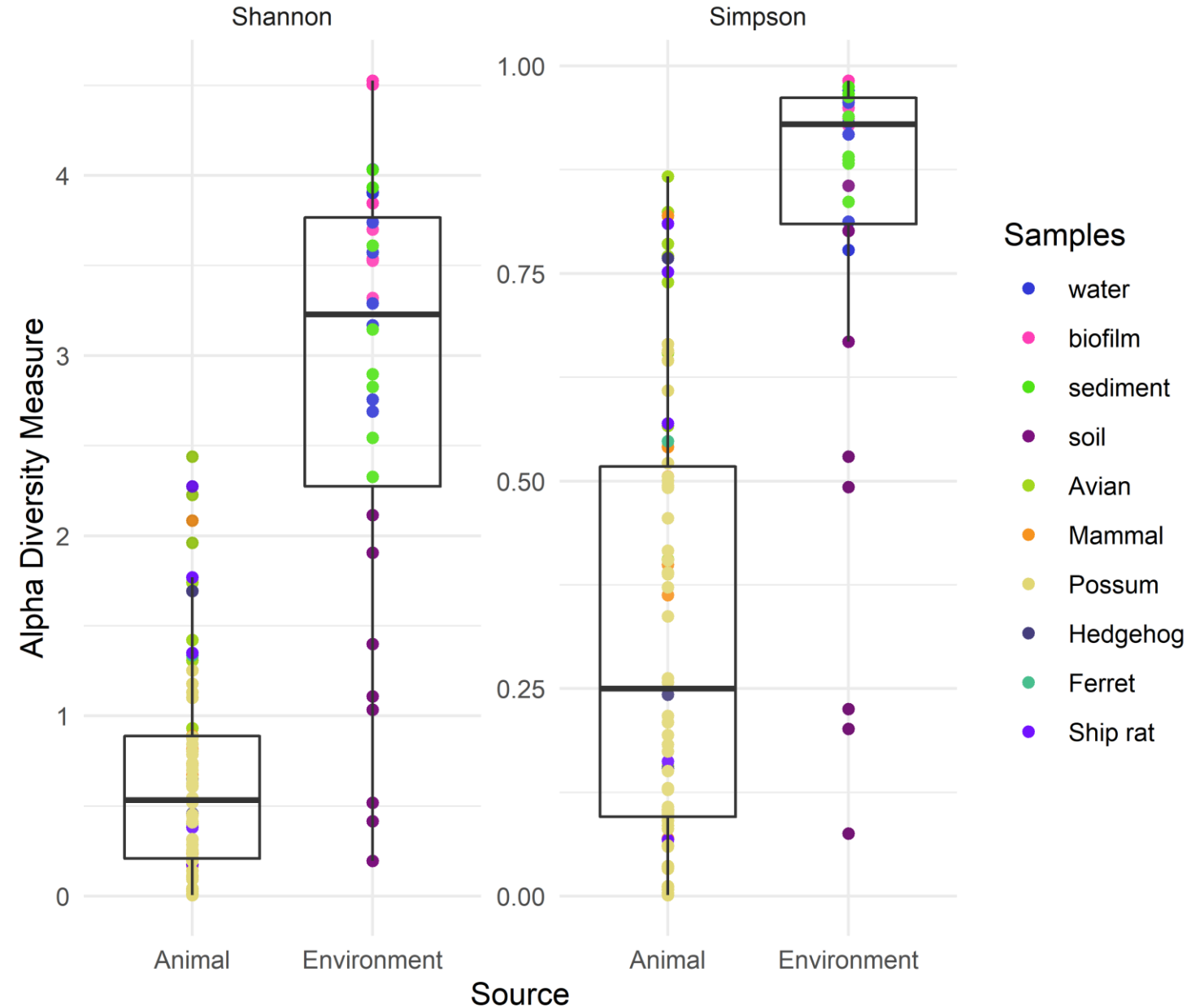
- gST535
  - 2 water
  - 1 soil
  - 3 mammal
  - 17   +1

Community composition (*gnd* metabarcoding)

SAMPLE TYPE		NO.	NUMBER OF gSTs DETECTED	% OF TOTAL	MEAN NUMBER OF gSTs PER SAMPLE +/- SD
Water		8	267	52%	87.5 +/- 14
Sediment		8	207	40%	55.6 +/- 31
Soil		8	84	16%	13.5 +/- 6.5
Biofilm		8	301	58%	131.5 +/- 34.9
Faeces		16	196	38%	16.2 +/- 11.3
	Avian	9	131	25%	21.4 +/- 10.3
	Mammal	7	45	9%	9.4 +/- 9.1
Gut content		58	173	34%	7.1 +/- 8.8

