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High perennial ryegrass seeding rates do not negatively impact pasture sward persistence

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Abstract

Poor persistence of perennial ryegrass swards is a common problem; however, there is a lack of long-term studies to understand the mechanisms associated with poor persistence. This study describes an experiment to test the hypothesis that high ryegrass seeding rates (>18 kg seed per ha) reduce long-term population persistence because of smaller plant size and poorer survival during the first year after sowing. Four cultivars, representing four functional types of perennial ryegrass, were sown at five seeding rates (equivalent to 6, 12, 18, 24 and 30 kg seed per ha) with white clover in three regions of New Zealand. Swards were monitored for 5 years. No evidence was found to indicate a lack of persistence of ryegrass-based swards sown at higher seeding rates. During the first year, swards sown at higher seeding rates had greater herbage accumulation (except at the Waikato site), greater ryegrass tiller density and greater ryegrass content. This initial impact of high seeding rates had largely dissipated by the fourth year, resulting in swards with similar annual herbage accumulation, tiller density and botanical composition. Similarly, there were relatively few differences among cultivars for these variables. Although high seeding rates did not negatively impact sward persistence, geographical location did, with strong evidence of ryegrass population decline at the Waikato site for all treatment combinations, some decline in Northland, and stable populations in Canterbury. It is possible that productive perennial ryegrass pastures can only be sustained for 4–5 years in some situations, even when the best ryegrass technology and management practices are used.

KEYWORDS
invertebrate pests, moisture deficit, pasture sward persistence, plant survival, sowing rate

1 | INTRODUCTION

Poor persistence of perennial ryegrass (*Lolium perenne* L.)-dominant swards is an important on-farm issue in some areas of New Zealand and Australia (Nie, Chapman, Tharmaraj, & Clements, 2004; Tozer, Bourdot, Edwards, & Mercer, 2011). While many farmers expect newly sown swards to persist for more than 10 years (Daly, Fraser, Perkins, & Moffat, 1999), the level of farmer satisfaction with swards has been reported to decline progressively during the first 3 years after renewal (Kelly, Smith, & Brazendale, 2011; Rijswijk & Brazendale, 2016), which is much sooner than anticipated.

Although persistence is a relative term invoking expectations of how swards should perform over time, the overall outcome desired by farmers is that the dry-matter (DM) yield advantage of the new sward compared with the previous sward persists for as long as possible. Thus, "persistence" is defined here as the persistence of yield. The failure of a new sward to maintain its yield advantage could be due to a loss of ryegrass plants or a loss of the overall yielding ability of the plants because yield-related traits are not expressed, or are lost altogether (Parsons et al., 2011). Consequently, to understand persistence, information is required on individual plants (the genotype and phenotype of the sown species), the population (the density of plants/tillers of the sown species) and the sward community as a whole (the presence and abundance of all species present). Few long-term studies have characterized herbage accumulation or other sward attributes in different environments after sowing, yet such systematic studies are crucial to understanding the mechanisms associated with poor persistence of perennial species.

The recommended seeding rates for perennial ryegrass swards in New Zealand are currently 20–25 kg seed per ha for diploid cultivars and 25–30 kg/ha for tetraploids. In Ireland, the recommended rates are higher (35 kg/ha for diploids; Teagasc, 2014). Previous research on the impact of ryegrass seeding rate on herbage accumulation has produced inconsistent results. Some studies reported greater herbage accumulation from higher seeding rates (>18 kg seed per ha) for the first 2 years after sowing (Creighton et al., 2012; Culleton & Murphy, 1987; Venuto, Redfearn, Pitman, & Alison, 2004), while others reported that the yield advantage from higher seeding rates dissipated after the first year (Culleton, Murphy, & O'Keefe, 1986; Keane, 1980). Conversely, others reported similar total herbage accumulation across a range of seeding rates for 1–3 years after sowing (Brougham, 1952; Frame & Boyd, 1986; Heddle & Herriott, 1954). It is accepted that the initial yield advantage of higher seeding rates often dissipates due to greater plant mortality in swards sown with higher seeding rates resulting from greater interplant competition for light, nutrients and water, while at lower seeding rates, plants initiate more tillers in a high-light environment to compensate for the reduced plant numbers (Donald, 1951; Heddle & Herriott, 1954). The result of these compensatory processes is that the long-term tiller density is similar for swards sown at very different seeding rates. Greater interplant competition in swards sown at higher seeding rates (>18 kg/ha) reduced plant size and survival during the first year after sowing, resulting in swards sown with a large variation in seeding rate (12–30 kg/ha) having similar tiller densities after 12 months (Lee et al., 2017). However, it is not known whether the resulting depleted population of smaller plants may be less able to resist abiotic or biotic stressors (e.g., invasion by volunteer weed species into sward gaps) leading to loss of yield potential and failure of the new sward to meet farmer expectations for long-term yield and persistence.

The hypothesis of this study is that high perennial ryegrass seeding rates (>18 kg seed per ha) reduce long-term pasture sward persistence because of reductions in plant size and survival during the first year after sowing. To test the hypothesis, four perennial ryegrass cultivars were sown at five different seeding rates and monitored for 4 years in three different environments in New Zealand. All four cultivars of one seeding rate treatment (18 kg/ha) were monitored for a fifth year at each site. Intensive data from the first year of this experiment have been reported previously by Lee et al. (2017).

2 | MATERIALS AND METHODS

2.1 | Experimental sites

The experiment was conducted in three dairying regions in New Zealand: Northland and Waikato in the North Island and Canterbury in the South Island. The experimental sites were located at Fonterra's Jordan Valley Farm in Northland (−35.612, 174.262; 96 m a.s.l.), DairyNZ's Scott Farm in the Waikato (−37.772, 175.378; 40 m a.s.l.) and the Lincoln University Research Dairy Farm in Canterbury (−43.638, 172.462; 10 m a.s.l.). The soil types at the three sites, respectively, were Wairua clay, Matangi silt loam and Wakanui silt loam over a mottled sandy loam phase. These are classified as an Orthic Gley, a Typic Sandy Gley and a Mottled Immature Pallic soil respectively (Hewitt, 1998). Both the Northland and Waikato sites were dry land while the Canterbury site was irrigated (as standard for dairy farms in each region).

The number of germinable buried seeds estimated from soil samples (25 mm diameter × 75 mm depth) collected before establishment of the experiment at each site revealed proportionately lower numbers of perennial ryegrass (394, 81 and 27 seeds/m^2 at the Northland, Waikato and Canterbury sites, respectively) and white clover seeds (122, 407 and 54 seeds/m^2, respectively) compared with other grass (13,622, 3,640 and 3,382 seeds/m^2, respectively) or weed seeds (1,589, 4,971 and 1,141 seeds/m^2 respectively).

2.2 | Experimental design and treatments

At each of the three sites, four perennial ryegrass cultivars, each representing different functional types within the species, were sown in plots at five nominal seeding rates (6, 12, 18, 24 and 30 kg seed per ha). Main treatment plots (cultivar, 540 m^2) were divided into subplots (seeding rate, 108 m^2), arranged in a randomized split-plot design with five replicates. All seeding rate treatment subplots were sampled for the herbage-related variables for the first 4 years of the experiment; however, from June 2015, only the 18 kg/ha treatment subplots were sampled as the effects of seeding rate had dissipated during 2014/2015.

The perennial ryegrass cultivars were "Grasslands Nui," "Commando," "Alto" and "Halo." In terms of their functional types, Nui is a diploid cultivar with a mid-season flowering date of approximately 22 October in NZ (Lee, Matthew, Thom, & Chapman, 2012). Nui was selected from plants collected from 40-year swards at a dairy farm in South Auckland in the 1960s and released commercially in 1976 (Armstrong, 1977). The other three cultivars were released commercially after 2000. Commando, a diploid with a similar flowering date to Nui, was bred from New Zealand ecotypes. Alto, a diploid that flowers 14 days later than Nui, is based on late heading material from Nui and material from north-west Spain. Halo is a tetraploid classified as very late season flowering (25 days later than Nui; Lee et al., 2012). The four cultivars were included to ensure that any conclusions based on seeding rate were applicable across the range of perennial ryegrasses currently available to farmers.

All cultivars were infected with the endophytic fungus (*Epichloë festucae* var. *lolii* (formerly *Neotyphodium lolii*); Leuchtmann, Bacon, Schardl, White, & Tadych, 2014). Nui was naturally infected with the wild or standard strain of this endophyte (denoted SE) while the other three cultivars were infected with the novel endophyte strain “AR37.” The effects of these endophytes on plant–insect–animal interactions in New Zealand grazing systems are described by Thom, Popay, Hume, and Fletcher (2012). Pre-sowing tests for endophyte viability confirmed endophyte infection frequencies of 87%, 74%, 84% and 78% of seed for Nui, Commando, Alto and Halo respectively.

Seed germination percentages and 1,000 seed weights were measured for each cultivar to ensure a similar number of viable seeds were sown per hectare. This resulted in the 6 kg/ha treatments being sown at 6.0, 7.3, 8.5 and 6.7 kg seed per ha for Alto, Commando, Halo and Nui, respectively, with the remaining seeding rate treatments increasing proportionally. Alto was considered to be a useful benchmark to “standardize” seeding rate against as it represented the modern diploid ryegrass type that breeders have focussed on recently in New Zealand (i.e., later heading, good early season growth, strong total annual yield).

2.3 | Site preparation and establishment

At all sites, the existing sward was sprayed in March 2011 with Glyphosate herbicide (2.7, 3.2 and 2.2 kg a.i. per ha in Northland, Waikato and Canterbury, respectively) plus Pulse® organo-silicone penetrant (100 ml in 100 L water). In Northland and Canterbury, the existing pasture sward also received a broadleaf weed herbicide (30 and 16.5 g/ha of tribenuron-methyl respectively). In Northland and Waikato, a second application of Glyphosate herbicide (2.7 and 2.2 kg a.i. per ha, respectively) and Pulse® was applied 3–4 weeks later. The ryegrass seed was direct-drilled in late March/early April using a Renovator MK3 drill (Duncan Ag, Timaru, New Zealand) or a John Deere 750A drill (John Deere International GmbH, Schaffhausen, Switzerland) respectively. In Canterbury, the site was surface rotary hoed and harrowed before seedbed preparation by ploughing, harrowing and rolling. Seed was drilled using a cone seeder in late March. All sites received slug bait (0.4 kg metaldehyde per ha) in the week before sowing. Ryegrass seed was Ultrastrike®-treated providing a fungicide to prevent “damping-off” disease and systemic insecticides to deter insect attack on seedlings before endophyte alkaloid production commenced. All plots were oversown with 8 kg/ha of Superstrike®-coated Tribute white clover seed (*Trifolium repens* L.; equivalent to about 5 kg/ha of bare seed). Post-emergence weed control was achieved at all sites within 8 weeks of sowing by the application of Preside® herbicide (40–52 g flumetsulam per ha) plus Uptake™ surfactant (500 ml in 100 L of water).

