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ORIGINAL PAPER

Cost of root disease on white clover growth in New Zealand dairy pastures

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Abstract The cost to clover growth of soil-borne root disease was measured in ten New Zealand dairy pasture soils. The average increase clover growth (weight) after soil pasteurisation was 28.5 %, but ranged from a 64 % increase (Whataroa soil) to a decrease of 11.9 % (Ruakura soil). The economic cost of reduced clover growth was determined using the Farmax Dairy Pro decision support system. In Southland and Canterbury, clover root disease was estimated to cost \$750 and \$715 ha^{-1} $year^{-1}$ respectively, and in the Waikato region \$1506 ha^{-1} $year^{-1}$. DNA-based testing of the soils detected the presence of diverse fungal, oomycete and nematode pathogen populations. A significant linear relationship was found between reduction in clover growth and group F *Pythium* spp. (P = 0.0177). The DNA-based assay indicated that *Aphanomyces trifolii*, a root pathogen of subterranean clover, may be present in the dairy-pasture soils. As this pathogen is currently not recognised as present in New Zealand, a definitive determination of its presence is required. Based on the high economic costs of diseases, the control of soil-borne root pathogens in New Zealand pasture is a direct means to increase profitability.

Keywords Pythium · Clover root disease · Economic cost · Pasture · *Aphanomyces trifolii*

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Introduction

A diverse range of pathogens are known to cause disease on the roots of forage plants in New Zealand pastures (Harvey and Harvey 2009). These include nematodes, fungi, and Oomycetes (Falloon 1985; Skipp and Christensen 1989; Waipara et al. 1996; Watson and Mercer 2000; Sarathchandra et al. 2000), which vary from highly host-specific through to broad-range generalists. In most cases, however, root diseases are often caused by a suite (disease complex) of pathogens (Skipp and Watson 1996). For example, disease maybe initiated through infection of the root tissue by a virulent host-adapted species, with subsequent colonisation of the compromised tissue by a range of secondary (generalist) pathogens.

Although the importance of soil borne disease to pasture productivity is widely recognised, there has been relatively little fundamental or applied research effort aimed at understanding or controlling disease complexes. In New Zealand, the bulk of research efforts were conducted in the mid 1980's through to the early 2000's (Falloon 1985; Skipp and Christensen 1989; Waipara et al. 1996; Watson and Mercer 2000; Sarathchandra et al. 2000), with relatively little work conducted for the past 15 years. This is at odds with the trend for increased value of pasture production on a per hectare basis. In the 1990's, the export value of pasture production in New Zealand was approximately \$585 per ha (Statistics New Zealand 1999). This has grown to the current level of \$2007 per ha, a nearly 241 % increase (Ministry of Primary Industries 2013). A portion of this increase can be attributed to land use conversion from sheep and beef grazing to dairy. In the period between 1990 and 2014, the area used for dairy

pasture grew from 1.57 million ha to 2.42 million ha (Statistics New Zealand 1999, 2014). However, the direct value of dairy as a land use has also grown, with total export values of $2.98 billion in 1996 from 1.76 million ha, to $15.82 billion in 2014 from 2.42 million ha.

The high return per ha of dairying (export value of ~$6500 per annum; 2014 data calculated from above values) is an outcome of high inputs (costs) of fertilisers, irrigation of dryland areas to escape summer drought conditions (e.g. Canterbury), close management of pasture weeds and pests, and many other factors. As such, there is an urgent need to define the base-line of soil biological constraints to pasture production. Furthermore, by determination of the economic cost of root diseases, appropriate decisions can be made for prioritisation of areas for research and development. This also provides an economic base (value proposition) for decisions associated with on-farm control of soil-borne diseases.

The maintenance of clover in pastures is central to the profitability and sustainability of New Zealand's pasture systems (Caradus et al. 1996). Clovers provide biological nitrogen fixation into pasture soils, thus lifting total system fertility and productive capacity (Ledgard 2001), and have high forage quality (nutritive value and intake characteristics) (Harris et al. 1997). Thus the value of clover for milk production in New Zealand is unequivocal (Woodfield and Clark 2009).

