Do cov**e**r **c**rops manage free-**l**iv**i**ng **ne**matodes? (DECLINE) - Final Report

Image courtesy of Damian Bienkowski

Introduction

Free-living and plant-parasitic nematodes

Soil-borne pests such as nematodes are major constraints to profitable crop production in the UK. The historical focus for nematology research in the UK is potato cyst nematode (PCN) that represents two species, *Globodera pallida* and *G. rostochiensis*, that significantly impact potato seed and ware production. Both PCN species are so-called endoparasites with a component of their life cycle within their plant host. Consideration of PCN is out of scope of this report.

In contrast, the term free-living nematode (FLN) encompasses numerous nematode species (more than 20,000 species) that complete their whole life cycle in soil or another substrate. Collectively these species form the vast majority of the soil nematode community (Figure 1), representing different trophic groups (bacterivores, fungivores, omnivores, predators and herbivores) and with the exception of herbivores are beneficial to production systems.

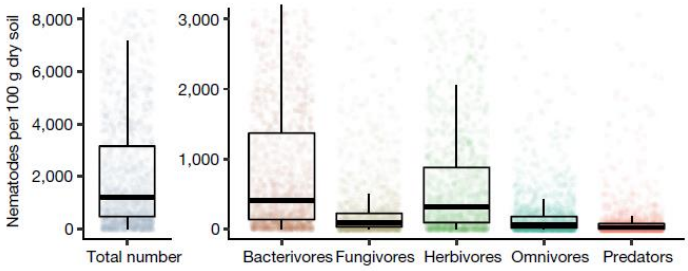


Figure 1. Typical composition of a soil nematode community based on 6759 global nematode datasets (van den Hoogen et al., 2019).

However, while globally FLN represents primarily beneficial nematode species, the acronym FLN has become synonymous in UK agronomy as being associated with nematode species that constrain crop production either by reducing yield or quality or both. Such a loose terminology can cause confusion to non-specialists (and sometime specialists) as FLN in a plant pathology context unlike PCN, does not consistently represent the same nematode species thus crop context is crucially important.

For clarity, in this report, three terms will be used throughout as follows:

PPN: *plant-parasitic nematodes which can feed on plants using a specialised stylet or spear (Yeates et al., 1993).*

FLN: *those nematodes which complete their life cycle in soil (or another substrate) and represent bacterivores, fungivores, omnivores, and predators (Yeates et al., 1993).*

Total nematode community: *all nematodes (FLN & PPN) present in a soil sample*

How did PPN become a problem?

In the UK, arguably the adoption of set-aside in the 1990s provided a stable habitat with a diverse host range for PPN to thrive over a 1,3- or 5-year period (Boag et al., 1998) that resulted in increased PPN abundance. Once land in set-aside was returned to production, the increased PPN abundance coupled with reduced and inappropriate rotations (including susceptible host crops) further exacerbated PPN

numbers. The lack of comparative focus on PPN, compared to PCN, and serendipitous secondary management through treatment of other pests and pathogens (e.g., nematocidal application for PCN) rarely brought PPN into sharp focus unless symptoms were extreme. However, a legislative focus on significant reductions in pesticide use coupled with a societal push for sustainable production practices has reduced the number of available active ingredients to manage PPN and effective replacement management strategies are unclear.

Which PPN are important to Scottish/UK potato production?

Root-lesion nematodes of the genus *Pratylenchus* are migratory endoparasites with a global economic impact on several important crops including reducing yield and quality of potato tubers. Moreover, root-lesion nematodes interact with fungi such as *Verticillium dahliae*, resulting in disease complexes. Orlando et al., (2020) has recently reviewed the status of *Pratylenchus* in the context of potato production.

Apart from the direct feeding/physical damage to the roots of plants including potatoes, a few species of Trichodorid nematodes are vectors of Tobacco rattle virus (TRV), a Tobravirus which causes corky ringspot or ‘spraing’ disease (Dale & Neilson, 2006). TRV can cause several different symptoms in potato plants including necrotic arcing (known as spraing, corky ringspot) in the tuber flesh, and stem-mottle (distortion, stunting and mottling) and aucuba in the foliage. Tubers of spraing susceptible varieties contain corky layers of tissue interspersed with rings of healthy tissue and brown flecks distributed throughout the tuber (Dale & Neilson, 2006).

Ditylenchus dipsaci known as stem and bulb nematode is a pathogen of potato leading to poor plant growth, and tuber quality issues including surface lesions and internal tuber rot. *Longidorus* if sufficiently abundant can occasionally lead to direct damage to potato leading to reduced yields.

Alternative agronomic practice to manage PPN

In stark contrast to Europe and North America, UK agriculture has been slow to consider alternative forms of PPN management. Only in recent years, once it was clear that legislation would continue to focus on reducing active ingredient use has the pace of exploration to find effective alternative practices to manage PPN increased. However, there is a risk that developing a potential single solution for PPN management replicates the previous situation of sole reliance on synthetic active ingredients and is therefore equally unsustainable I n the long-term. Thus, a range of achievable strategies are required to maintain long-term sustainable agricultural production.

In the short-term, the use of cover crops, a collective descriptor that represents several plant species has been viewed as a potential for PPN management. Cover crops are plant species grown between cash crops normally between a winter and a spring crop, providing ground cover during the autumn and winter months and thus, aligning with one of the fundamental principles of regenerative agriculture of minimising bare soil and retaining living roots all year round. Where the production system allows, spring-sown cover crops are also an option, as too is planting ‘catch

crops' which provide short-term cover for approximately 6-10 weeks between cash crops.

Agronomically, the growing of cover crops between two cash crops as part of an annual rotation was originally implemented to both protect soil from erosion and deliver a perceived benefit to soil quality. However, over and above erosion protection there are recognised multiple benefits of growing cover crops including enhancing nutrient and moisture retention, suppressing pests and diseases, and improving soil aggregation (Couëdel et al., 2019). Moreover, additional benefits to soil, which might encourage nematode suppression, include soil structural effects, plant cover effects and organic matter addition, which create microenvironments favourable to antagonistic flora and fauna (Wang et al., 2001). Furthermore, a recent review reported that the use of cover crops can result in a 4% reduction in cereal yields, though this can be both mitigated and transformed into an > 10% overall yield increase through the adoption of mixed cropping (Abdalla et al., 2019).