2.4 | Grazing management

Plots were rotationally grazed by dairy cows at all sites, with all plots at a site grazed at the same time. The timing of the first grazing after sowing was determined by the “pluck” test, which involves grasping ryegrass seedlings firmly between the thumb and forefinger and tugging in a single quick movement to mimic the biting action of a cow. If the leaves broke and the roots remained in the ground, the sward was considered ready for grazing. The first grazing took place 96 (20 July 2011), 57 (25 May 2011) and 158 days (4 September 2011) after sowing at the Northland, Waikato and Canterbury sites respectively. Subsequent grazing took place when the herbage biomass reached 2,500–3,500 kg DM per ha, with a target post-grazing residual of 1,600 kg DM per ha (approximately 5 cm residual stubble height). Over the 5 years, each site was grazed between nine and 12 times per year, resulting in a total of 50, 48 and 54 grazings at the Northland, Waikato and Canterbury sites respectively (Table S1). At each grazing, a discrete group of cows was allocated to each main plot; the number of cows in each group was determined by pasture availability above the desired residual and expected cow intake.

During the peak of seedhead production (late spring/early summer), when clumps of herbage were left ungrazed around dung pats and urine-affected areas, plots were mown to a stubble height of ~7 cm after grazing if required. Over the 5 years, this occurred five times each at the Northland and Canterbury sites and 19 times at the Waikato site.

2.5 | Fertilizer

Before sowing, soil cores (25 mm diameter) were taken across each replicate at each site to 150 mm depth and analysed for pH, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and sulphur (S). This revealed suboptimal K and S concentrations for pasture sward growth at the Waikato site. To rectify the deficiencies, 8, 50, 9, 3 and 19 kg/ha of P, K, S, Mg and Ca, respectively, were applied on 23 March 2011 at this site. Soil nutrient testing was subsequently conducted to 75 mm depth at all sites annually. To maintain nutrient levels in the optimum range, maintenance fertilizer was applied at each site as specified in Table S2. Nitrogen (N) fertilizer was also applied as urea at all sites (Table S2), spread over two to nine applications per year. This resulted in total annual applications of 105, 146 and 238 kg N/ha/year at the Northland, Waikato and Canterbury sites, averaged over the 5 years.

2.6 | Climate and irrigation

Weather records were obtained from Fonterra’s Kauri processing plant 4 km from the Northland site (rainfall only), the Ruakura climatological station 5 km from the Waikato site and from the Lincoln climatological station 2 km from the Canterbury site (Figure 1). No temperature gauge exists near the Northland site; therefore, temperature data were obtained from the National Institute of Water and Atmospheric Research virtual climate database (e.g., Tait,

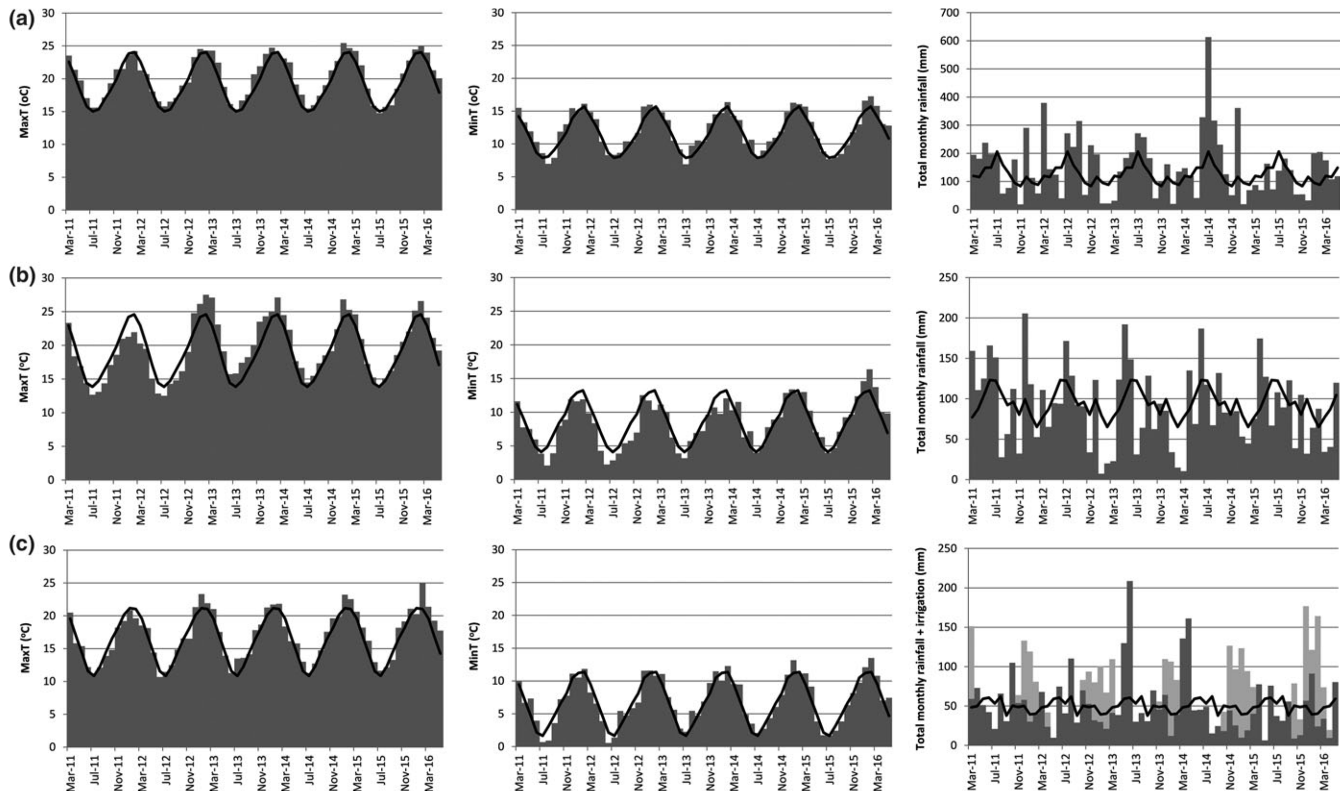

FIGURE 1 Average monthly maximum and minimum temperatures (°C; MaxT and MinT) and total monthly rainfall (■) plus irrigation water applied (■; Canterbury site only) at the (a) Northland, (b) Waikato and (c) Canterbury sites from March 2011 to May 2016. The grey bars represent data during the experiment while the black lines represent the 25-year (Northland) or 30-year (Waikato and Canterbury) means

Henderson, Turner, & Zheng, 2006; Tait & Woods, 2007). These estimates are produced daily, based on the spatial interpolation of actual data observations made at climate stations located around New Zealand.

Water was applied from spring to autumn each year using either a lateral move or centre pivot irrigator at the Canterbury site only. Before sowing, a total of 92 mm was applied over three applications in March 2011, with subsequent irrigation water applications of 232, 287, 194, 400 and 430 mm/year during years one to five after sowing (Figure 1). Irrigation could not be applied for approximately 6 weeks during mid- to late spring 2013 (year three) when the lateral move irrigator was replaced by a centre pivot.

2.7 | Herbage accumulation

Pasture sward biomass was estimated by quadrat cuts on the day before grazing at each site. This started at the second grazing at the Northland and Waikato sites (September and June 2011, respectively) and at the third grazing at the Canterbury site (October 2011). Herbage samples (n = 2) were cut to the approximate post-grazing height (40 mm) within square quadrats (0.2 m^2) placed randomly within each subplot in three of the five replicates. Samples or 100 g blended subsamples were oven-dried at 95°C (Northland and Waikato) or 65°C (Canterbury) to constant weight (approximately 48 or 72 hr, respectively) to allow calculation of herbage biomass and herbage accumulation rate per hectare. Seasonal herbage accumulation for each subplot was calculated from the herbage accumulation rate data for individual harvests partitioned within the following periods: winter, 1 June to 31 August; spring, 1 September to 30 November; summer, 1 December to 28/29 February; and autumn, 1 March to 31 May. Total annual herbage accumulation was also calculated for the period 1 June to 31 May.

The method remained consistent at the Northland site for the 5 years. At the Waikato and Canterbury sites, the method of estimating herbage accumulation, however, changed in December 2012 and August 2013 respectively. The new method involved cutting one 5-m strip from the centre of each subplot in all five replicates using a Haldrup F-55 forage harvester (Haldrup GmbH, Ilshofen, Germany) of cut width 1,500 mm set to a height of 55 mm above the ground (minimum cut height available). The fresh weight of the cut herbage was recorded using the on-board weighing system. A representative sample (~1,000 g fresh weight) was then taken before the cut herbage was ejected. In the laboratory, the sample was blended and duplicate subsamples (~150 g fresh weight) were oven-dried at 95°C to constant weight (~48 hr) to allow calculation of seasonal and total annual herbage accumulation as specified above. Annual herbage accumulation at the Canterbury site during 2013/14 was slightly underestimated because herbage accumulation before the May 2014 grazing could not be estimated as the harvester was out of action. During April 2015 (Canterbury) and September 2015 (Waikato), the lowest cut height on the harvester was modified from 55 mm residual height to 40 and 45 mm at the Canterbury and Waikato sites respectively.