PCA
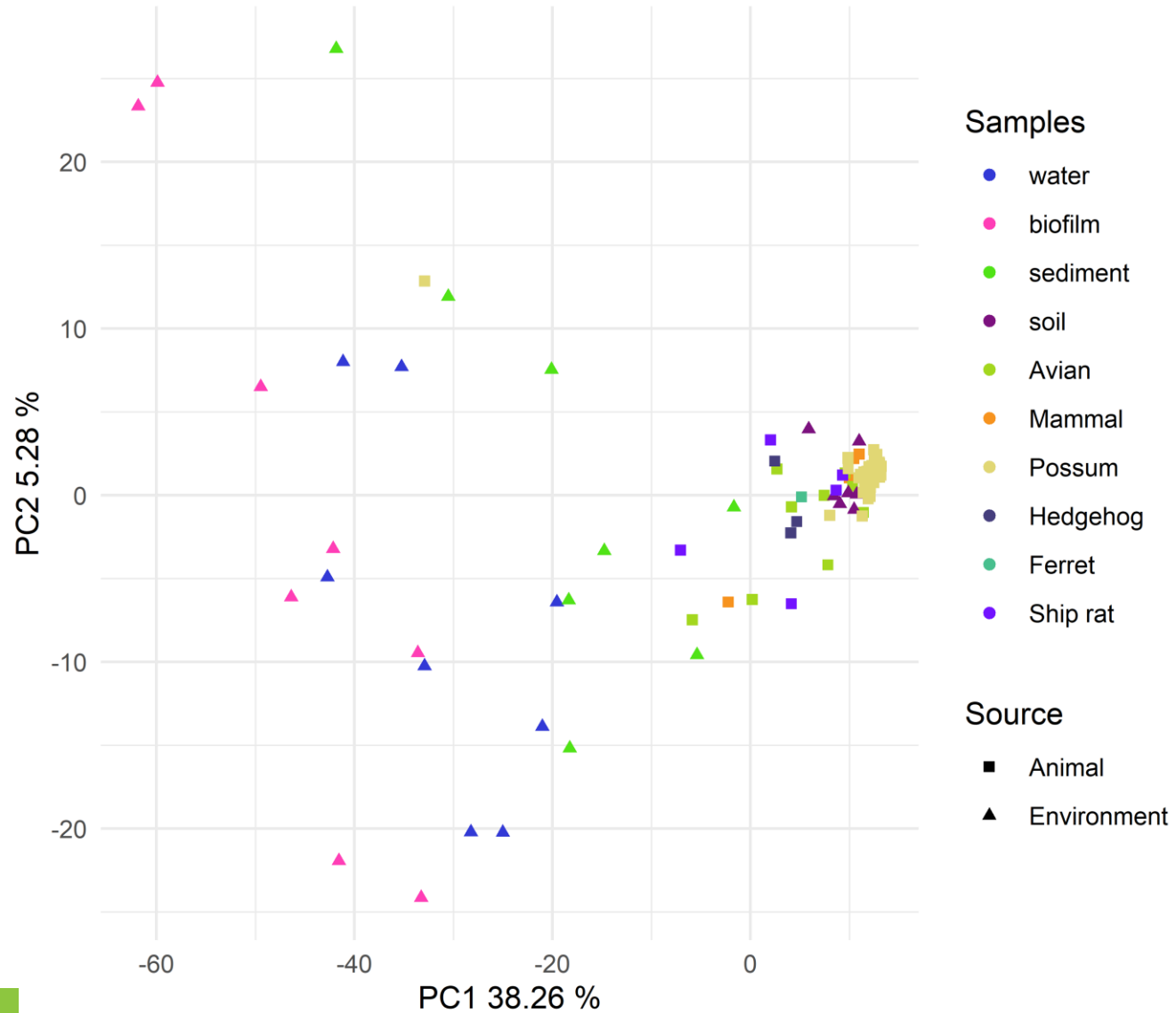
Most frequent gSTs (with ≥10 reads)

Most frequent gSTs	in Animal samples	in Environmental samples	Overall
gST535	62 (84%)	28 (88%)	90 (85%)
gST258	55 (74%)	20 (63%)	75 (71%)
gST522	12 (16%)	26 (81%)	38 (36%)
gST152	11 (15%)	22 (69%)	33 (31%)
gST514	11 (15%)	22 (69%)	33 (31%)
gST308	14 (19%)	17 (53%)	31 (29%)
gST587	13 (18%)	15 (47%)	28 (26%)
gST231	5 (7%)	22 (69%)	27 (25%)