Cover crops have potential to manage PPN through different mechanisms such as allelochemical production, poor/non-host effects, trap cropping and (partial) biofumigation. Plants from different families produce biocidal secondary metabolites (allelochemicals), which kill PPNs. Several plant families produce a range of allelochemicals that have potential nematocidal properties. Asteraceae produce polythienyls, alkaloids and polyacetylenes; Brassicaceae produce isothiocyanates; Fabaceae and Boraginaceae exude alkaloids; and Poaceae releases glucosides (Thoden et al., 2009); and *Sorghum bicolor* and *S. bicolor* subsp. Sudanense produce a toxic secondary metabolite (Vetter, 2000). Non/poor host cover crops reduce nematode abundance by starvation. Trap crops allow nematodes to feed but are unable to subsequently reproduce, and allelopathic crops produce nematocidal compounds (Ntalli and Caboni, 2017). Some cover crops may suppress nematode populations by multiple mechanisms, independently or simultaneously (Hooks et al*.,* 2010; Grabau et al*.,* 2017). For example, marigolds reduce populations by being poor or non-host plants, through allelochemical production, by trapping nematodes, or by favouring the growth of nematode antagonistic flora and fauna (Pudasaini *et al*., 2008; Wang *et al*., 2001). The mechanisms can occur separately or together, causing enhanced nematode suppression.

Biofumigation refers to the process by which soil-borne pests and pathogens are suppressed through hydrolysis of toxic metabolites called glucosinolates (GSL) produced by Brassicaceous or related plant species once after they are flailed *in-situ* and incorporated into the soil (Kirkegaard and Sarwa, 1998). Upon tissue damage of *Brassica* spp., glucosinolates are hydrolysed by the enzyme myrosinase, and the end products are sulphur-containing biocidal compounds called isothiocyanates (ITCs), nitriles and epithionitriles (Matthiessen and Kirkegaard, 2007). Furthermore, during plant growth, Brassicaceae roots release glucosinolates into the soil in a process known as "partial biofumigation". Both practices, partial biofumigation and biofumigation, can result in a suppressive effect on a range of soil-borne pests and diseases, for example, nematodes (Brennan et al., 2020; Waisen et al., 2020). The mechanisms of action of different ITCs and potential suppressive effects on PPN have recently been considered by Chekanai et al. (2024).

Do cover crops increase rather than suppress nematode abundance?

One consequence of growing cover and companion crops is an increase in soil organic matter through crop residue retention and/or crop incorporation. In addition to GSL release, the plant residues release nutrients, providing substrate for a range of beneficial soil biota such as bacteria, fungi, earthworms, and nematodes (Hao et al., 2023).

In a recent meta-analysis (Puissant et al., 2021) with 414 global observations that included cover crops, representing 31% of all observations in the meta-analysis, reported that cover crops increased the abundance of the total nematode community by 45.3%, and bacterivores by 101.0%. However, and in contrast to some individual studies, cover crops were found to increase the abundance of PPN by 79.6% and had a positive interaction with mean annual precipitation, i.e., the wetter the soil, the greater the PPN abundance. It should be noted that only a few of the 414 observations included in the meta-analysis were from the UK.

Notwithstanding the imperative of identifying an efficacious agronomic practice to manage FLN, field-based research studies in the UK are rare. Holland et al. (2021) reported that seven different cover crop treatments including radish and mustard had no effect on two PPN *Pratylenchus* and *Trichodorus*, thus contrasting with that reported in the global meta-analysis (Puissant et al., 2021) and *in vitro* studies (Chekanai et al., 2024; Mwangi et al., 2024) of which the latter reported PPN suppression. Moreover, emerging anecdotal evidence from practitioners has suggested that outcomes of growing cover crops resulted in a broad spectrum of outcomes including PPN suppression, PPN enhancement and no effect with no clear pattern regarding cover crop, soil type or PPN species. The single constant to emerge was that sampling typically reflected that used for PCN, i.e., a Pi/Pf model of sampling soon after cover crop sowing and then either at pre- or post-incorporation. While this is a proven strategy for PCN, Paterson et al. (2011) demonstrated under controlled conditions that nematodes mineralised nutrients exuded by plants in under 30 days. Thus, there is a potential that a Pi/Pf sampling model is not fit-for-purpose for PPN when exploring the effects of cover crops.

Thus, the overall aim of the project was to determine under Scottish conditions whether cover crops are a viable agronomic strategy to manage PPN and would be delivered by addressing three cognate objectives:

Objective 1 - conduct a multi-year temporal assessment of the impact of cover crop treatments on both PPN and beneficial FLN.

Objective 2 - from a panel of cover crop treatments understand the optimal cover crop treatment for control of PPN.

Objective 3 - understand whether under Scottish conditions, autumn sowing of cover crops is viable.

Methods

Experimental Design

Each experiment was a randomised block design comprising of six cover crop treatments, Defender Oil radish, Bento Oil radish, Vetch/Rye mixture, Viterra Intensiv, *Phacelia* and a Biofumigation mix (Ethiopian mustard, radish and white mustard) and a barley control sown with an Amazone seed drill, with 5 replicate 3m strips of each treatment/control. At the agronomically correct point, the cover crop was terminated by flailing followed by incorporation.

Soil samples were collected prior to sowing of the experiment, thereafter every 2 or 3 weeks until cover crop incorporation with a single sampling post incorporation. A ~1.5 kg composite soil sample was collected from each plot using a grass plot sampler (internal diam. 2.3 cm, Eijkelkamp, Giesbeek, The Netherlands) and by collecting cores at random points along a “W” shape (Marshall et al., 1998). Each composite sample consisted of approximately 24 random cores to a depth of 10 cm. Soil samples were individually bagged, labelled, transported in a cool box to the laboratory and stored at 4 ^{o}C until processing.

From each soil sample, nematodes were extracted from a 200 g subsample of soil (Wiesel et al., 2015) using a modified Baermann funnel extraction method (Brown and Boag, 1988). After *ca.* 48 h, extracted nematodes were collected in 20 ml of water and left to settle for *ca.* 2 hours. Thereafter, the water volume was carefully reduced to approximately 1.5 ml using a modified pipette tip connected to a vacuum pump, and frozen at -20 ^{o}C prior to freeze-drying for 72 h.