2.8 | Genotypic analysis

Individual tillers ($n = 90$) were collected at random from separate plants in the Alto and Nui plots for the 12 and 30 kg/ha treatments at the Waikato site in autumn 2015, 4 years after trial establishment. Tillers were excised basally to ensure sampling of fungal endophyte *in planta* from the leaf sheath region. Plant and endophyte whole-genomic DNA isolation was completed using the FastDNA® Kit (Q-Biogene Inc., Carlsbad, CA, USA) for plant tissue as per manufacturer's instructions. The DNA extracts were typed for endophyte strain using simple sequence repeat (SSR) marker B11 (Moon, Tapper, & Scott, 1999), which discriminates the AR37 endophyte strain from standard endophyte (SE) and other commercial endophyte strains. Samples were also fingerprinted using 14 ryegrass SSR markers distributed across the genome (Sartie, Matthew, Easton, & Faville, 2011). Polymerase chain reaction (PCR) for marker B11 was performed as described by Moon et al. (1999), except that a 10 μl reaction volume was used, and for ryegrass SSR markers according to Sartie et al. (2011). For all SSR markers, capillary electrophoresis of PCR products was conducted using an ABI3130xl (Applied Biosystems, Foster City, CA, USA) as described by Sartie et al. (2011), and electropherograms were analysed and fragments were sized using GeneMarker v1.75 (SoftGenetics, LLC, PA, USA).

2.9 | Perennial ryegrass tiller density

Perennial ryegrass tiller population densities were calculated from the total number of ryegrass tillers counted in five randomly placed frames (5 × 20 cm) per subplot in autumn 2011, spring, summer and autumn 2012, and every autumn thereafter.

2.10 | Endophyte infection frequency

Fifty tillers per subplot were randomly sampled from three of the five replicates at each site in autumn every year. Tillers were cut with a scalpel at ground level. The sap from the cut base of each tiller was squeezed onto nitrocellulose blotting paper before colour development that confirmed the presence/absence of endophyte (Hahn, Huth, Schoberlein, & Diepenbrock, 2003).

2.11 | Botanical composition and nutritive value characteristics

At all sites, representative samples of herbage from each subplot were collected the day before grazing in late winter/early spring, late spring, summer and autumn (August/September, November, January and April respectively). Hand shears were used to cut six 0.5-m strips per subplot to the approximate post-grazing height (40 mm). All five replicates were sampled in Northland and Canterbury, while only three replicates were sampled at the Waikato site. In the laboratory, samples were blended and a subsample dissected into the following categories: perennial ryegrass leaf, perennial ryegrass reproductive stem (including seed head), white clover, unsown species and dead material. Dissected subsamples were oven-dried at 95°C to constant weight (approximately 48 hr) and weighed to determine botanical composition as a proportion of above-residual herbage biomass on a DM basis. At the Northland site in late winter 2011, volunteer ryegrass plants were identified that appeared visually different from the sown ryegrass. Tillers from a number of these plants were genotyped using single sequence repeat (SSR) markers (Moon et al., 1999) and a large proportion identified as being infected with *Neotyphodium occultans*, the endophyte that frequently infects annual ryegrass species, indicating the volunteers were annual ryegrass types. Therefore, samples from Northland also included the category, annual ryegrass, as identified by the following criteria: leaves ≥6 mm wide, large clasping auricles and a rolled emerging leaf. Seedhead with awns were also included in this category.

From the Northland and Waikato late winter/early spring 2011 samples, and for all seasons at all three sites during the three subsequent years, a second representative subsample (~150 g fresh weight) of the undissected herbage from the 12 and 24 kg/ha seed treatments of all cultivars was oven-dried at 60°C and ground to pass through a 1-mm sieve (Christy Mill, Suffolk, UK). Dried samples were analysed for crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and organic matter digestibility (OMD) content using near-infrared spectroscopy (Corson, Waghorn, Ulyatt, & Lee, 1999).

2.12 | Invertebrate pest populations

In March 2011, the invertebrate populations at each site were assessed before sowing. At each site, four spade squares (200 × 200 × 200 mm deep) were removed on a transect across each replicate; these were crumbled by hand in the field, and all soil macro-invertebrates identified and counted. Ten soil cores (25 × 100 mm depth) were also taken on the same transect for nematode extraction. These were bulked per replicate, mixed and hand-crumbled, and a 100 g subsample was used for extraction using the method of Bell and Watson (2001). Total nematodes were counted and plant feeders were identified to the genus level. A modified blower vacuum with a 100-mm-diameter collection sleeve was used to estimate aboveground invertebrate densities along two transects (50 m) across the centre of each trial site. Collected invertebrates were kept in cool storage until identification in the laboratory.

Following establishment of the plots, invertebrate populations in the 6 and 30 kg/ha subplots of cv. Alto, Halo and Nui were assessed annually at the Northland and Waikato sites. The intended timing of sampling was autumn each year; however, on occasion, this was delayed to early winter due to drought/low summer rainfall conditions. At the Canterbury site, the 6 and 30 kg/ha subplots of cv.

Alto, Halo and Nui were sampled in April 2014, May 2015 and June 2016. Before that, sampling had been restricted to the 6 and 30 kg/ha subplots of Nui, with samples taken in May and October 2012 and October 2013. At each site, 10 soil cores (100 mm diameter × 140 mm depth) were taken in each of the specified subplots. The top 20 mm of the cores were taken from each subplot, bulked per replicate and subsequently placed in a Berlese funnel to extract invertebrates found in the litter. The remainder of the cores were crumbled by hand, and all soil invertebrates were identified and counted. Likewise, 10 soil cores (25 mm × 100 mm depth) were taken in each of the specified subplots for extraction of nematodes. These were processed in the same way as the pre-sowing samples.

2.13 | Statistical analysis

Data were analysed using Restricted Maximum Likelihood (REML) in GenStat 14.1 (VSN International Ltd., 2011) with cultivar (main plot), seeding rate (subplot), site and their interactions as fixed effects, and block, main plot within block and subplot within main plot as random effects. For botanical composition data, both untransformed and angular transformed data were analysed; similar conclusions were drawn from both analyses; therefore, untransformed data are presented here. Fisher's protected least significant difference test was used to identify significant differences between treatment means. Analysis of total herbage accumulation over the 4 years was performed using the three replicates that were sampled each of the 4 years.

In the genotypic analysis, a binomial test implemented in GenStat v18 (2015) was used to test the null hypothesis, that the proportion of endophyte in the 12 kg/ha treatment was equal to that in the 30 kg/ha treatment, within a cultivar. Ryegrass SSR data were used in GenAlEx 6.5 software (Peakall and Smouse, 2006, 2012) to calculate gene diversity (H_E) within populations and G'_{ST} (Hedrick, 2005), an analogue of Wright's F_{ST} (Wright, 1969), as an estimate of differentiation among populations.

Analyses of the invertebrate populations used a generalized estimating equations (GEE) approach, to model potential within-plot correlation among counts of individual species. The groups were defined by combination of two factors: cultivar (Alto, Halo or Nui) and seeding rate (6 or 30 kg/ha), with analysis examining effects of cultivar and seeding rate, and cultivar × seeding rate interactions. Soil invertebrate pest numbers were analysed using statistical software SAS version 9.3 (SAS, 2011), while nematodes numbers were analysed using R (R Core Team, 2014).

3 | RESULTS

3.1 | Climate

Conditions following the sowing of the trials in autumn 2011 were generally favourable for germination and sward establishment. Rainfall in autumn 2011 at all sites was similar to, or greater than, the long-term average, while winter rainfall was similar to, or slightly lower than, the long-term average (Figure 1). Significant climatic events during the 5 years included flooding at the Northland site in July 2014 (503 mm rainfall in 4 days; 144% more than the long-term average for July), which left swards under water for approximately 3 days. At the Waikato site, significant periods of low summer rainfall were experienced in two of the 5 years: in year two from January to March 2013 (3-month total rainfall of 50 mm; 23% of the long-term average, most severe drought in 40 years; Mullen & Porteous, 2013) and in year three from January to March 2014 (3-month total rainfall of 59 mm; 27% of the long-term average). Summer in years four and five were also drier than normal, though not to the same extent as the previous 2 years (3-month total rainfall of 182 and 184 mm, respectively; 75% of the long-term average). These periods of low rainfall at the Waikato site were combined with warmer than average (by 1–4°C) monthly maximum temperatures.

3.2 | Evidence of interactions

There were few significant seeding rate × cultivar × site interactions or cultivar × site interactions identified within the herbage-related measurements (Table 1). There were, however, seeding rate × site interactions evident within the herbage accumulation, tiller density and botanical composition data (Table 1). In these cases, individual site data are presented. In addition, there were very few seeding rate × cultivar interactions evident in the herbage-related measurements; thus, this paper will focus solely on the effects of seeding rate and cultivar.

3.3 | Herbage accumulation

At the Northland site, seeding rate effects on annual herbage accumulation were evident for the first 3 years after sowing, with the 6–24 kg/ha treatments yielding less than the 30 kg/ha treatment during the first and second years ($p < .001$), and the 12 kg/ha treatment yielding less than the 24 and 30 kg/ha treatments during the third year ($p < .05$; Table 2). At the Waikato site, annual herbage accumulation was similar for all seeding rates for all 4 years ($p > .05$). At the Canterbury site, the low seeding rates (6 and 12 kg/ha) yielded less than the 24 and 30 kg/ha treatments during the first year after sowing, with the 18 kg/ha treatment intermediary ($p < .01$). All treatments had similar herbage accumulation during the second year, but during the third year, the 6 kg/ha treatment yielded less than all others ($p < .001$). At the Northland and Canterbury sites, significant seeding rate effects on annual herbage accumulation were a result of significant effects during each season, with the exception of summer 2012/13, winter 2013 and autumn 2014 at the Northland site.