The aim of this work was to determine the magnitude of soil biological constraints to pasture production, with a focus on clover within dairy-based systems. Given the relatively high value of dairy-based pastoral land use, we aimed to calculate a conservative economic cost of root diseases to system profitability. As dairying is intensively managed, and typically conducted on accessible terrain, this land use provides greater opportunity for disease control than hill or high country pastoral systems (Dignam et al. 2016). As such, dairy-based production is best positioned to provide disease management practices to reduce soil biological constraints to pasture production. This is directly in line with industry-led targets to 'lift production while reducing the environmental impacts of dairy farming' (DairyNZ 2009). Alleviation of soil biological constraints (i.e. reduction of pathogens or supply of beneficial symbionts) is identified as a key path towards achieving this goal (Wakelin et al. 2013).

Materials and methods

Soil collection and handling

Soils were collected from 10 dairy farms across a broad geographic range. The sites are listed in Table 1. Approximately 12 kg of soil was collected from a single paddock at each site. Sampling was made to the approximate rooting depth of the pasture (~10–15 cm). On return to the laboratory, the samples were sieved to 2 mm (removing stones and other bulky material) and stored at 4 °C until use. Soil physicochemical properties were determined at Hill Laboratories Ltd. (Christchurch), using well characterised methods (e.g. as described in Wakelin et al. 2013).

For each soil, the moisture content was determined using loss of mass (water) after drying a sample at 105 °C for 24 h (θ_d); soils that were >40 % moisture were air-dried until this level was obtained. The maximum water holding capacity (MWHC) of each soil was determined by calculating the mass of water hold under gravity (i.e. no suction; Jenkinson and Powlson 1976).

Disease release assay

The growth of white clover (*Trifolium repens*) was assessed in non-sterilised and sterilised soils. For each soil, 1 kg (dry weight equivalent) of soil (at 40 % θ_d) was weighed into 8 polyethylene bags. Four of these bags were microwave irradiated for 4 min at 850 W. While these are hereafter referred to as 'sterilised' experimental treatments, it is highly likely the soil was pasteurised to an extent and certainly colonised by microorganisms over time. The remaining four bags of soil were not treated (non-sterilised).

For both sterile and non-sterile treatments, 24 small pots were set up with 130 g soil (dry wt) in 65 mm diameter plastic (non-draining) disposable cups. Ten seeds of white clover (cv. Tribute) were placed on the surface and covered with a further 20 g of soil. The samples, in replicates of 6 pots, were then brought to 80 % of the MWHC using either water (0 × fertiliser), 'Thrive' all-purpose soluble fertiliser (Yates, NZ) at 0.22 g l^{-1} (1 × fertiliser), double-rate Thrive (2 × fertiliser), or triple-rate Thrive (3 × fertiliser). The pots were placed in a controlled environment room (12 h photoperiod; 20 °C). Pots were maintained at 80 % MWHC by addition of water or soluble fertiliser to the respective treatments. After 6 weeks, the clover plants in each pot were excised at their base (soil level) and the fresh weight per pot immediately determined.

DNA isolation and pathogen testing

Samples of soil were sent to the root disease testing service (RDTS; Primary Industries and Regions SA; PIRSA, Australia) for pathogens DNA detection (Ophel-Keller et al. 2008). This service involves the extraction of DNA from bulk samples of soil, and qPCR-based detection of a suite of pathogenic taxa in the samples. This included fungi (*Didymella pinodes*, *Rhizoctonia solani* AG groups 2.1, 2.2, and 8), Oomycetes (*Pythium* clades F and L, *Phytophthora clandestina*, *Aphanomyces trifolii*), and nematode pathogens (*Pratylenchus neglectus*, *Pr. penetrans*, and *Pr. quasitereoides*). Details associated with these DNA tests are given in Simpson et al. (2011).