Total nematode DNA was extracted using a Purelink 96 Genomic DNA kit (Invitrogen, Paisley, UK). The region of the 18s Ribosomal RNA gene covering the two Variable Regions V7 and V8 were amplified by first-round primers NF1 and 18Sr2v (Treonis et al., 2018) using KAPA taq. This was done in triplicate, the PCR products pooled, and then purified using AMpure XP beads (Beckman Coulter). The cleaned first-round PCR products were then attached to indexes in combinations unique to each sample (Nextera XT Index Kits, Illumina USA) via a further 8 rounds of PCR as per the manufacturer’s instructions. Indexed PCR products were again purified using AMpure XP beads before quantifying to determine their molarity. Equimolar pools of 75 samples were combined for each Miseq run and these libraries were sequenced on the Miseq sequencing platform (Illumina, USA) at the James Hutton Institute in-house sequencing facility.

Bioinformatics

All data was processed using the Qiime2 environment (version 2023.7) (Bolyen *et al* 2019). Raw data were assessed for read count and quality to inform denoising parameters.

The Dada2 plugin was used to denoise all datasets (Callahan *et al* 2016). Specifically, reads were truncate based on average quality dropping below a Phred score of 20 and adaptors were trimmed. Dada2 was used to dereplicate, merged and

removed chimeric reads for all amplicon data sets. The downstream analysis of all data was processed as amplicon sequence variants (ASV).

Taxonomic assignments of ASV's were performed using a Qiime2's Naive Bayes classifier using the Nemataxa database (Baker *et al* 2023). The taxonomic classifier was trained on extracted regions based on primer sequences as this can improve data classification (Werner *et al* 2012). ASV abundance tables were then output into the BIOM format, reformatted into a tab separated file for downstream analysis.

Graphs and visualisations were made using the python libraries seaborn, matplotlib and pandas (Waskom 2021; Barrett et al 2005; McKinney 2011).

Results

Overall, the study comprised of six cover crop experiments, three summer and three winter cover crop experiments each with the same cover crop treatments (and control) and level of replication. Five of the six experiments successfully established and were sampled. The autumn sown cover crop experiments (referred to as winter 1-3) were considered as a risk being highly dependent on harvest date of the previous crop to enable rapid access for sowing coupled with suitable weather conditions to enable growth and establishment. Winter 1 and 2 successfully established and where appropriate were sampled. Unfortunately, due to the poor summer weather conditions, harvest was late and as a result, Winter 3 was sown late and did not establish sufficiently and was not sampled.

Across the different summer and winter cover crop experiments, nine plant-parasitic nematode genera were detected (Table 1).

PPN genus	**Typical UK crop host**
Bitylenchus	Cereals, potato
Ditylenchus	Potato
Globodera	Potato
Helicotylenchus	All crops but only relevant at high numbers
Heterodera	Sugar beet, cereals
Longidorus	Potato, root vegetables, grass
Nanidorus	Potato, root vegetables
Pratylenchus	All UK crop rotation
Trichodorus	Potato, root vegetables

Table 1. ASVs identified as the following PPN genera with their associated typical host. Genera in bold are those that include potato as a major host.

Relative PPN abundance during cover crop growing season

Under all cover crop treatments including the barley control, *Bitylenchus* increased from a low initial relative abundance and peaked approximately during the mid-point of the growing season (Figure 2). The Vetch/Rye mix was comparable to the barley control regarding *Bitylenchus* relative abundance. However, all other cover crops

increased *Bitylenchus* relative abundance in the region of 150-170% (Figure 2). By the time the cover crop was terminated and then incorporated, *Bitylenchus* relative abundance returned close to the Barley control. Under all cover crop treatments and the barley control, the relative abundance of *Trichodorus* reduced early in the growing season, especially so for Vetch/Rye and the Biofumigation Mix followed by a short-term increase in relative abundance prior to cover crop termination but the increase in all treatments mirrored that of the barley control (Figure 2) and returned to low relative abundance post termination/incorporation. *Ditylenchus* was mostly at very low relative abundance throughout the summer growing season with the exception of clear short-term peaks of increased relative abundance at mid-season under Defender Oil Radish (~350%), Vetch/Rye (~150%) and Viterra Intensiv (~400%) compared to the Barley control (Figure 2). In contrast, the relative abundance of *Ditylenchus* under Bento Radish, Bio Fumigation Mix and Phacelia did not change compared to the Barley control. The only other PPN to show any clear change in relative abundance was Helicotylenchus which under all cover crop treatments and the barley control significantly reduced in relative abundance soon after sowing and remained low.

In winter conditions, all PPN with the exception of *Bitylenchus* and *Helicotylenchus* remained low and constant throughout (Figure 3). In general, the relative abundance of both *Bitylenchus* and *Helicotylenchus* increased in relative abundance for all cover crops but reflected the pattern observed under the Barley control, though arguably the duration of the peaks was marginally longer than the control (Figure 3). The one exception is a short-term sharp increase in relative abundance of *Bitylenchus* under Viterra intensiv (Figure 3).

Alpha diversity of the total nematode community

Alpha diversity measures the diversity of species within a specific area which in this study is field scale. Overall, the three summer cover crop trials, alpha diversity of the total nematode community was highly variable with few differences from the barley control. During summer 1, alpha diversity remained constant until cover crop termination where the alpha diversity under Defender and Bento Oil Radish, and Phacelia decreased compared to the Barley control and remained lower through to post incorporation (Figure 4). During summer 2, alpha diversity remained similar throughout again until cover crop termination where all cover crop treatments with the exception of Vetch/Rye increased in diversity compared to the Barley control (Figure 4). In general, the alpha diversity of the total nematode community under summer 3 followed the same trend throughout the growing season (Figure 4).

There is no clear pattern to alpha diversity of either winter 1 or 2 (Figure 5). However, around late October of winter 2, perhaps around the time of the first air frost, a clear reduction in alpha diversity occurred for all treatments and the Barley control.