Herbage accumulation over the first 4 years when totalled was similar across seeding rate treatments at the Waikato site ($p > .05$; Table 2). At the Canterbury site, the 18 and 30 kg/ha treatments yielded more than the 12 kg/ha treatment, with the 24 kg/ha treatment intermediary ($p < .001$). The 6 kg/ha treatment yielded less than all other seeding rate treatments. At the Northland site, the

TABLE 1 Evidence of regional differences in the herbage-related data

	Statistical significance		
	Seeding rate × site	Cultivar × site	Seeding rate × cultivar × site
Annual herbage accumulation			
2011/12	n.s.	n.s.	n.s.
2012/13	†	n.s.	n.s.
2013/14	**	n.s.	n.s.
2014/15	n.s.	n.s.	n.s.
Four-year total	***	n.s.	n.s.
2015/16	–	n.s.	–
Tiller density			
Autumn 2011	***	n.s.	*
Spring 2011	***	n.s.	n.s.
Summer 2011/12	**	n.s.	n.s.
Autumn 2012	*	n.s.	n.s.
Autumn 2013	n.s.	n.s.	n.s.
Autumn 2014	**	n.s.	n.s.
Autumn 2015	n.s.	*	n.s.
Autumn 2016	–	n.s.	–
Endophyte infection frequency			
Autumn 2012	n.s.	n.s.	n.s.
Autumn 2013	n.s.	**	n.s.
Autumn 2014	n.s.	n.s.	n.s.
Autumn 2015	n.s.	n.s.	n.s.
Autumn 2016	–	n.s.	–
Botanical composition[a]			
Perennial ryegrass content	* (5 occasions) n.s. (11 occasions)	* (10 occasions) n.s. (10 occasions)	n.s. (all 16 occasions)
White clover content	* (6 occasions) n.s. (10 occasions)	* (2 occasions) n.s. (18 occasions)	* (3 occasions) n.s. (13 occasions)
Unsown species content	* (4 occasions) n.s. (12 occasions)	* (6 occasions) n.s. (14 occasions)	* (1 occasion) n.s. (15 occasions)
Nutritive value characteristics[a]			
Crude protein	* (2 occasions) n.s. (10 occasions)	n.s. (all 12 occasions)	n.s. (all 12 occasions)
Acid detergent fibre	n.s. (all 12 occasions)	* (4 occasions) n.s. (8 occasions)	* (1 occasion) n.s. (11 occasions)
Neutral detergent fibre	* (1 occasion) n.s. (11 occasions)	* (3 occasions) n.s. (9 occasions)	n.s. (all 12 occasions)
Organic matter digestibility	* (2 occasions) n.s. (10 occasions)	* (4 occasions) n.s. (8 occasions)	n.s. (all 12 occasions)

– = only the 18 kg/ha seeding rate treatment was measured in 2015/16.
[a]Botanical composition data were available on 16, 20 and 16 occasions (i.e., seasons × years) for the Northland, Waikato and Canterbury sites respectively. Nutritive value data were available on 12 occasions for each site. For each variable, the number of occasions for which a significant or not significant interaction was detected is shown.
$^{\dagger}p < .1$; $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; n.s., not significant ($p > .05$).

30 kg/ha treatment yielded more than the 24 kg/ha treatment, which in turn yielded more than the 12 kg/ha treatment, with the 6 and 18 kg/ha treatments intermediary ($p < .001$).

No cultivar effects were evident in the total annual herbage accumulation data across the three sites in any of the 5 years ($p > .05$; data not shown).

TABLE 2 Seeding rate effects on annual herbage accumulation (t DM per ha) at three sites in New Zealand. Annual data are from 1 June to 31 May each year

	Seeding rate treatment (kg/ha)						
	6	12	18	24	30	*SED*	Statistical significance
Northland							
2011/12	13.2	13.5	14.3	13.8	16.7	0.72	***
2012/13	15.8	15.7	16.8	17.1	18.6	0.65	***
2013/14	16.5	15.8	16.3	17.5	17.3	0.55	*
2014/15	12.6	13.0	12.9	12.8	13.9	0.54	n.s.
Four-year total	58.1	57.9	60.2	61.3	66.4	1.53	***
2015/16[a]			14.9				
Waikato							
2011/12	17.2	16.6	17.5	16.9	17.7	0.73	n.s.
2012/13[b]	8.9	9.4	9.8	9.3	9.4	0.43	n.s.
2013/14	6.7	6.8	6.8	7.6	7.1	0.40	n.s.
2014/15	8.2	7.8	7.5	8.3	7.9	0.39	n.s.
Four-year total[c]	41.7	40.2	43.0	42.7	42.5	1.15	n.s.
2015/16[d]			10.7				
Canterbury							
2011/12	14.7	14.7	16.2	16.4	17.7	0.76	**
2012/13	20.5	21.7	22.1	22.1	22.5	0.80	n.s.
2013/14[b,e]	12.1	14.1	14.3	14.4	14.8	0.46	***
2014/15	9.3	9.8	10.1	10.4	9.8	0.48	n.s.
Four-year total[c]	57.2	60.9	64.4	64.1	66.3	1.63	***
2015/16[d]			15.6				

SED, standard error of the difference between means; *$p < .05$; **$p < .01$; ***$p < .001$; n.s., not significant ($p > .05$).
[a]Only the 18 kg/ha treatment was sampled in 2015/16.
[b]During December 2012 and August 2013 at the Waikato and Canterbury sites, respectively, the method changed from quadrat cuts to 40 mm residual height on three replicates to harvester cuts to 55 mm residual height on five replicates.
[c]The total herbage accumulation over the 4 years at the Waikato and Canterbury sites does not equal the sum of the four individual years because only data from the three replicates that were sampled for the entire 4 years could be used for analysis.
[d]During April 2015 and September 2015 at the Canterbury and Waikato sites, respectively, the lowest cut height on the harvester was modified from 55 mm residual height to 40 and 45 mm at the Canterbury and Waikato sites respectively.
[e]Annual herbage accumulation during 2013/2014 is underestimated as data from the May grazing could not be collected.

3.4 | Genetic characteristics

Using the SSR marker B11, tillers containing AR37 endophyte produced an amplicon of 132 ± 0.4 base pairs while those containing SE amplified a fragment of 179 ± 0.4 base pairs. Four years post-sowing, the frequency of the two strains was not significantly different between the 12 and 30 kg/ha seeding rate treatment populations within each cultivar population (Table 3).

TABLE 3 Level of endophyte infection by strain in Alto and Nui ryegrass populations at the Waikato site 4 years after sowing

Cultivar	Seeding rate treatment (kg/ha)	Infection by AR37 (%)	Infection by SE (%)	Nil endophyte (%)
Alto AR37	12	74[a]	12[a]	13[a]
Alto AR37	30	74[a]	7[a]	19[a]
Nui SE	12	2[b]	93[b]	5[b]
Nui SE	30	2[b]	91[b]	7[b]

Data with different letters in a column are significantly different ($p < .01$) as established by binomial proportion test.

TABLE 4 Pairwise genetic differentiation between Alto and Nui ryegrass populations at the Waikato site 4 years after sowing

	Alto AR37 12 kg/ha	Alto AR37 30 kg/ha	Nui SE 12 kg/ha	$H_E \pm$ SEM
Alto AR37 12 kg/ha	–			0.690 ± 0.025
Alto AR37 30 kg/ha	0.000	–		0.695 ± 0.025
Nui SE 12 kg/ha	0.194	0.199	–	0.677 ± 0.029
Nui SE 30 kg/ha	0.194	0.198	0.003	0.664 ± 0.032

A value of 0 indicates identical populations and a value of 1 indicates no alleles in common (complete differentiation). Within-population gene diversity (H_E), averaged across loci for each population, is shown in the rightmost column.

Genotyping the four population samples with 14 ryegrass SSR markers yielded a total of 152 polymorphic alleles across all populations. Genetic differentiation between populations was assessed using the statistic G'_{ST} and was found to range from 0.194 to 0.199 between Alto AR37 and Nui SE cultivar populations and 0.000–0.003 when comparing seeding rate treatments within the respective cultivars (Table 4). These data indicate moderate-to-strong differentiation due to cultivar ($p < .05$) but no differentiation due to seeding rate treatment ($p > .05$). Within-population genetic variation was estimated using Nei's gene diversity (H_E). Mean H_E across all loci ranged from 0.664 to 0.695, with higher values observed for the Alto AR37 populations compared with the Nui SE populations (Table 4), although differences were not significant by t test. There was no significant difference in H_E between seeding rate treatments within either cultivar.

3.5 | Population characteristics

3.5.1 | Perennial ryegrass tiller density

In autumn 2011 (7 weeks after sowing), increases in perennial ryegrass tiller density with increasing seeding rate were detected at all three sites ($p < .001$; Figure 2). This seeding rate effect had dissipated by autumn 2012 at the Waikato site and by autumn 2013 at the Canterbury site. At the Northland site, no significant seeding rate effect was detected in autumn 2012 ($p > .05$); however, in autumn 2013, 2014 and 2015, the 6 kg/ha treatment had lower tiller density

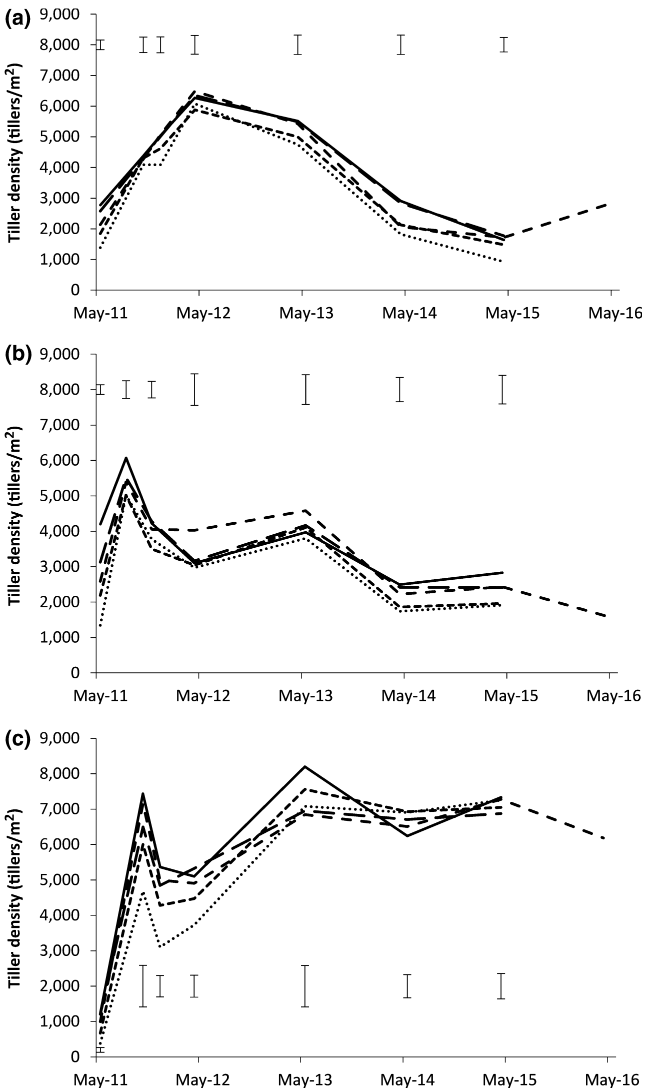

FIGURE 2 Perennial ryegrass tiller density in swards sown at different seeding rate treatments in late March/early April 2011 at the (a) Northland, (b) Waikato and (c) Canterbury sites. Seeding rate treatments were 6 (dotted line), 12 (short dashed line), 18 (medium dashed line), 24 (long dashed line) and 30 (solid line) kg seed per ha. Bars are $LSD_{0.05}$ values. Only the 18 kg/ha treatment was sampled in autumn 2016

than the 24 and 30 kg/ha seeding rate treatments (p = .06, p < .01 and p < .01; Figure 2a).