WGS analysis

gST 258

7 SNPs between AGR5279 (avian) and AGR5791

95 SNPs between AGR5585 and AGR5791

38k SNPs between AGR5695 and AGR5424 (avian)

Cryptic clades

gST 535

0-3 SNPs between AGR5247, AGR5371,

Reference from 2018, and AGR5683

3 SNPs between AGR5850 and AGR5595
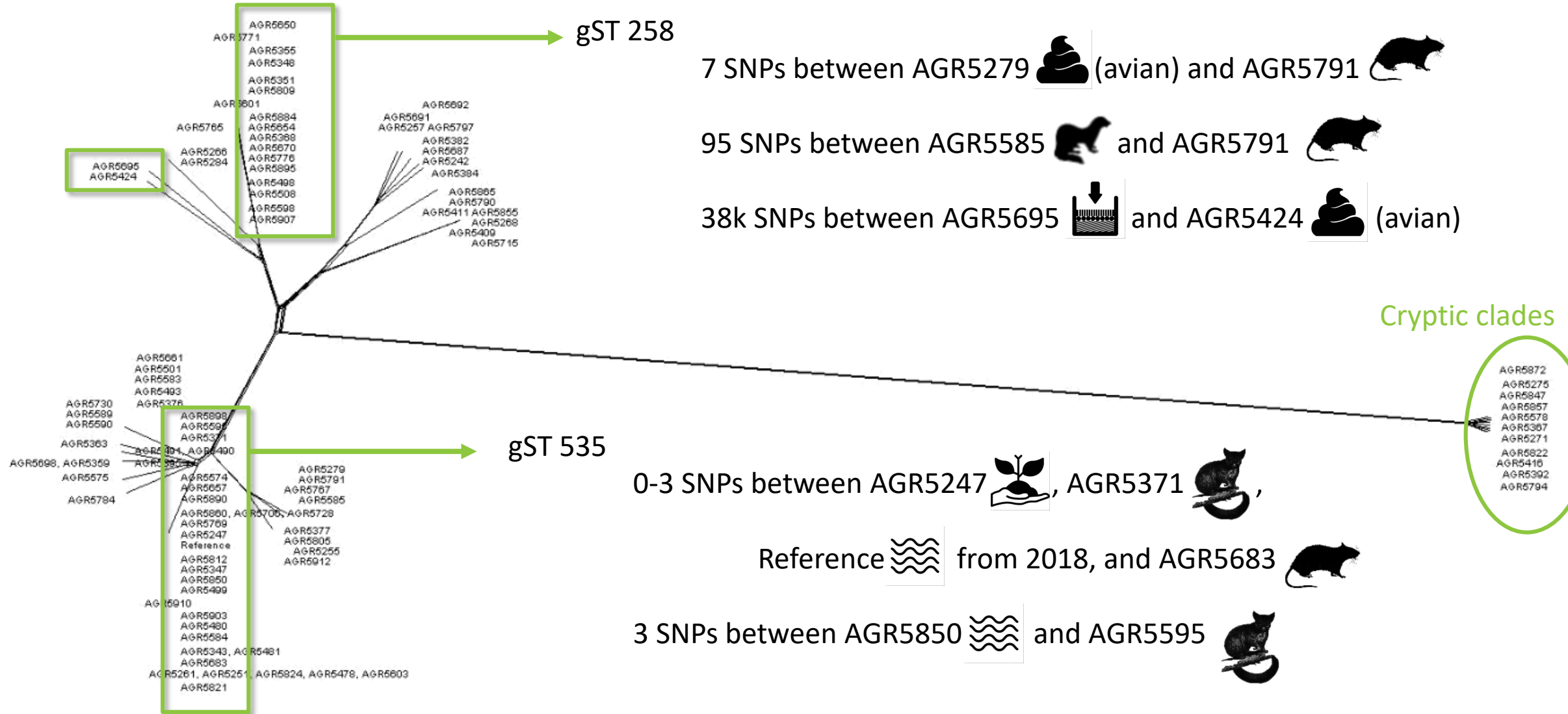
→ **Stable and long-lasting clone in the environment**

Conclusions

- gSTs shared between animal and environmental samples
- Wildlife = source (among others) of environmental contamination
- Cryptic clades found only in low abundance

- Traps were gifted to local hapū at the end of the project to continue trapping

→**Pest management could conceivably improve water quality (as well as improve biodiversity)**

Acknowledgements

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Questions?

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