Summer

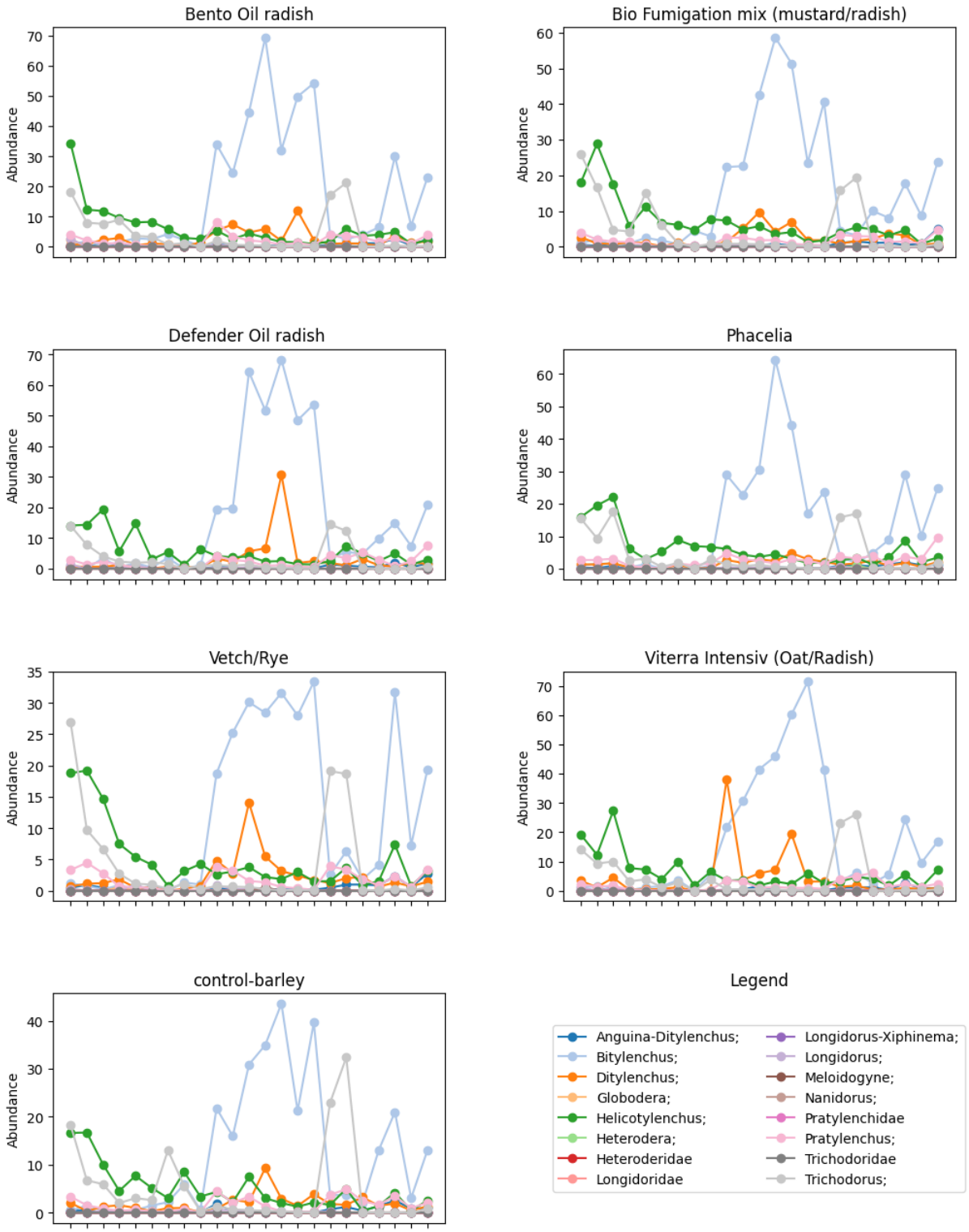


Figure 2. Relative abundance of plant-parasitic nematodes with time, all summer experiments combined. Colours are representative of different PPN as explained in the accompanying key.