Cultivar effects on tiller density were evident for the first 4 years of the experiment, with the exception of summer 2011/12 (Table 5). Commando consistently had the greatest tiller density, while Nui was among the lowest.

3.5.2 | Endophyte infection frequency

Endophyte infection level in autumn each year was similar across seeding rate treatments (p < .05), averaging 83%, 86%, 90% and 86% in May 2012, 2013, 2014 and 2015 respectively. In autumn 2016, endophyte infection level in the 18 kg/ha treatment remained at 86%.

TABLE 5 Cultivar effects on mean perennial ryegrass tiller density (tillers/m^2) across three sites in New Zealand

	Cultivar					Statistical
	Alto	Commando	Halo	Nui	*SED*	significance
Autumn 2011	1,749	2,111	1,850	1,934	113	*
Spring 2011	5,239	5,665	5,182	4,662	312	*
Summer 2011/12	4,755	4,514	4,358	4,318	331	n.s.
Autumn 2012	5,153	5,602	4,622	4,430	222	***
Autumn 2013	6,026	6,835	5,072	5,198	377	***
Autumn 2014	4,181	4,750	3,599	3,312	229	***
Autumn 2015	3,864	4,826	3,484	3,287	198	***
Autumn 2016	3,508	4,196	3,175	3,235	501	n.s.

SED, standard error of the difference between means; *p < .05; ***p < .001; n.s., not significant (p > .05).

During the first 4 years across all sites, Commando had a lower endophyte infection level than the other three cultivars (p < .01; average of 81% vs. 88%). These cultivar effects had dissipated by autumn 2016 (p > .05), where all cultivars averaged 86% across the three sites.

3.6 | Sward community characteristics

3.6.1 | Botanical composition

Overall, at the two North Island sites, there were times when the content of weed species or clover was similar to, or greater than, the content of perennial ryegrass (e.g., at the Northland site in summer 2013/14 and 2014/15 and at the Waikato site in summer 2014/15, autumn 2015, summer 2015/16 and autumn 2016; Figures 3–5). In comparison, the predominant species in all treatments at the Canterbury site over the 5 years was perennial ryegrass (Figure 3c).

At the Northland site, herbage from the 6 kg/ha treatment generally contained less perennial ryegrass than the 18–30 kg/ha treatments throughout the 4 years (p < .05; Figure 3a), with the exceptions of late spring 2012 and late winter/early spring 2014 (p > .05). This trend was also evident (p < .05) at the Waikato and Canterbury sites to a lesser extent (i.e., only significant during nine and seven seasons of the 16 seasons analysed, respectively; Figure 3b,c). There were no significant effects of seeding rate on perennial ryegrass content beyond late winter/early spring 2014 at the Waikato site or beyond late spring 2013 at the Canterbury site.

At all three sites, herbage from the 6 kg/ha treatment contained more white clover than the 18–30 kg/ha treatments throughout the first year after sowing (p < .01; Figure 4). After this, seeding rate differences were less consistent. Herbage from the 6 kg/ha treatment contained more white clover than the 18–30 kg/ha treatments during six seasons at the Waikato site (p < .05) and five seasons at the Canterbury site (p < .05). Herbage from the 6 kg/ha treatment also contained more white clover than the 30 kg/ha treatment during four seasons at the Northland site (p < .05), one season at the Waikato site (p < .01) and three seasons at the Canterbury site (p < .05).

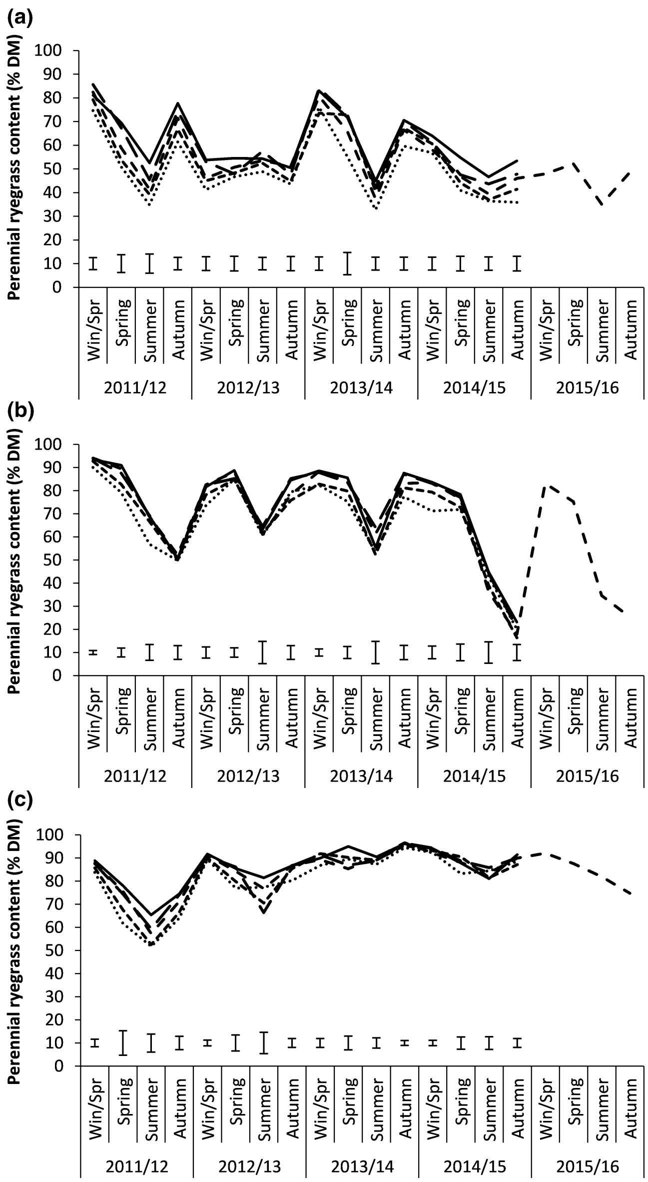

FIGURE 3 Seasonal perennial ryegrass content (% DM) in herbage above 40 mm in swards sown at different seeding rate treatments at the (a) Northland, (b) Waikato and (c) Canterbury sites. Seeding rate treatments were 6 (dotted line), 12 (short dashed line), 18 (medium dashed line), 24 (long dashed line) and 30 (solid line) kg seed per ha. Bars are $LSD_{0.05}$ values. Only the 18 kg/ha treatment was sampled in 2015/16

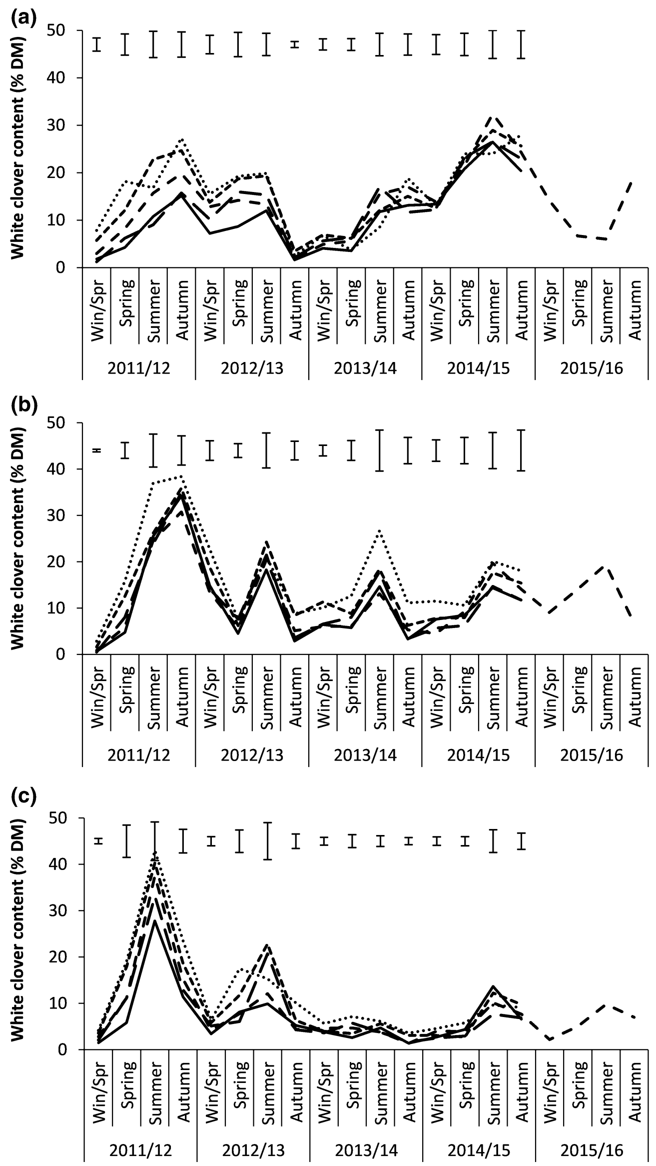

FIGURE 4 Seasonal white clover content (% DM) in herbage above 40 mm in swards sown at different seeding rate treatments at the (a) Northland, (b) Waikato and (c) Canterbury sites. Seeding rate treatments were 6 (dotted line), 12 (short dashed line), 18 (medium dashed line), 24 (long dashed line) and 30 (solid line) kg seed per ha. Bars are $LSD_{0.05}$ values. Only the 18 kg/ha treatment was sampled in 2015/16

No significant seeding rate effects on white clover content were recorded at any site during summer 2014/2015 or autumn 2015.

