New test for biological soil health

A quick way to assess the biological health of soil has been developed using molecular techniques to measure earthworm activity, but this needs more work before it's ready for use on New Zealand farms.

Biological test of soil health using molecular techniques

Why: To develop a molecular test to measure the number of earthworms within the soil profile as an indicator of soil health.

Where: On three properties in King Country, Waikato and Canterbury. We thank Ngāi Tahu Farming for allowing the use of their whenua.

Who: Roger Hill (Hill Laboratories), Lisa Hsu (Hill Laboratories), Sara Loeffen (Hill Laboratories), Nicole Schon (AgResearch) and Bob Longhurst (Pastoral Nutrient Management).

What:

- This study has shown that earthworm eDNA in the soil can be measured successfully and, in many cases, correlate well with the earthworm counts by traditional visual assessment.
- The project identified suitable primers for the most common earthworms found within each earthworm ecological group in New Zealand.
- The test was sensitive to changes in earthworm abundance at individual sites. Further data needs to be collected prior to the test becoming commercially available to ensure confidence in the test developed, and the ability to predict earthworm abundance and diversity.

Read more: *Biological test of soil health using molecular techniques*: ourlandandwater.nz/RPF2022

Roger Hill of Hill Laboratories imagines a day when farmers can not only test the fertility of their soil but, using the same samples, also get an assessment of their soil health based on the level of earthworm activity from traces of their DNA found in the soil.

"Currently earthworms are assessed by going out to a field and taking a spade-square down to the depth of the spade, taking that soil, breaking it up and counting the earthworms. We realised that perhaps we could use our capability with DNA testing to develop a faster and more convenient earthworm test," Roger says.

He also says that interest in the health of our soils, beyond the common measures of fertility, is growing both among farmers and consumers.

Soil is one of our most important natural resources and is essential for a range of soil functions and ecosystem services, such as sustainable plant production, nutrient cycling, water purification and regulation, carbon sequestration and greenhouse gas regulation, and the maintenance of soil biodiversity.

Earthworms are a key component of the soil biology and abundant populations are recognised as a sign of a healthy soil. Current methods used to identify earthworm populations are labour-intensive, requiring soil to be physically broken up and earthworms collected.

Roger says that while much is known about how to test the chemical and physical properties of soil, assessing its biological properties is less well developed.

"From what I've read, less than 5% of the organisms that live in the soil have been identified. But when you talk about soil biological health, one of the first things that comes to mind is earthworms, and people associate good earthworm activity with healthy soil," he says.

Roger Hill (Hill Laboratories) collecting worms from a spade-square sample

The research process

This study involved three different species of common New Zealand pasture earthworms – *Lumbricus rubellus* (epigeic), *Aporrectodea caliginosa* (endogeic) and *Aporrectodea longa* (anecic) – being hand sorted from soil samples (20 × 20 × 20 cm) collected from farms in Waikato, King Country and Canterbury.

Hill Laboratories' scientists Lisa Hsu and Sara Loeffen worked with AgResearch scientist Nicole Schon on the project, funded by Our Land and Water via its Rural Professionals Fund.

"There was published information about DNA for those three types of worms, but when we took the literature data and designed a test around those it wasn't completely successful," Roger says.

"So our scientist Dr Hsu got some earthworms from Dr Schon and sent samples away for the DNA to be characterised and we went right back to first principles to develop the tests."

Next was to develop a reliable way to assess actual worm populations from the DNA test results. Spade-square samples were taken, the worms counted and then DNA levels in adjoining core samples measured. The intention was to establish a correlation, and therefore derive a conversion formula that could then convert the DNA result into units that people are used to (i.e. numbers of earthworms per sqm).

Soil samples collected from five sites showed a good correlation between molecular and morphological assessment, good extraction efficiency and precision.

Further samples analysed during the validation stage of the project reduced the strength of correlation between the molecular and morphological assessment of earthworms. Investigation into the factors causing this are ongoing.

"Initially the first few samples looked promising, but then as we added more soils from different areas the correlation weakened significantly, to the point that as we finished the project we thought, 'This isn't good enough'," says Roger.

Next steps

Work is continuing beyond the initial trial to improve that correlation, self-funded by Hill Laboratories and with the support of AgResearch scientists.