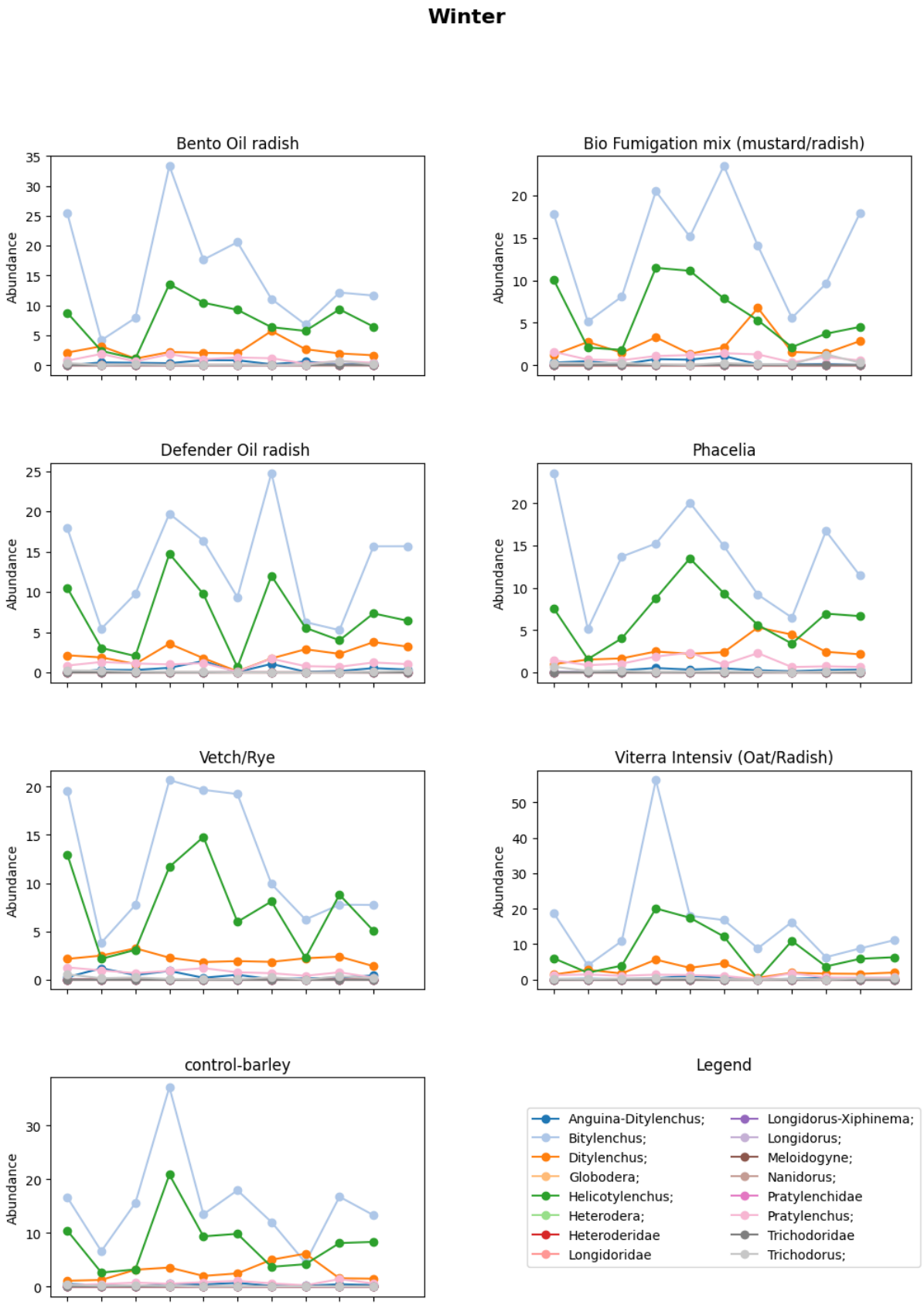
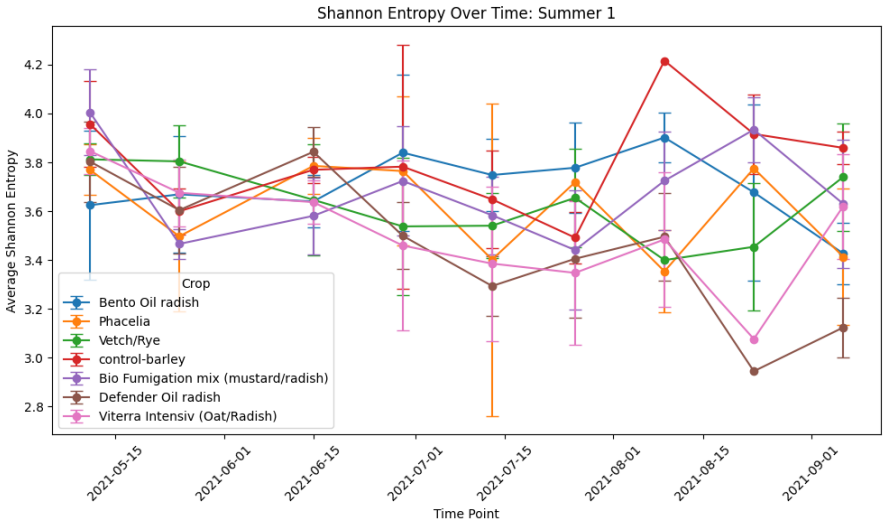
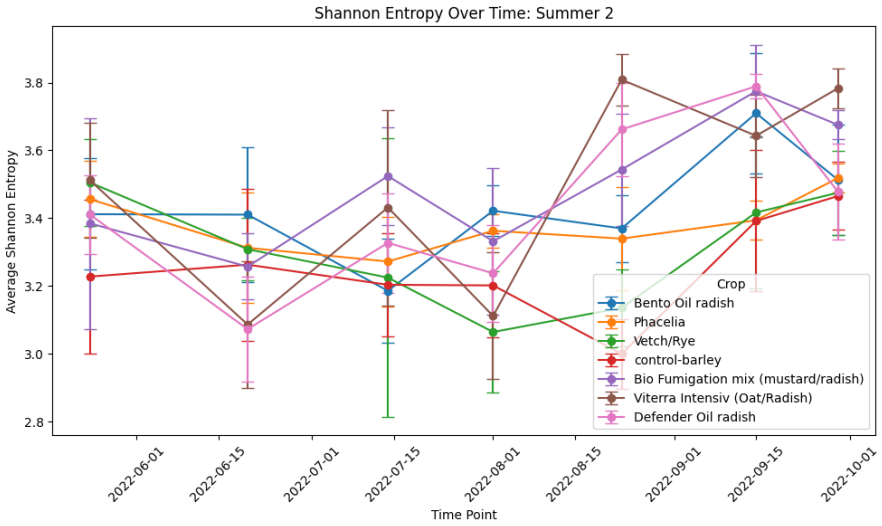


Figure 3. Relative abundance of plant-parasitic nematodes with time, all winter experiments combined. Colours are representative of different PPN as explained in the accompanying key.





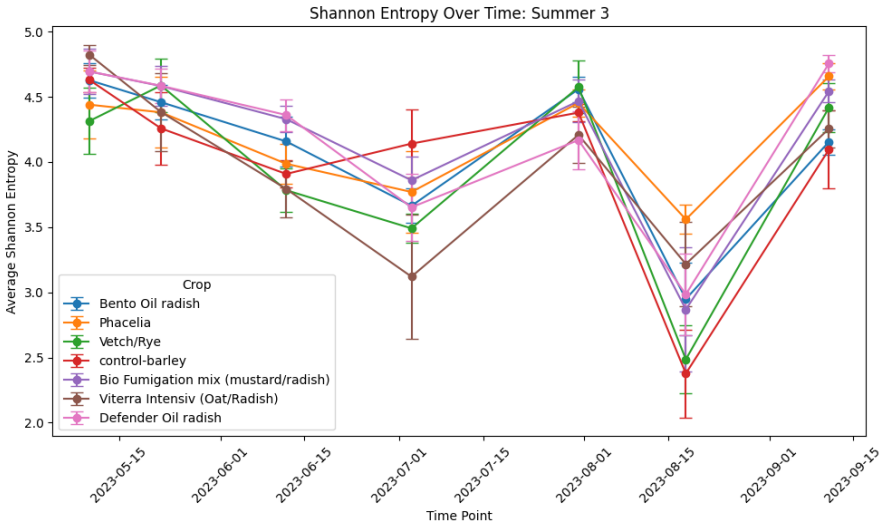
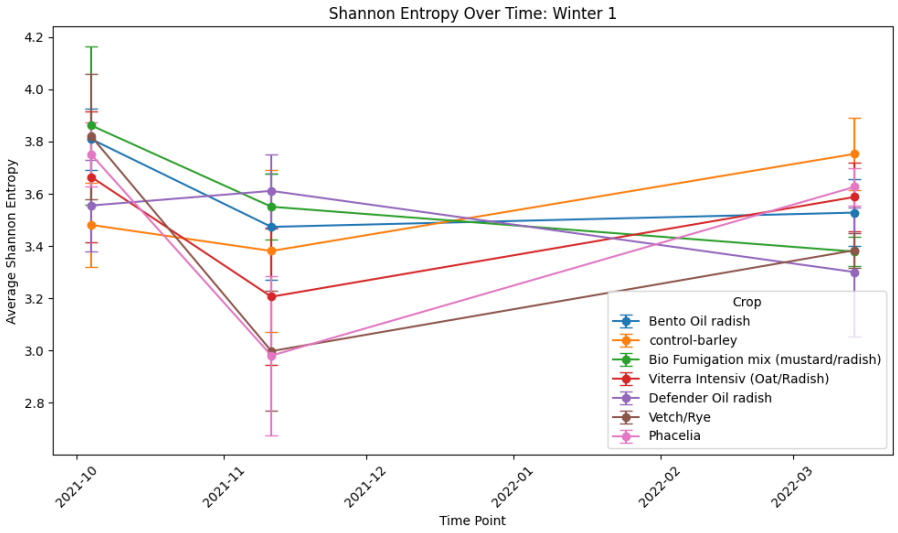


Figure 4. Alpha diversity of the total nematode community for each summer cover crop experiment by time. Colour is indicative of cover crop treatment/barley control as explained in the accompanying key.