Table 1 Soils: origin, soil type, and physicochemical properties

Site	Soil type[a]	pH	Olsen phosphorus[b]	Sulphate sulphur[b]	Nitrate-N[c]	Total nitrogen[d]	CEC[e]	Total base saturation[d]	Volume weight[f]
Taieri	Gley	7.2	92	36	44	0.37	24	100	0.91
Kurow	Recent	6.3	12	4	38	0.31	15	77	1.01
Inchbonnie	Recent	5.6	29	13	34	0.28	14	44	0.91
Gordonton	Organic (peat)	5.5	57	44	121	1.68	81	55	0.54
Rotorua	Allophanic	5.7	8	13	16	0.44	15	29	0.74
Eyrewell	Brown	6.1	24	50	72	0.17	13	71	1.02
Whataroa	Recent	5.3	30	23	33	0.32	15	36	0.75
Springston	Gley	6.1	45	35	52	0.49	30	79	0.81
Ruakura	Podzol	6.3	26	75	72	0.69	31	66	0.72
Woodlands	Brown	6	35	19	31	0.47	20	58	0.83

[a] New Zealand soil classification order (Hewitt 1988),[b] mg/L, [c] mg/kg, [d] %, [e] cation exchange capacity in me/100 g, [f] g/ml

Analysis of data

Disease pressure in the soils was calculated based on the percentage difference in plant growth (fresh weight; *fwt*) between the sterilised (microwave pasteurised) and non-sterilised soils:

$$\%\ \text{growth change} = \frac{(\mathit{fwt}\ \text{plants sterile soil}) - (\mathit{fwt}\ \text{plants}\ \mathit{non}\text{-sterile soil})}{\mathit{fwt}\ \text{plants sterile soil}} \times 100$$

For each soil, the influence of sterilisation on plant growth was tested using t-tests (two tailed). Each soil was tested individually (i.e. we did not assume consistent variance among the soils), and corrections were made for multiple comparisons using the Holm-Šídák method (Holm 1979) at $\alpha = 0.05$. The average disease pressure across the ten soils was calculated and linear regression used to determine if relationships existed to soil physiochemical properties or soil pathogen levels (RDTS pathogen - DNA quantification).

The potential influence of nutrient mobilisation (following soil sterilisation) on increased clover growth was assessed by response of plants to soluble fertiliser addition. For each soil, the plant growth across the four fertiliser levels was assessed in the sterile and non-sterile soil using linear regression. Within each soil, the sterile and non-sterile fertiliser responses (slope of regression lines) were tested for response to fertiliser addition, and then for then for equality of responses (i.e. response of fertiliser addition in non-sterile soil ≠ to response in sterile soil). This was conducted by comparing slopes of the linear regressions (two-tailed t-test), with the null hypothesis that the slopes were parallel. A description of the rationale is given in the discussion.

Relationships between pathogen abundance values and soil disease were explored using linear regression. Each of the above analyses was performed in Prism7 (GraphPad Software Inc., CA).

Economic modelling of clover disease cost

Production and financial costs of clover soil disease were analysed using Farmax Dairy Pro decision support models (Bryant et al. 2010) of the Southland, Canterbury, and Waikato-BOP Monitor Dairy farms of Year 2012/13. The model assumed that removing clover soil disease constraints would increase average clover yield by 28.5 %. In turn, this would impact ryegrass-clover pasture yield, seasonally adjusted pasture metabolisable energy (ME), clover-rhizobia N_2 fixation, and milk solid production. From these, effects on farm revenue, expenditure, and gross margin were calculated.

Underlying data in the models were based on appropriate Ministry of Agriculture and Fisheries MAF/ Ministry of Primary Industries monitor farms for each region. Pasture growth profiles and clover content were determined from Woodlands and Mona Bush (representing Southland), Lincoln and Winchmore (representing Canterbury), and Tirau and Ruakura (representing Waikato). DairyNZ farm expenses, milk price and levy costs for year 2012/13 were used. N fertiliser use, and feed supplementation were based on best practice for the regions, and the models considered stock wintered with complete reconciliation of stock for the year.