Volunteer weeds or unsown species, including *Poa annua*, summer-active C_4 annuals and broadleaf species such as *Taraxacum officinale*, *Crepis capillaris*, *Plantago lanceolata* and *Rumex obtusifolius* were present at all sites, particularly the North Island sites. In late winter/early spring 2011, the content of unsown species was greatest in the 6 kg/ha treatment compared with the 24 and 30 kg/ha treatments at all sites ($p < .05$; Figure 5). This effect was still evident in late spring 2011 at the Northland and Canterbury sites ($p < .05$), but not the Waikato site. At the Northland site, seeding rate effects were detected on the content of unsown species for the remainder of the first 4 years, with herbage from the 6 kg/ha treatment containing more unsown species than all other treatments ($p < .001$; three occasions), more than the 18–30 kg/ha treatments ($p < .05$; two occasions) or more than the 30 kg/ha treatment alone ($p < .05$; five occasions). At the Waikato site, seeding rate effects on the content of unsown species were detected on two further occasions; summer 2013/14 ($p < .05$; 12 and 30 kg/ha contained more than 6 and 24 kg/ha) and late winter/early spring 2014 ($p < .05$; 6 kg/ha contained more than 24 and 30 kg/ha). At the Canterbury site after

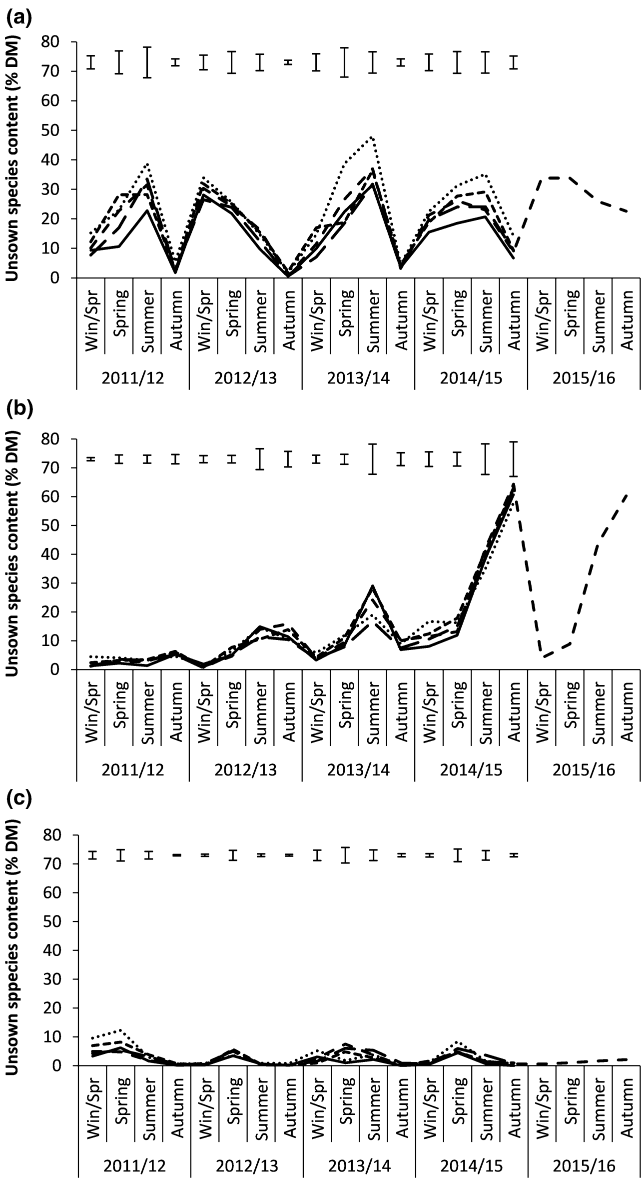

FIGURE 5 Seasonal content of unsown species (% DM) in herbage above 40 mm in swards sown at different seeding rate treatments at the (a) Northland, (b) Waikato and (c) Canterbury sites. Seeding rate treatments were 6 (dotted line), 12 (short dashed line), 18 (medium dashed line), 24 (long dashed line) and 30 (solid line) kg seed per ha. Bars are $LSD_{0.05}$ values. Only the 18 kg/ha treatment was sampled in 2015/16

late spring 2011, no further effect of seeding rate on unsown species content was detected ($p > .05$).

The greatest component of the unsown species at the Northland site was the volunteer annual ryegrass (45%–100% of the unsown species component), making up, on average, 15%, 21%, 25% and 3% of total herbage above 40 mm during late winter/early spring, late spring, summer and autumn respectively. During the first 4 years, content of the volunteer annual ryegrass was greater (47% more on average) in herbage from the 6 kg/ha treatment compared with the 30 kg/ha treatment for nine of the 16 seasons analysed ($p < .05$; data not shown).

Over the 5 years of the experiment, the greatest variation in botanical composition occurred at the Waikato site. During the last 2 years in particular, these swards alternated from being perennial ryegrass dominant through winter and spring (≥75%) to being dominated by unsown species during autumn (≥60%). These unsown species largely consisted of *Paspalum dilatatum*, *Crepis capillaris* and *Digitaria sanguinalis*.

Cultivar effects on ryegrass content across the three sites were evident during 11 of the 20 seasons, whereby pastures sown with Nui generally had lower ryegrass content than those sown with one or more of the other cultivars ($p < .05$; data not shown). Conversely, during 10 of the 20 seasons, pastures sown with Nui generally had greater white clover content than those sown with one or more of the other cultivars ($p < .05$; data not shown). The cultivar effects were generally less than those of seeding rate.

3.6.2 | Nutritive value characteristics

During late winter/early spring 2011, herbage from the 6 and 12 kg/ha treatments had greater OMD and CP content than the 24 and 30 kg/ha treatments, as well as lower ADF and NDF content (Table 6). After the first year, differences between the 12 and 24 kg/ha treatments were sporadic (Table 6). Herbage from the 12 kg/ha treatment had lower fibre (ADF and NDF) content compared with 24 kg/ha in late spring 2012 and greater fibre content in summer 2013/14. Herbage from the 12 kg/ha treatment had greater OMD compared with the 24 kg/ha treatment during late spring 2012 and late winter/early spring 2013 and lower OMD during summer 2012/13 and 2013/14.

3.7 | Invertebrate pest populations

Soil macro-invertebrate pest populations at all sites pre-sowing were low (<10 per m^2), substantially below the thresholds considered to cause damage to new pasture swards. Soil nematode populations were 4,370, 3,018 and 1,580 per 100 g dry soil for the Northland, Waikato and Canterbury sites, respectively, and included a range of plant-feeding genera (root knot, cyst, lesion, pin and spiral).

At the Northland site, clover root weevil (*Sitona obsoletus*, formerly *S. lepidus*) populations increased after sowing, with populations towards the end of the fourth year (191 per m^2) approaching damaging levels (~300 per m^2; Gerard, Hackell, & Bell, 2007). Towards the end of the fourth year, the population of root-knot nematodes (*Meloidogyne* spp.; one of the most damaging plant-feeding nematode genera; Perry, Moens, & Starr, 2009) was also greater at the Northland site compared with the other two sites (41, 24 and 4 per 100 g of dry soil for the Northland, Waikato and Canterbury sites, respectively), with a different species composition at each site (*M. naasi* and *M. trifoliophila* in Northland; *M. hapla* and *M. trifoliophila* in Waikato and *M. naasi* in Canterbury, as determined by 18S DNA sequence matches >99%).

At the Waikato site, grass grub (*Costelytra zealandica*) populations increased after sowing, reaching significantly damaging levels of

TABLE 6 Seeding rate effects on mean seasonal nutritive value characteristics across three sites in New Zealand

	Seeding rate treatment (kg/ha)						Statistical significance
	6	12	18	24	30	*SED*	
Crude protein (% DM)							
Late winter/early spring 2011	19.9	18.8	18.0	18.0	17.5	0.33	***
Late spring 2012		18.9		18.3		0.33	n.s.
Summer 2012/13		21.1		21.3		0.23	n.s.
Autumn 2013		20.8		20.7		0.24	n.s.
Late winter/early spring 2013		24.4		24.1		0.30	n.s.
Late spring 2013		20.5		21.0		0.38	n.s.
Summer 2013/14		20.2		20.8		0.40	n.s.
Autumn 2014		26.6		26.5		0.22	n.s.
Late winter/early spring 2014		23.6		23.3		0.32	n.s.
Late spring 2014		17.7		18.1		0.36	n.s.
Summer 2014/15		18.8		18.6		0.33	n.s.
Autumn 2015		22.0		21.6		0.35	n.s.
Acid detergent fibre (% DM)							
Late winter/early spring 2011	20.1	20.4	20.7	21.0	21.3	0.16	***
Late spring 2012		24.4		25.0		0.19	**
Summer 2012/13		25.2		24.9		0.23	n.s.
Autumn 2013		24.2		24.0		0.39	n.s.
Late winter/early spring 2013		21.1		21.2		0.18	n.s.
Late spring 2013		23.6		23.6		0.17	n.s.
Summer 2013/14		25.1		24.4		0.29	*
Autumn 2014		23.1		23.2		0.15	n.s.
Late winter/early spring 2014		21.1		21.4		0.13	n.s.
Late spring 2014		23.7		23.9		0.15	n.s.
Summer 2014/15		24.3		24.3		0.21	n.s.
Autumn 2015		23.6		23.7		0.30	n.s.
Neutral detergent fibre (%DM)							
Late winter/early spring 2011	35.6	36.6	37.5	38.5	39.1	0.48	***
Late spring 2012		45.8		47.2		0.54	*
Summer 2012/13		40.2		39.9		0.47	n.s.
Autumn 2013		42.0		42.5		0.44	n.s.
Late winter/early spring 2013		40.7		41.6		0.40	*
Late spring 2013		43.3		43.1		0.39	n.s.
Summer 2013/14		45.5		44.1		0.54	*
Autumn 2014		42.1		42.5		0.37	n.s.
Late winter/early spring 2014		42.1		42.5		0.37	n.s.
Late spring 2014		47.5		48.0		0.34	n.s.
Summer 2014/15		49.0		49.9		0.47	n.s.
Autumn 2015		51.2		52.1		0.67	n.s.
Organic matter digestibility (% DM)							
Late winter/early spring 2011	94.1	93.7	92.9	92.0	90.8	0.46	***
Late spring 2012		86.6		85.5		0.47	*
Summer 2012/13		79.3		80.6		0.43	*
Autumn 2013		73.9		73.8		0.48	n.s.
Late winter/early spring 2013		91.2		90.5		0.29	*

(Continues)

TABLE 6 (Continued)

	Seeding rate treatment (kg/ha)						
	6	12	18	24	30	*SED*	Statistical significance
Late spring 2013		88.5		88.4		0.40	n.s.
Summer 2013/14		79.2		80.8		0.44	***
Autumn 2014		88.0		87.6		0.35	n.s.
Late winter/early spring 2014		88.4		88.2		0.27	n.s.
Late spring 2014		85.0		84.8		0.24	n.s.
Summer 2014/15		79.2		79.0		0.41	n.s.
Autumn 2015		75.6		75.2		0.55	n.s.