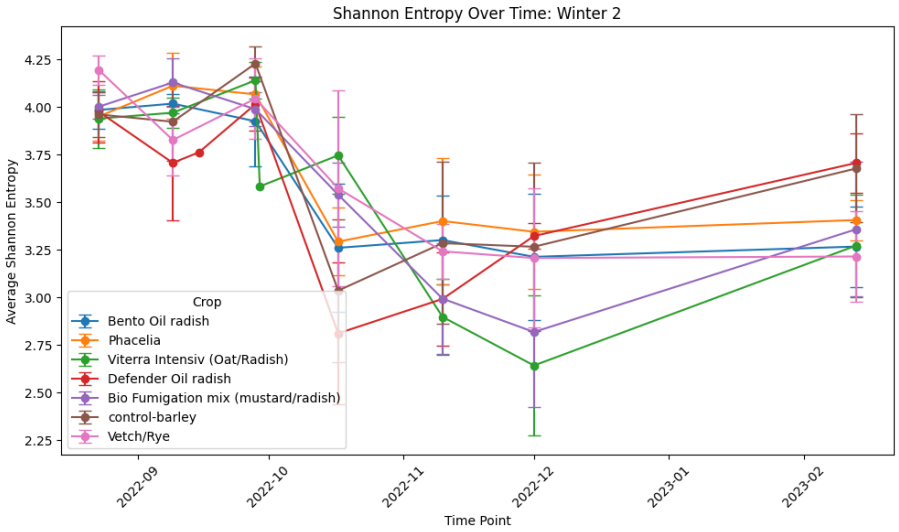


Figure 5. Alpha diversity of the total nematode community for each winter cover crop experiment by time. Colour is indicative of cover crop treatment/barley control as explained in the accompanying key.

Beta diversity of the total nematode community

Beta diversity is a measure of how the composition of species, i.e., the total nematode community, varies across different areas, which in this study are the different fields. Under summer cover crop trials, not only is there a clear separation between the three summer trials, but all three trials appear to split into two discrete clusters (Figure 6). While perhaps a similar pattern also exists for winter, any potential effect is considerably more discrete (Figure 6).

To try and better understand the drivers of the clustering pattern, a deep statistical dive of the data is required which given the time required to conduct such a comprehensive study is out of scope of this study. However, a starting point has been to visualise the similarity between samples based on presence/absence data which has potential to tease out whether certain nematode genera drive clustering by cover crop (Figure 7). While some patterns can be seen, there is no consistent pattern (Figure 7).

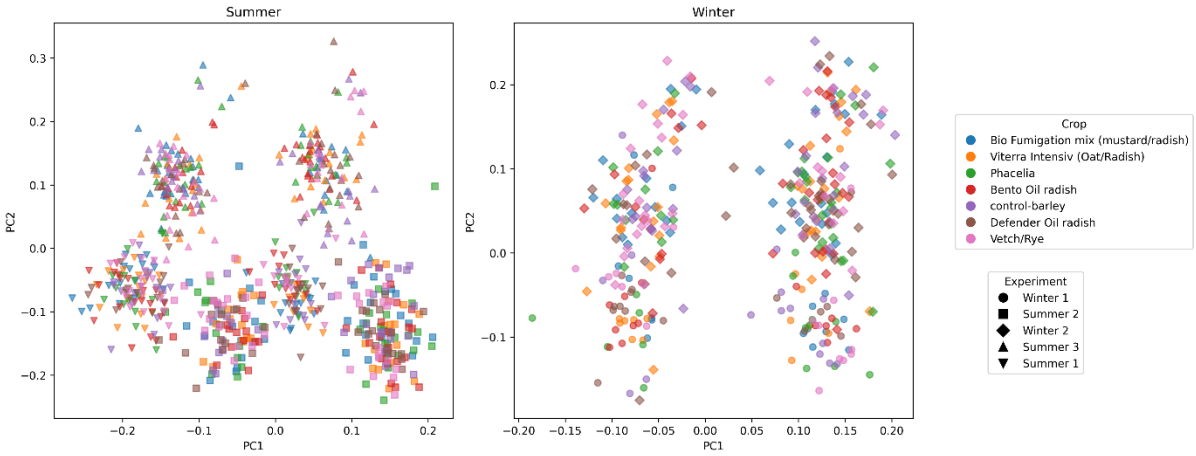


Figure 6. Principal coordinate analysis showing beta diversity of the total nematode community for summer and winter cover crop experiments, each respectively combined. Shapes and colours of symbols are explained in the accompanying key.

Discussion

Do cover crops impact both pathogenic and beneficial FLN? (Objective 1)

In short, yes and no. With a couple of exceptions there appears to be minimal impact of different cover crop treatments, on total nematode communities (PPN & FLN) in terms of alpha diversity regardless of whether the cover crop was spring, or autumn sown, i.e., a summer or winter cover crop. There are perhaps hints of some minor

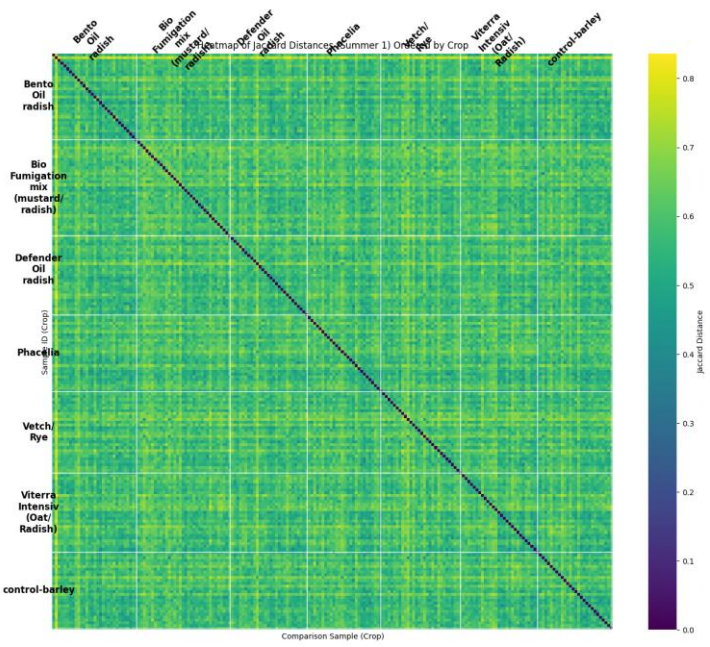


Figure 7. A heatmap of pairwise Jaccard distances between all samples from cover crop trial, summer 1. 0 = both samples share exact the same species; 1 = both samples have no species in common. Thus, a bluer hue = more similar; a more yellow hue = more different. The diagonal blue line represents is where a sample is being compared to itself.

change in alpha diversity at point of cover crop termination and incorporation, but such a change may be due to the disturbance of the soil rather than any direct impact of the individual cover crops.