Models required that monthly pasture cover on the farm should exceed minimum pasture cover to ensure feed intake and pasture quality meets desired livestock numbers and performance. From this, scenarios for each region were generated that encompassed change of stock rate wintered at a maximum feasible level (current farm potential). Each of these were then adjusted to account for an additional 28.5 % increase in clover growth (fresh weight). The cost of clover root disease was taken as the difference between the scenarios for each respective region.

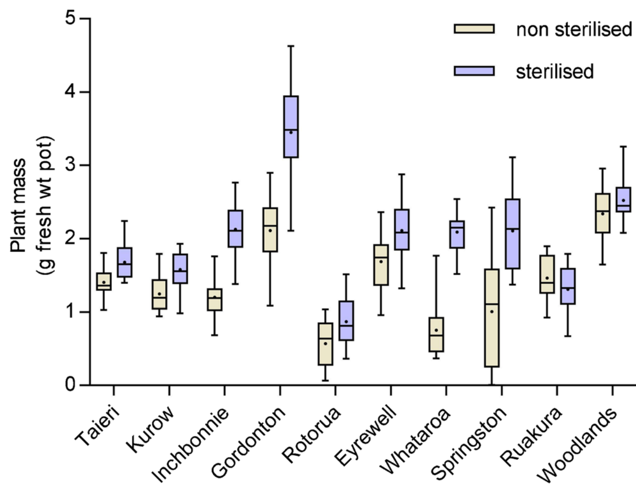

Fig. 1 Clover growth in ten microwave sterilised and non-sterile soils. Boxes extend from the 25th to 75th percentiles and whiskers from the smallest to the largest values. The solid line indicates the median value and the floating '·' symbol the mean

Results

Clover disease pressure in dairy soils

Sterilisation of soils significantly (P < 0.05) increased clover growth in all soils except for Ruakura (Fig. 1). When calculated as percentage growth change, microwave-treatment of soil resulted in clover growth increases from 64 % (Whataroa) to −11.9 (Ruakura). An average 'disease pressure' of +28.55 % was calculated across the 10 soils, and this value was used for the economic modelling for each of the three regional scenarios.

Influence of soluble fertiliser on plant growth in sterile and non-sterile soils

Linear regression was used to determine relationships between fertiliser addition and soil sterilisation on clover growth. The regression slope summary data are given in Table 2. With the exception of soil from Eyrewell, the responses to fertiliser addition between the sterile and non-sterile soils were not different (P > 0.05; Table 2). There was no evidence that nutrient released from the sterilisation process significantly contributed to the plant response between the sterile and non-sterile treatments. For the Eyrewell soil, there was no fertiliser response in the non-sterile soil, however, in the sterile soil there was (P < 0.001).

Soil pathogens

A DNA-based test was used to detect for the presence of a suite of pathogens in the pasture soils; the set of species in which positive DNA-based tests were found are given in Fig. 2a. Across these, positive DNA-based 'detection' of multiple fungal and Oomycete pathogens were found in most soils (Fig 2a). However, the most commonly detected pathogen was *Pythium* clade F, with positive results returned for all soil samples. *Rhizoctonia solani* AG2.1 and 2.2 were mutually exclusive among the soils (correlation $r = -0.68$; $P = 0.029$), with the exception of Ruakura where both were present. The nematode species *Pratylenchus neglectus* and *Pr. penetrans* had sparse occurrence among the soils, while *Pr. quasitereoides* and *Pr. thornei* were not detected. The pathogenic fungi *Didymella pinodes*, *Rhizoctonia solani* AG8, and the Oomycete *Phytophthora clandestina* (Fig 2a), were not detected in the dairy pasture soils. *Aphanomyces trifolii*, a relatively recently

Table 2 Clover response to fertiliser addition in sterile and non-sterile soils. All data are probability estimates from linear regression