SED, standard error of the difference between means; *$p < .05$; **$p < .01$; ***$p < .001$; n.s., not significant ($p > .05$).

>200 per m^2 (Ferguson, Popay, & Barrett, 2012) by the start of the fifth year after sowing. There was also likely to be a damaging effect of black beetle (*Heteronychus arator*) at the Waikato site. Although the population, on average, remained below damaging levels of 20 per m^2 (Watson & Marsden, 1982), in years three, four and five, about 10% of the subplots had more than 20 black beetle present per m^2.

At the Canterbury site, clover root weevil populations approached damaging levels in May 2012 (240 ± 73.7 larvae per m^2 in the sampled Nui plots). By October 2012, clover root weevil densities had increased (consistent with overwintering recruitment of larvae), with greater populations in the 6 kg/ha treatment compared with the 30 kg/ha treatment ($p < .001$; 430 vs. 189 larvae per m^2 respectively). Subsequent sampling in autumn 2014 and 2015 and early winter 2016 indicated that clover root weevil larval populations had declined to below damaging levels (McNeill, van Koten, Cave, Chapman, & Hodgson, 2016). By early winter 2016, grass grub densities were generally high (154 ± 190.3 across all sampled subplots), reaching damaging levels in a number of subplots. The other invertebrate pests generally associated with damage and pasture sward yield losses generally occurred at densities unlikely to impact markedly on production during the 5 years.

Seeding rate effects on macro-invertebrate and nematode populations were relatively minor. At the Waikato site, clover root weevil larvae were more abundant in the 6 kg/ha treatment compared with the 30 kg/ha treatment 1 year after sowing ($p < .05$; 111 vs. 81 per m^2). This was also evident at the Canterbury site in April 2014 and April 2015 ($p < .05$; 75 vs. 40 per m^2 in April 2014 and 47 vs. 34 per m^2 in April 2015). Over the first 4 years after sowing, there was also a consistent trend for greater grass grub populations in the 6 kg/ha treatment compared with 30 kg/ha at the Waikato site ($p = .058$; 106 vs. 86 per m^2); an effect which was also evident at the Canterbury site in April 2015 ($p < .01$; 75 vs. 42 per m^2).

At both the Waikato and Canterbury sites, cyst nematode populations (likely *Heterodera trifolii*) were greater in the 6 kg/ha treatment compared with the 30 kg/ha treatment during most years ($p < .05$; 58 vs. 24 per 100 g dry soil at the Waikato site and 55 vs. 32 per 100 g dry soil at the Canterbury site, averaged across the 4 years after sowing). In addition, the mean number of lesion nematodes (*Pratylenchus*) at the Canterbury site was significantly larger for Nui in the 30 kg/ha treatment compared with 6 kg/ha ($p < .05$; 1.2 vs. 0.7 per 100 g dry soil respectively).

Likewise, cultivar effects were also relatively minor. At the Northland site, a weak cultivar effect became evident towards the end of the fourth year, with more clover root weevil found beneath Nui than Commando (234 vs. 152 per m^2) with Alto and Halo (166 and 213 per m^2, respectively) intermediate ($p = .097$). At the Waikato site, combined data from the first 4 years showed a significant cultivar effect on black beetle populations, with significantly more beneath Commando than Halo (7.6 vs. 1.3 per m^2) with Alto and Nui (4.8 and 4.1 per m^2, respectively) intermediate ($p < .01$). There were also more lesion nematodes found beneath Halo than Alto ($p < .001$; 190 vs. 101 per 100 g dry soil respectively). This cultivar effect on lesion nematodes was also seen at the Canterbury site with more found under Halo than Nui in the 6 kg/ha treatment ($p < .05$; 1.2 vs. 0.7 per 100 g of dry soil respectively). *Helicotylenchus* (spiral nematodes) also showed a cultivar effect, with significantly larger populations under Alto and Halo than Nui ($p < .05$; 2.9, 4.2 and 1.5 per 100 g of dry soil for Alto, Halo and Nui respectively).

4 | DISCUSSION

No evidence has been found from this study to support the hypothesis that high perennial ryegrass seeding rates reduce long-term pasture sward persistence. There are numerous ways in which a lack of persistence of ryegrass-based swards sown at higher seeding rates could have been identified, including reduced annual herbage accumulation compared to the lower seeding rate treatments, reduced ryegrass tiller density and reduced survival of the original population of sown plants. Changes in botanical composition can also be a good indicator of trends in perennial ryegrass persistence in sown swards, as any diminution in the intensity of competition from ryegrass allows other species (such as white clover) to increase their presence in the sward. There was no evidence of any of these effects. Seeding rate effects on annual herbage accumulation had dissipated at all sites by the end of the third year after sowing, resulting in similar herbage accumulation across seeding rate treatments at each site during year four. Similarly, during the fourth year, there was no negative impact of higher seeding rates on tiller density or ryegrass content, or positive effect on the content of unsown species or white clover.

Population genetic analysis based on DNA markers has been used for a variety of applications in forages, including the discovery of genetic shifts in populations exposed to specific environments over time (Collins et al., 2012; Ghesquiere, Baert, Malengier, & De Riek, 2010). In the current study, genetic analysis by SSR markers of selected populations at the Waikato site 4 years after sowing found no differentiation between populations due to different seeding rates (comparing 12 and 30 kg/ha), nor any change in the genetic diversity within populations attributable to this treatment. These results suggest that no shift in population genetic structure or diversity occurred over 4 years as a consequence of applying different seeding rates.

To our knowledge, no other studies have investigated the effect of seeding rate on long-term pasture sward persistence. The longest studies (3 years) are consistent with trends reported here, with no negative impact of higher seeding rates on herbage accumulation, ryegrass tiller density or botanical composition of perennial ryegrass swards during the 3 years (Culleton et al., 1986; Heddle & Herriott, 1955; Keane, 1980). Although 4 years of study is shorter than farmers expect new swards to persist, the absence of significant seeding rate effects in the fourth year across all three sites (particularly in the range of seeding rates commonly used on farm; 12–30 kg/ha) indicates little value in continuing to compare all five seeding rate treatments.

While there was little evidence that the range of seeding rates commonly used on farm affects pasture sward persistence, there were other effects of seeding rate on pastures. Swards in the higher seeding rate treatments (24–30 kg/ha) initially had a greater density of perennial ryegrass tillers than those in the 6 kg/ha treatment and contained more perennial ryegrass, and less clover and unsown species. Herbage from these swards was also less digestible, with lower CP and fibre content than that from the lower seeding rate (6–12 kg/ha) treatments, likely due to differences in clover content. Tiller density differences generally dissipated by the start of the second (Northland and Waikato) or third (Canterbury) year after sowing, at least between treatments within the range of 12–30 kg/ha. This is likely a result of self-thinning processes (Harper & White, 1970), which are described in greater detail for this study by Lee et al. (2017). The length of time that the effects on botanical composition remained differed across the sites, with differences dissipating much earlier at the Waikato site than the Northland site. This is also true for differences in herbage accumulation which were never identified at the Waikato site, but remained for 3 years after sowing at the Canterbury and Northland sites. One important difference between the sites was the substantial ingress of the volunteer annual ryegrass at the Northland site. Several studies have concluded that one advantage of higher seeding rates is the greater initial ground cover to reduce weed ingress (Armstrong, Harrington, & Seefeldt, 2002; Culleton & Murphy, 1987; Heddle & Herriott, 1955). This was evident at all sites during the first year, although at the Waikato and Canterbury sites, even the 6 kg/ha treatment generally still had low (<10%) weed content. The benefit of a higher seeding rate lowering weed content only remained at the Northland site beyond the first year, with the greatest contributor being the volunteer annual ryegrass. Thus, in areas where there is greater potential for weed ingress (i.e., more weed/weed grass seeds in the seedbank and/or vigorous volunteer grasses), or where use of post-emergence herbicides has to be limited, a higher seeding rate may be beneficial in limiting ingress of unsown species. This certainly appears to be the case at the Northland site where the buried seed count of other grass seeds was extremely high (13,622 seeds per m^2), numbers which unfortunately are not unheard of in other regions of New Zealand (Bell, 1996).

No evidence was found that perennial ryegrass functional type affected pasture sward persistence based on the four cultivars tested. Pastures sown with Nui generally had less perennial ryegrass, both in terms of tiller population and contribution towards herbage harvested, at least compared with the diploids Alto and/or Commando. It is not known whether this may make swards more vulnerable in the future; thus, the 18 kg/ha seeding rate treatment swards will continue to be monitored to identify whether ryegrass functional type is a factor in long-term pasture sward persistence.

Persistence of the perennial ryegrass-based swards did differ across the three sites. At the Canterbury site, swards persisted well over the 4 years (i.e., relatively stable ryegrass tiller density and content and low content (<10%) of unsown species). This is not surprising as the Canterbury site represents a relatively benign environment for ryegrass where irrigation was used to minimize the impact of low summer soil moisture and the invertebrate pest burden was low for the first 4 years after sowing, at least for invertebrates that damage perennial ryegrass.

In comparison, there was evidence of ryegrass persistence failure at both the Northland and Waikato sites. At the Northland site, presence of volunteer annual ryegrasses was first observed 4 months after sowing. It was originally thought that a relatively high content (>15% of herbage above 40 mm from late winter to summer) of the short-term species would make swards more vulnerable to poor persistence, i.e., if the space occupied by the annual ryegrass plants was then taken over by undesirable weed or grass species instead of perennial ryegrass or white clover. However, annual ryegrasses continued to contribute strongly to the swards for the 5 years after sowing. When combined, both ryegrass species contributed 80%, 78%, 67% and 61% of the herbage above 40 mm during late winter/early spring, late spring, summer and autumn respectively. From a practical viewpoint, this situation at the Northland site does not necessarily have serious consequences for productivity as the volunteer species is productive with high nutritive value compared with other species such as kikuyu which are often components of Northland swards (Crush & Rowarth, 2007; Stewart, Kerr, Lissaman, & Rowarth, 2014). Together, the two ryegrass species retained dominance of the sward year-round.