In terms of beta diversity of the total nematode communities, there is a clear impact of cover crops in the summer trials, i.e., those that are spring sown. This is demonstrated that consistently for each of the three years, the community breaks into paired clusters or groupings. For the two winter trials (autumn sown), although not clear there is also a hint of the total nematode communities separating into different clusters.

We have focussed our effort on trying to understand the driver of this clustering pattern in the summer cover crop trials. We returned to the raw data and reanalysed the data to ensure that the clustering was not a result of a methodological artifact. For example, do the samples cluster based on different DNA sequencing runs? Thankfully, this was not the case primarily because best practice was followed where we archive samples collected throughout the year and then do a single sequencing run where we randomise samples on each sequencing plate, and in doing so mitigates any potential sequencing run/plate effect. We then reanalysed the data at the resolution of month to explore whether there was a fine scale temporal driver that was generating the paired clustering for each year. The results of this analysis demonstrated that at a monthly level, there was no obvious driver (data not shown). With potential methodological artifacts discounted as a driver of the clustering pattern, it is clear that a deep statistical dive is required to determine the driver or drivers of the clustering pattern. Although out of scope of this project, such an analysis has been initiated (see Figure 7). It is evident that complex interactions exist between cover crops and nematodes.

Is there an optimal cover crop for management of PPN? (Objective 2)

Based on PPN data under the studied cover crops it is not obvious that there is a *de facto* optimal cover crop for the management of PPN. A concern of using the common Pf/Pi sampling approach for PPN which may mask within growing season increases in PPN was to an extent upheld with the relative abundances of *Bitylenchus*, *Trichodorus* and *Ditylenchus* increasing by 150-400% depending upon cover crop compared to a barley control. The within growing season increase in relative abundance is important as both *Trichodorus* and *Ditylenchus* are important pathogens of potato. Some species of *Trichodorus* can vector Tobacco Rattle Virus which impacts tuber quality (Taylor & Brown, 1997), and a sufficiently high *Trichodorus* abundance can impact yield this is especially true given that the point of increase within the growing season aligns with tuber formation. Some species of *Ditylenchus*, although relatively uncommon, are associated with potato rot (Abrantes et al. 2023). Like *Trichodorus*, the timing of the increase in relative abundance is arguably optimal for impact on potato. *Bitylenchus* is relatively common in the UK and is a significant PPN of cereals, however, it is known to be a PPN of potato but only when present in very high abundance. Whether the increase in *Bitylenchus* relative abundance across all studied cover crops, except the Vetch/Rye mix, would be sufficient to have an impact on tuber yield is unknown. However, a precautionary

principle should be observed, if PPN testing highlights the presence of *Bitylenchus* in a field used in a rotation which includes potato and a cover crop.

For all three nematodes, by the time of cover crop incorporation and termination, relative abundance of these nematodes had returned to control levels. Unknown is whether this reduction in relative abundance is a direct result of soil perturbation as part of the incorporation process. A future research consideration would be to evaluate whether incorporation is an important component of PPN management.

Is autumn sowing of cover crops viable in Scotland? (Objective 3)

In short, potentially but it is highly dependent on weather conditions both during late summer and winter. We planned three winter cover crop trial, but the third trial had to be abandoned due to poor weather conditions during late summer which resulted in a delayed harvest and prevented access for a sufficiently early cover crop sowing date. As a result, the trial did not establish well.

While we sampled two winter cover crop trials it should be noted that due to a reasonably cold winter period, winter 1 had restricted sampling as once the cover crops had established, the cold weather restricted crop growth during winter months. In contrast, winter 2 benefitted from favourable weather conditions at harvest, allowing an early sowing date leading to early establishment and more or less continued growth during a predominantly mild winter period.

Thus, one of the three autumn sowing cover crop trials was viable and one other marginal.

Acknowledgements

The following are thanked for their input during the project. Alan Johnson (Kings Seeds) for the provision of seed. Andrew Christie and colleagues from the Hutton farm staff who prepared, maintained and incorporated the experimental trials. David Roberts and Susan Mitchell who collected and processed all samples. Pete Hedley and Jenny Morris from the Hutton sequencing unit. Victoria Buswell who led the bioinformatics analyses.

References

Abrantes, I., Teresa Almeida, M., Luci Conceição, I., Esteves, I., & Maleita, C. (2023). Nematodes of potato and their management, Editor(s): Mehmet Emin Çalişkan, Allah Bakhsh, Khawar Jabran, Potato Production Worldwide, Academic Press, pp. 213-240.

Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R. M., & Smith, P. (2019). A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. Global Change Biology, 25, 2530–2543.

Baker, H.V., Ibarra Caballero, J.R., Gleason, C., Jahn, C.E., Hesse, C.N., Stewart, J.E. & Zasada, I.A. (2023). NemaTaxa: A new taxonomic database for analysis of nematode community data. Phytobiomes Journal, 7, 385-391.

Barrett, P., Hunter, J., Miller, J.T., Hsu, J.C. & Greenfield, P. (2005). December. matplotlib--A Portable Python Plotting Package. In *Astronomical data analysis software and systems XIV*, 347, 1-91.

Boag, B., Hebden, P.M., Neilson, R. and Rodger, S.J. (1998). Observations on the effect of different management regimes of set-aside land on nematode community structure. Applied Soil Ecology, 9, 339-343.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37, 852-857.

Brennan, R.J.B., Glaze-Corcoran, S., Wick, R., & Hashemi, M. (2020). Biofumigation: an alternative strategy for the control of plant parasitic nematodes. Journal of Integrative Agriculture, 19, 1680-1690.