Soil	Regression slope		Test for slope ≠ 0		Test for equal slopes
	Non-sterile	Sterile	Non-sterile	Sterile	
Taieri	0.065	0.018	0.058	0.669	0.364
Kurow	0.063	0.120	0.152	**0.003**	0.306
Inchbonnie	0.039	−0.024	0.412	0.713	0.431
Gordonton	−0.016	−0.228	0.853	0.065	0.155
Rotorua	0.161	0.172	**0.003**	**0.001**	0.866
Eyrewell	−0.085	0.239	0.189	**<0.001**	**<0.001**
Whataroa	−0.063	−0.033	0.381	0.523	0.745
Springston	−0.054	−0.023	0.587	0.865	0.847
Ruakura	0.050	−0.052	0.350	0.380	0.199
Woodlands	0.159	0.046	**0.009**	0.358	0.135

Values in bold-type are significant at P < 0.05

described multi-host root-rot pathogen (O'Rourke et al. 2010), was detected in 7 of the 10 soils (Fig 2a).

For each of the pathogens, regressions were made between abundance (DNA quantification) and clover growth reduction across the soils. A significant positive association was found between abundance of *Pythium* clade F pathogens and clover disease pressure ($P = 0.0177$; $R^2 = 0.525$); this relationship is shown in Fig. 2b. Disease pressure was not positively related ($P < 0.05$) to the occurrence of any of the other pathogens detected.

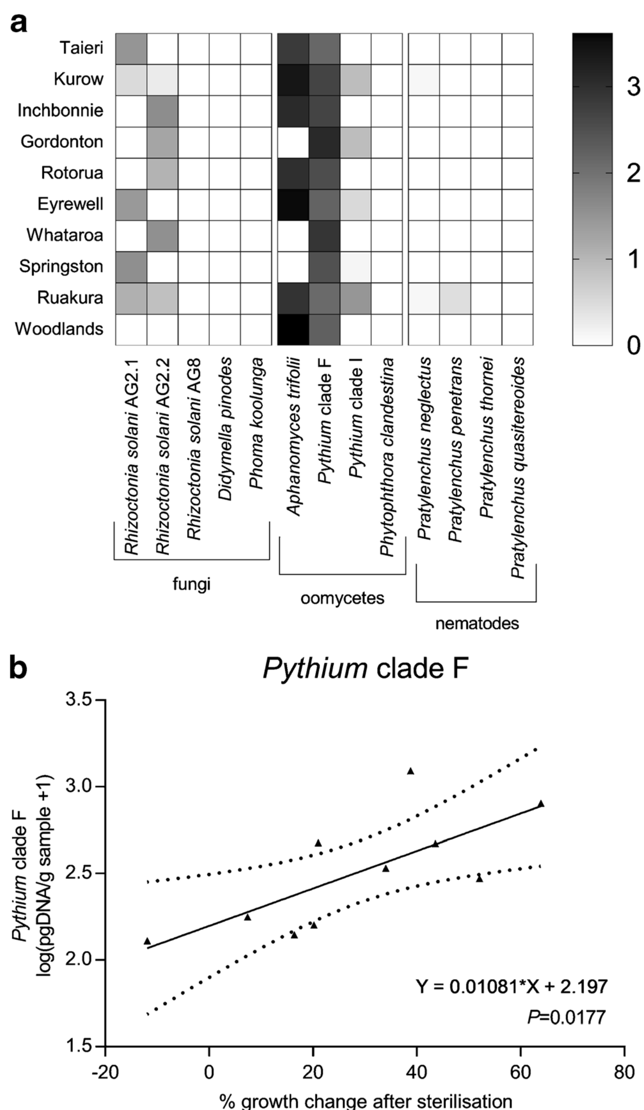

Fig. 2 **a** Heat-map of soil DNA testing for pathogenic fungi, oomycetes, and nematodes. All fungi and oomycete data were pg DNA detected/g sample, except for *Aphanomyces trifolii* (copies/g sample). Nematodes are given as counts/g soil. All data were log-transformed. Several pathogens were not detected (0-values). **b** Relationship between clover growth after soil sterilisation (release from disease pressure) and quantity of *Pythium* clade f DNA. Solid line is the linear regression (equation given) and dashed lines the 95 % confidence intervals

Economic modelling of clover disease cost

Economic cost of soil disease on clover growth was determined separately for the Canterbury, Southland, and Waikato regions, as each has varying seasonal pasture growth rates, percentage clover composition, and input costs. In Southland and Canterbury the costs of root disease to potential production were similar, at \$750 and \$715 ha^{-1} $year^{-1}$ respectively, but were much greater in the Waikato at \$1506 ha^{-1} $year^{-1}$.

