Technical Note

Soil Test for Earthworms

The presence of earthworms in soil is widely regarded as evidence of a healthy soil (Schon *et al* 2022), as they require favourable soil conditions to thrive, while also imparting direct benefits (aeration and mixing of nutrients). Methods currently used to assess earthworm types and densities are labour intensive and require specialist knowledge. Also, the number of samples being assessed is usually limited, raising issues as to how representative the results are for the whole area.

Hill Labs (HL) has investigated developing a molecular (DNA) test utilising quantitative polymerase chain reaction (qPCR) to identify populations of specific earthworm species (Hsu *et al* 2022). It measures their environmental DNA (eDNA) which are the tiny traces of genetic material left behind as they pass through the soil. This work was undertaken in collaboration with Dr Nicole Schon, an AgResearch scientist whose experience and knowledge of earthworms was an important contribution. The key drivers for undertaking this development were the rapidly growing interest in soil health, the convenience and labour savings compared to the current approach, and that the representativeness of the sample should be greatly improved if the current soil fertility samples (20 plugs along a sampling transect) are used.

What do we know about earthworms?

New Zealand does have native earthworms but the predominant species in our agricultural soils have been introduced during European settlement. As well as numbers of earthworms present, ecological diversity of species is also important as they each perform different functions in the soil. The three main ecological groups of earthworms are:

a) *Epigeic, e.g. Lumbricus rubellus*. These earthworms live near the soil surface and are important for decomposition of organic matter accumulating on the soil surface, e.g. dung patches.
b) *Endogeic, e.g. Aporrectodea caliginosa*. These are earthworms that burrow extensively throughout the topsoil, forming semi-permanent burrows, feeding on the organic matter within the soil and enhancing nutrient availability. This functional group is predominant, usually accounting for 70 – 80% of the total earthworms present.
c) *Anecic, e.g. Aporrectodea longa*. These are larger and form semi-permanent burrows which extend to depth but remain open to the soil surface; these earthworms incorporate organic material deeper into the soil profile and enhance water infiltration via macropores (Shipitalo 2004).

All ecological groups of earthworms benefit pasture production (van Groenigen *et al* 2014), with the greatest benefits realised where all three functional groups are present (Schon *et al* 2021).

The numbers of earthworms present varies during the year due to seasonal fluctuations. When the soil is very dry earthworms move to greater depths and hibernate, and so there is little value testing at that time. Sampling during the wetter months is recommended, i.e. winter and early/mid spring when earthworms are more active. More information is at www.earthworms.nz

How are earthworms currently assessed?

The current approach to assess earthworm populations is a **field visual assessment**, which involves taking a spade square (20cm×20cm×20cm) of soil and carefully removing all earthworms present. These earthworms are sorted into the three main ecological groups and counted. This approach is very time consuming, requires expert knowledge for species identification, and needs a moderate number of samples collected to be representative of the area.

Developing the qPCR test

HL staff recognised the potential to utilise molecular testing to develop a convenient and efficient test for earthworms. An initial literature survey provided useful information but further in-house research was necessary before settling on the most appropriate primers to use. Tests for all three classes of earthworms were developed, but the two less populous species (*L. rubellus* and *A. longa*) suffered from sensitivity limitations. Consequently, the initial routine test offering is for *A. caliginosa* only. This is still very useful as this is the predominant species present, typically 70 -80% of the total earthworm population. Research is ongoing to try to develop a cost effective way to routinely analyse for all three species.

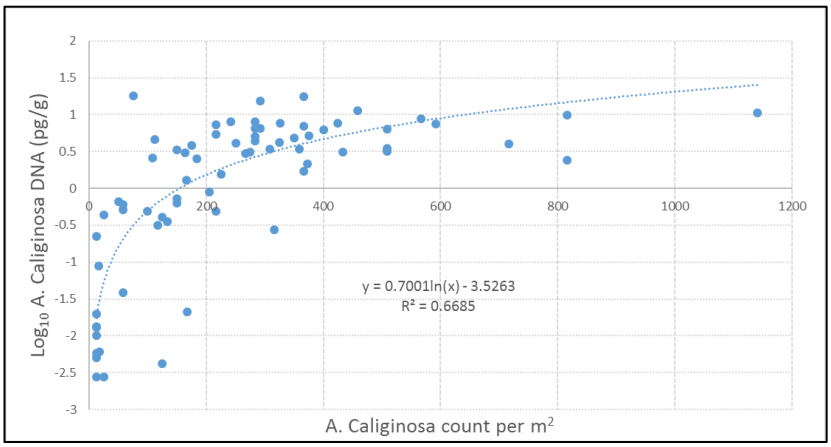
Analytical precision (repeatability) was fully characterised and compared to that observed with the field visual assessment. Both were very similar, with the new test being marginally better (a relative standard deviation of 28%, compared to 30%).

It was also found that in fresh moist soil, the eDNA is prone to degradation, and therefore the sample should be received at the lab as quickly as possible, i.e. ideally within 24 hours of sampling. Sending samples close to a weekend should be avoided.

Interpreting the results

The ideal interpretation approach would be to convert the qPCR result into a number of earthworms per square meter, the way the field visual assessment is reported. Pairs of samples were collected along sampling transects (typically 100 m long). One sample was a standard fertility sample (20 x 7.5 cm cores) and the other was comprised of three spade squares, and the earthworm counts being averaged.

This data showed reasonably good agreement between the two testing approaches (see Fig 1). However, because of the 'noise' associated with both methods, we are reluctant to produce an actual number of earthworms present from the DNA result. While both tests are assessments of the earthworm population, they are different. The new test measures earthworm DNA present in the soil and results are expressed as pg/g of the eDNA sequence for *A.caliginosa*, whereas the field visual assessment is measuring the earthworms in the spade square at that moment. Recognising that earthworms move freely through the soil, it is not surprising if occasionally the agreement is poor. At this point in time, it is not possible to claim if one approach is superior to the other.



Log_{10} (DNA pg/g)	DNA (pg/g)	Count per m^2
-2.3	0.005	6
-0.6	0.25	65
-0.3	0.5	100
0.4	2.75	289
0.7	5	418
1.1	12.5	738
1.3	20	988

Fig 1. Relationship between the eDNA result and the Field Visual Count for *A. caliginosa*.

This initial field calibration was undertaken on pastoral soils only, and it is quite possible that arable or horticultural soils may naturally contain lower earthworm eDNA levels (as the plant and animal residues would encourage earthworm activity.) Also, those samples are taken to a greater depth, at which *A. caliginosa* are less likely to be found.

Conclusions

This Note describes an exciting new test that provides a convenient way to assess earthworm populations. Instead of spending significant time performing field visual assessments across the property, the farmer can now simply request this test be included on the routine soil fertility sample. We believe it will be a powerful way to assess earthworm populations in NZ farms.

This initial study has been completed using pastoral soils (different soil types, predominately dairy but also sheep and beef), but the test should work equally well with horticultural and arable soils. We intend to investigate the interpretation criteria for these other agricultural soils due to deeper sampling depth and the absence of pasture and grazing animal.

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