Brown, D.J.F., & Boag, B. (1988). An examination of methods used to extract virus-vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. Nematologia Mediterranea, 16, 93-99.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13, 581-583.

Chekanai, V., Neilson, R., Roberts, D., Edwards, S., and Back, M. (2024). *In vitro* nematicidal efficacy of brassica-derived isothiocyanates against root lesion nematodes (*Pratylenchus penetrans*). Nematology, in press.

Couëdel, A., Kirkegaard, J., Alletto, L., & Justes, É. (2019). Crucifer-legume cover crop mixtures for biocontrol: Toward a new multi-service paradigm. Advances in Agronomy, 157, 55–139.

Dale, M.F.B. & Neilson, R. (2006). Research review: free living nematodes and spraing. British Potato Council Research Review, pp. 1-62.

Grabau, Z.J., Maung, Z.T.Z., Noyes, D.C., Baas, D.G., Werling, B.P., Brainard, D.C., & Melakeberhan, H. (2017). Effects of cover crops on *Pratylenchus penetrans*

and the nematode community in carrot production. Journal of Nematology, 49, 114-123.

Hao, X., Najm, M.A., Steenwerth, K.L., Nocco, M.A., Basset, C., Daccache, A. (2023). Are there universal soil responses to cover cropping? A systematic review. Science of The Total Environment, 861, 160600.

Holland, J., Brown, J.L., MacKenzie, K., Neilson, R., Piras, S., & McKenzie, B.M. (2021). Over winter cover crops provide yield benefits for spring barley and maintain soil health in northern Europe. European Journal of Agronomy, 130, 126363.

Hooks, C.R.R., Wang, K.H., Ploeg, A., & McSorley, R. (2010). Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant-parasitic nematodes. Applied Soil Ecology, 46, 307–320

Kirkegaard, J.A., & Matthiessen, J.N. (2004). Developing and refining the biofumigation concept. Agroindustria, 3, 233–239.

Kirkegaard, J.A., & Sarwar, M. (1998). Biofumigation potential of brassicas. Plant and Soil, 201, 71–89.

Marshall, B., Boag, B., McNicol, J.W. & Neilson, R. (1998). A comparison of the spatial distribution of three plant-parasitic nematode species at three different scales. Nematologica, 44, 303-320.

Matthiessen, J.N., & Kirkegaard, J.A. (2006). Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Critical Reviews in Plant Sciences, 25, 235-265.

McKinney, W. (2011). pandas: a foundational Python library for data analysis and statistics. Python for High Performance and Scientific Computing, 14, 1-9.

Mwangi, N.G., Stevens, M., Wright, A.J.D., Edwards, S.G., Hare, M.C. and Back, M.A. (2024) Sensitivity of stubby root nematodes (*Trichodorus* and *Paratrichodorus spp*.) to isothiocyanates associated with Brassicaceae in an in vitro assay. Nematology, 26, 203-210.

Ntalli, N., & Caboni, P. (2017). A review of isothiocyanates biofumigation activity on plant parasitic nematodes. Phytochemistry Reviews, 16, 827–834.

Orlando, V., Grove, I.G., Edwards, S.G., Prior, T., Roberts, D.M., **Neilson, R.** and Back, M. (2020). Root lesion nematodes of potato: current status of diagnostics, pathogenicity and management. Plant Pathology, 69, 405-417.

Paterson, E., Neilson, R., Midwood, A.J., Osborne, S.M., Sim, A., Thornton, B. and Millard, P. (2011). Altered food web structure and C-flux pathways associated with mineralisation of organic amendments to agricultural soil. Applied Soil Ecology, 48, 107-116.

Pudasaini, M.P., Viaene, N., & Moens, M. (2008). Hatching of the root-lesion nematode, *Pratylenchus penetrans*, under the influence of temperature and host. Nematology, 10, 47–54.

Puissant, J., Villenave, C., Chauvin, C., Plassard, C., Blanchart, E., & Trap, J. (2021). Quantification of the global impact of agricultural practices on soil nematodes: A meta-analysis. Soil Biology & Biochemistry, 161, 108383.

Qiime2 documentation (2023) docs.qiime2.org. Training feature classifiers with q2-feature-classifier — QIIME 2 2023.9.2 documentation. [online] Available at: https://docs.qiime2.org/2023.9/tutorials/feature-classifier/ [Accessed 15 Dec. 2023].

Taylor, C.E., & Brown, D.J.F. (1997). Nematode vectors of plant viruses. Wallingford, Oxon, UK: CABI Publishing.

Thoden, T.C., Boppré, M., & Hallmann, J. (2009). Effects of pyrrolizidine alkaloids on the performance of plant-parasitic and free-living nematodes. Pest Management Science, 65, 823–830.

Treonis, A.M., Unangst, S.K., Kepler, R.M., Buyer, J.S., Cavigelli, M.A., Mirksy, S.B., & Maul, J.E. (2018). Characterization of soil nematode communities in three cropping systems through morphological and DNA metabarcoding approaches. Scientific Reports **8**, 2004.

van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., et al., (2019). Soil nematode abundance and functional group composition at a global scale. Nature, 572, 194-198.

Vetter, J. (2000). Plant cyanogenic glycosides. Toxicon, 38, 11–36.

Waisen, P., Cheng, Z., Sipes, B.S., DeFrank, J., Marahatta, S.P., & Wang, K.-H. (2020). Effects of biofumigant crop termination methods on suppression of plant-parasitic nematodes. Applied Soil Ecology, 154, 103595.

Wang, K.H., Sipes, B. S., Schmitt, D. P., & Wang, K. H. (2001). Suppression of *Rotylenchulus reniformis* by *Crotalaria juncea*, *Brassica napus*, and *Tagetes erecta*. Nematropica, 31, 235–250.

Waskom, M.L. (2021). Seaborn: statistical data visualization. Journal of Open Source Software, 6, 3021.

Werner, J.J., Koren, O., Hugenholtz, P., DeSantis, T.Z., Walters, W.A., Caporaso, J.G., Angenent, L.T., Knight, R. & Ley, R.E. (2012). Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. The ISME Journal, 6, 94-103.

Wiesel, L., Daniell, T.J., King, D., Neilson, R., 2015. Determination of the optimal soil sample size to accurately characterise nematode communities in soil. Soil Biology & Biochemistry, 80, 89-91.

Yeates, G.W., Bongers, T., de Goede, R.G.M., Freckman, D.W., and Georgieva, S.S. (1993). Feeding habits in soil nematode families and genera - an outline for soil ecologists. Journal of Nematology, 25, 315-331.