Discussion

The average disease pressure in New Zealand's dairy soils is estimated to cost 28.5 % of clover growth, however, this is variable and can exceed 60 % growth in some soils (e.g. Whataroa). These are slightly lower than estimates of pasture production losses of 40–50 % based on a consensus of studies (Skipp and Watson 1996) but reflect that the focus of this study was solely on clover. Furthermore, the estimates of disease pressure in the assays conducted are conservative. Pasteurisation of the soil not only removes deleterious microorganisms, but also beneficial taxa that are required to support plant growth. Notably, these include *Rhizobium* bacteria (N-fixing symbionts of clover) and mycorrhizal fungi. Indeed, in the absence of significant disease pressure, pasteurisation of soil can be expected to have no direct benefit (as measured in Ruakura soil) or a nett negative effect (removal of beneficial symbionts) on plant health. Regardless, our findings reinforce the high burden that soil biological constraints have on clover growth in New Zealand's pastoral systems, and the urgent need to be able to accurately assess pathogen populations and predict and manage disease, particularly in high-input, high-value pastoral systems.

The disease burden on clover has implications for potential productivity of the pastoral systems. On a per hectare basis, the highest cost of disease was in the Waikato region with the reduction in clover growth estimated to cost \$1506 to potential gross margins per year. In Canterbury and Southland, these margins were approximately half that of Waikato, yet still represent a very large potential loss in the farming systems. By establishing these base line costs, and appreciating that they are ongoing in perennial systems, an accurate value proposition associated with control of these diseases is evident.

In addition to a direct cost on plant productivity, root-disease pressure also contributes indirect costs associated with reduced nutrient and water-use efficiency (Baligar et al. 2001; Angus and Van Herwaarden 2001; Angus et al. 2015). However, much of the work conducted in this area has been in arable cropping systems, and the indirect cost of root disease on nutrient and water use efficiency in pastoral systems

represents a significant knowledge gap (Unkovich 2012). For pasture legumes, such as white clover, these costs may be particularly high when root nodules are diseased, resulting in reduced biological nitrogen fixation and greater requirement to access N from the soil mineral N pool (Unkovich 2012).

Pythium clade F contains a group of highly virulent plant pathogens with worldwide distribution, such as *P. irregulare* and *P. debaryanum* (Lévesque and de Cock 2004). Indeed, both species have been recorded as agricultural pathogens in New Zealand (Robertson 1973; Falloon 1985; Waipara and Hawkins 2000), along with other pathogenic species such as *P. ultimum* (Robertson 1973; Falloon 1985). The association found between clade F *Pythium* and clover disease is supported by work in Australia. Using the same detection system, Simpson et al. (2011) found the DNA concentration of clade F *Pythium* to have a linear relationship with damage to *Trifolium subterraneum* (subterranean clover) across multiple sites in Australia. This reinforces the importance of these pathogens in limiting production in pastoral agroecosystems.

Plant pathogenic *Pythium spp.* often have a broad host range, causing diseases ranging from asymptomatic root invasion to highly devastating damping-off root rot (Christensen et al. 1988; Falloon 1985), and often infect plants as multiple-pathogen disease complexes (e.g. Barbetti et al. 1987; Skipp and Watson 1987, 1996). As shown in this study, the control of these pathogens is highly desirable as it may significantly increase the productive capacity of agroecosystems. However, these attributes also make *Pythium* very difficult to control. In pastoral systems in particular, options for chemical control are currently limited to seed dressings (soil drenches being costly for broad-acre application), and plant breeding has yet to provide robust heritable resistance traits. However, the development of disease-suppressive soils offers one of the few approaches whereby gains in reducing disease in pastures may be achieved (Dignam et al. 2016). The application of DNA based tools that can quantify the pathogen loading in soils over time will be an important component of this approach (Dignam et al. 2016). In addition, by monitoring pathogen populations during changes in farm management, e.g. shift in stocking rate, alteration of fertiliser inputs, or change of pasture composition, insights in to the ecology of the pathogens in farming systems can be gained, and potential opportunities to control population sizes identified (Dignam et al. 2016).