"To work out why the correlation is not as good as we would have expected, we need to understand more about the stability of DNA residues in the soil and the

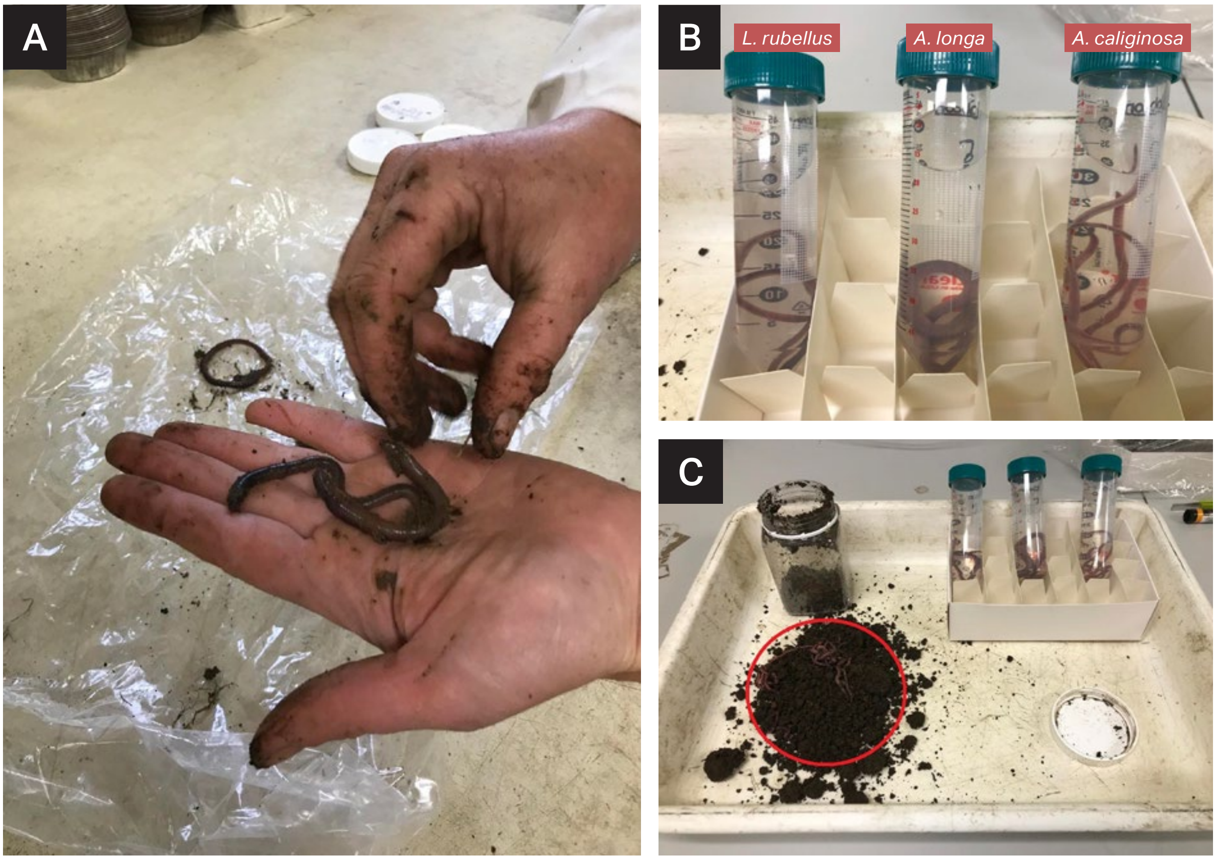

Figure 1: Earthworms were collected and sorted manually into three separate species – *Lumbricus rubellus* (epigeic), *Aporrectodea caliginosa* (endogeic) and *Aporrectodea longa* (anecic) (A & B). Two earthworms of each species were placed in 50 mL falcon tubes for 48h prior to DNA extraction (B). Soil around earthworms (red circle) was collected and the environmental DNA from soil was extracted before PCR and sequencing (C)

behaviour of earthworms," says Roger. "In particular, we need to quantify the uncertainties associated with each of the two approaches (DNA sampling and physical counting)."

Factors now being studied include the stability of DNA in fresh soils, earthworm behaviour after heavy rain compared with when soil is dry, and the accuracy of taking spade-square samples in a paddock for assessing worm populations. Several hundred samples will be taken to better understand those issues.

Roger says good progress was made in the Our Land and Water project, but not yet good enough for a reliable test that can be offered to farmers commercially, as was hoped.

"Because we do many thousands of samples a year for fertility testing, we would like to be able to do this test on the same soil sample as it has already been collected, dried and ground for the other tests. If it's just a tick box to say, 'I'd also like also an earthworm assessment', from a commercial point of view, that's very convenient for everybody."

What the uptake of such a test would be among farmers is difficult to estimate, Roger says, although he expects those interested in the biological health of their soils are likely to be very interested.

"With something like phosphate availability, a soil test may show it's very low, and if you put phosphate on you can expect a significant improvement in production. This new test won't be quite like that, it's more to provide insight about earthworm activity in the soil."

Roger believes that with our overseas clients showing increasing interest in whether their food is produced in a sustainable manner, there could also be marketing advantage for Aotearoa.

"To be able to say to our overseas customers that we are monitoring the soil's biological health with regular testing for earthworm activity, I think it could be useful as a marketing ploy, especially in terms of selling our produce at the top end of the market."

Tony Benny for the Our Land and Water National Science Challenge