The low levels of detection of the nematode species was not surprising. Many of these are primarily pathogenic to cereal crops and are included as part of the DNA-based pathogen testing provided to grain growers in southern Australia (Simpson et al. 2011). While some of the *Pratylenchus* spp. are present in New Zealand, they are of low abundance and importance in pasture systems. *Pratylenchus quasitereoides* a cereal pathogen described from Western Australia (Hodda et al. 2014) is not present in New Zealand. The nematode taxa of greatest destructive potential in New Zealand pasture systems are species of *Meloidogyne* (Watson and Mercer 2000). There is currently no DNA-based testing service to quantify populations of these in New Zealand pastures. Similarly, the lack of detection of the fungal pathogens *Rhiocotonia solani* AG8, *Didymella pinodes*, and *Phoma koolunga* in these samples was not surprising. *R. solani* AG8 is of primary importance in grain cereal systems, while *Phoma koolunga* and *Didymella pinodes* are involved in the Aschochyta-blight disease-complex of field pea (Davidson et al. 2009).

An unexpected finding was the detection of *Aphanomyces trifolii* DNA in all of the pasture samples tested. This Oomycete pathogen was recovered from diseased subterranean clover roots in Western Australia, and was subsequently found to be cause root rot on a wide range of plant species (O'Rourke et al. 2010). As *A. trifolii* has not been reported in New Zealand, the detection of this significant pathogen in this study has important implications. If the presence of the pathogen is validated, it will remain to be determined if it has recently invaded New Zealand or has been established for some time or is indigenous. The potential host-range of the pathogen is unknown, however, testing to date (O'Rourke et al. 2010) indicates strong pathogenicity to the forage legumes *T. subterraneum*, *Medicago polymorpha*, and *Medicago truncatula*. The pathogenicity of *A. trifolii* to forage legumes of importance to New Zealand, particularly *T. repens*, *T. pratense*, and *M. sativa* requires determination.

A concern when using soil pasteurisation (or sterilisation) to remove the biological background is the flush of nutrients that can occur through mineralisation of the microbial biomass (Jenkinson and Powlson 1976). Although these are immobilised as the microflora rapidly establishes, the early provision of nutrients to the seedling may account for some of the growth promotion observed in the sterilised treatments. An approach to address this is the use of a fertiliser dose-response treatment within both the sterile and non-sterile treatments, and the comparison of the fertiliser responses therein (Skipp and Sarathchandra 1999). However, we found no evidence that nutrient release from soil sterilisation contributed to the stimulation of clover growth (Table 2). As noted previously, in this study the context of 'sterilisation' is primarily in relation to alleviation of pathogen loads, and the soil microbiology can be more reasonably considered as 'pasteurised'. In such systems, we expect a rapid re-colonisation of the soil microbiota and immobilisation of nutrients which may have been plant-available (Jenkinson and Powlson 1976). This supports the findings of Skipp and Sarathchandra (1999), the 'microwaved soil pathogen potential assay', is not sensitive to the influence of nutrient release from the soil treatment.

Based on these findings, three primary recommendations can be made. Firstly, root diseases are causing a significant economic cost to farmers. Given the high costs and return of modern pastoral agriculture, research into identifying the

primary causal agents (and complexes) is needed as a basis to developing control options. Secondly, diagnostic tools such as DNA-based disease testing assays are required to establish the potential disease pressure in farming systems. Combined with modelling (such as described here), these can be used to enable informed decision making on the cost-return of disease control using various measures (chemical, cultural, or biological). Thirdly, there is currently no widespread monitoring of potential incursion of new soil-borne pathogens into New Zealand pastures that impose an additional direct burden on production and also reduce the efficiency of inputs of water and fertiliser.

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