Swards at the Waikato site, however, deteriorated to a state that was quite unstable. Unfortunately, due to the changes in the method for measuring herbage accumulation in December 2012 (2012/13 year) and September 2015 (2015/16 year), it is not possible to determine accurately the decline in annual herbage accumulation. However, by the fourth year after sowing, swards alternated between being dominated by perennial ryegrass in the winter/spring and by less productive, unsown species in the summer/autumn period. As the unsown species (largely hawksbeard and C_4 grasses) died in late autumn/early winter, the perennial ryegrass exploited the unoccupied space and returned to dominance. This presumably makes these swards more vulnerable, particularly if stressful events occur to prevent perennial ryegrass exploiting the unoccupied space.

Many interacting factors are likely to have contributed to the lack of ryegrass persistence at the Waikato site. During three of the 5 years, there were extended periods of very low summer rainfall. Combined with greater than average maximum temperatures and populations of grass grub and black beetle at damaging levels, this likely contributed to poor persistence. Notably, none of the treatments prevented this decline: pastures based on different seeding rates, ryegrass cultivars and ryegrass endophyte strains all showed the same trend. While the experimental design was not balanced for ryegrass cultivar and endophyte strain combinations, it is still possible to conclude that the ryegrass type generally expected to offer greatest persistence in this region (the old cultivar Nui, with standard endophyte SE) did not prevent population decline and weed ingress. Similarly, the novel endophyte AR37 which is considered equivalent to SE in protecting plants against insect damage (Thom et al., 2012) did not prevent population decline. Furthermore, recommended grazing management and fertiliser application practices for perennial ryegrass pastures were followed at all times, yet these did not prevent population decline either. Hence, it appears the environment (climate and pest populations, possibly interacting with soil type) dominated all other factors at this site and exceeded tolerance thresholds for the species. This has significant implications for the expectations of farmers and others for ryegrass persistence in locations across this region where similar environmental conditions occur. It is possible that productive perennial ryegrass pastures can only be sustained for 4–5 years in some situations, even when best ryegrass technology and management practices are used.

The negative impacts of factors such as soil moisture deficit (Boschma & Scott, 2000; Rawnsley, Donaghy, & Stevens, 2007), pest attack (Zydenbos et al., 2011) and weed ingress (Fulkerson, Slack, Moore, & Rolfe, 1993; Tozer et al., 2011) on the performance of ryegrass swards have been well established. Our findings regarding regional persistence issues are consistent with those published by Tozer et al. (2014), who undertook an *ex post* approach to "fast track" persistence outcomes and sampled survivor perennial ryegrass plants from paddocks of different age classes to generate a time contrast. Their survey was carried out in three regions of New Zealand, and they also reported improved pasture sward persistence in Canterbury compared with the upper North Island, likely due to irrigation and also greater herbicide use to control broad-leaved weeds.

5 | CONCLUSION

This experiment provides no evidence to support the hypothesis that high perennial ryegrass seeding rates reduce long-term pasture sward persistence. The initial impact of high seeding rates on swards had largely dissipated by the fourth year, resulting in swards with similar annual herbage accumulation, tiller density and botanical composition. While high seeding rates did not negatively impact sward persistence, geographical location did, with strong evidence of ryegrass population decline at the Waikato site for all treatment combinations, some decline in Northland and stable populations in Canterbury. It is possible that productive perennial ryegrass pastures can only be sustained for 4–5 years in some situations, even when best ryegrass technology and management practices are used.

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REFERENCES

Armstrong, C. S. (1977). 'Grasslands Nui' perennial ryegrass. *New Zealand Journal of Experimental Agriculture*, *5*, 381–384.

Armstrong, M. L., Harrington, K. C., & Seefeldt, S. S. (2002). Weed establishment in the second year after high pasture sowing rates. *New Zealand Plant Protection*, *55*, 116–120.

Bell, C. C. (1996). Subtropical seed-bank of a Bay of Plenty dairy farm. *Proceedings of the New Zealand Grassland Association*, *57*, 59–61.

Bell, N., & Watson, R. (2001). Optimising the Whitehead and Hemming tray method to extract plant parasitic and other nematodes from two soils under pasture. *Nematology*, *3*, 179–185.

Boschma, S. P., & Scott, J. M. (2000). Measuring and predicting the consequences of drought for a range of perennial grasses on the Northern Tablelands of New South Wales. *Australian Journal of Experimental Agriculture*, *40*, 285–297.

Brougham, R. W. (1952). Seeding rates of short rotation ryegrass. *Proceedings of the New Zealand Grassland Association*, *14*, 172–181.

Collins, R. P., Helgadottir, A., Frankow-Lindberg, B. E., Skot, L., Jones, C., & Skot, K. P. (2012). Temporal changes in population genetic diversity and structure in red and white clover grown in three contrasting environments in northern Europe. *Annals of Botany*, *110*, 1341–1350.

Corson, D. C., Waghorn, G. C., Ulyatt, M. J., & Lee, J. (1999). NIRS: forage analysis and livestock feeding. *Proceedings of the New Zealand Grassland Association*, *61*, 127–132.

Creighton, P., Gilliland, T. J., Delaby, L., Kennedy, E., Boland, T. M., & O'Donovan, M. (2012). Effect of *Lolium perenne* sward density on productivity under simulated and actual cattle grazing. *Grass and Forage Science*, *67*, 526–534.

Crush, J. R., & Rowarth, J. S. (2007). The role of C_4 grasses in New Zealand pastoral systems. *New Zealand Journal of Agricultural Research*, *50*, 125–137.

Culleton, N., & Murphy, W. E. (1987). Effects of seeding rates and of mixtures of *Lolium multiflorum* Lam. and *Lolium perenne* L. on productivity. *Irish Journal of Agricultural Research*, *26*, 93–103.

Culleton, N., Murphy, W. E., & O'Keefe, W. F. (1986). The role of mixtures and seeding rate in ryegrass productivity. *Irish Journal of Agricultural and Food Research*, *25*, 299–306.

Daly, M. J., Fraser, T., Perkins, A., & Moffat, C. M. (1999). Farmer perceptions of reasons for perennial pasture persistence and the relationship of these with management practice, species composition, and soil fertility. *Proceedings of the New Zealand Grassland Association*, *61*, 9–15.

Donald, C. M. (1951). Competition among pasture plants. I. Intraspecific competition among annual pasture plants. *Australian Journal of Agricultural Research*, *2*, 355–376.

Ferguson, C., Popay, A., & Barrett, B. (2012). Developing a value proposition for prediction, and minimisation, of invertebrate pest damage in New Zealand dairy systems. Report for the Pastoral 21 Project Team. RE400/2012/363, 24 pp.

Frame, J., & Boyd, A. G. (1986). Effect of cultivar and seed rate of perennial ryegrass and strategic fertilizer nitrogen on the productivity of grass/white clover swards. *Grass and Forage Science*, *41*, 359–366.

Fulkerson, W. J., Slack, K., Moore, K., & Rolfe, C. (1993). Management of *Lolium perenne/Trifolium repens* pastures in the subtropics. I. Effect of defoliation interval, seeding rate and application of N and lime. *Australian Journal of Agricultural Research*, *44*, 1947–1958.

Gerard, P. J., Hackell, D. L., & Bell, N. L. (2007). Impact of clover root weevil *Sitona lepidus* (Coleoptera: Curculionidae) larvae on herbage yield and species composition in a ryegrass-white clover sward. *New Zealand Journal of Agricultural Research*, *50*, 381–392.

Ghesquiere, A., Baert, J., Malengier, M., & De Riek, J. (2010). Botanical and genetic change in grass-clover based systems. In C. Huyghe (Ed.), *Sustainable use of genetic diversity in forage and turf breeding* (pp. 211–216). Dordrecht The Netherlands: Springer.

Hahn, H., Huth, W., Schoberlein, W., & Diepenbrock, W. (2003). Detection of endophytic fungi in *Festuca* spp. by means of tissue print immunoassay. *Plant Breeding*, *122*, 217–222.

Harper, J. L., & White, J. (1970). The dynamics of plant populations. *Proc. Adv. Study Inst. Dynamics Numbers Popul.(Oosterbeek)*, 41–63.

Heddle, R. G., & Herriott, J. B. D. (1954). The establishment, growth and yield of ultra-simple grass seeds mixtures in the south-east of Scotland. Part I. The effects of varying grass seed rates. *Grass and Forage Science*, *9*, 99–110.

Heddle, R. G., & Herriott, J. B. D. (1955). The establishment, growth and yield of ultra-simple grass seeds. Part II. The effects of varying grass and clover seed rates. *Grass and Forage Science*, *10*, 157–168.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution*, *59*, 1633–1638.

Hewitt, A. E. (1998). *New Zealand soil classification. Landcare research science series no. 1*. Lincoln: Manaaki Whenua, Landcare Research.

Keane, G. P. (1980). Effect of seeding rate on the production from new leys. *Irish Journal of Agricultural Research*, *19*, 141–145.

Kelly, S., Smith, E., & Brazendale, R. (2011). Pasture renewal in the Waikato and Bay of Plenty regions: An overview of farmer practice, experience and attitudes. In C. F. Mercer (Ed.), *Pasture persistence symposium. Grasslands research and practice series no. 15* (pp. 21–24). Hamilton, New Zealand: New Zealand Grassland Association.

Lee, J. M., Matthew, C., Thom, E. R., & Chapman, D. F. (2012). Perennial ryegrass breeding in New Zealand: A dairy industry perspective. *Crop and Pasture Science*, *63*, 107–127.

Lee, J. M., Thom, E. R., Chapman, D. F., Wynn, K., Waugh, D., & Rossi, L. (2017). High perennial ryegrass seeding rates reduce plant size and survival during the first year after sowing – Does this have implications for persistence? *Grass and Forage Science*, *72*, 382–400.

Leuchtmann, A., Bacon, C. W., Schardl, C. L., White, J. F., & Tadych, M. (2014). Nomenclatural realignment of *Neotyphodium* species with genus Epichloë. *Mycologia*, *106*, 202–215.

McNeill, M. R., van Koten, C., Cave, V., Chapman, D., & Hodgson, H. (2016). Does white clover (*Trifolium repens*) abundance in temperate pastures determine *Sitona obsoletus* (Coleoptera: Curculionidae) larval populations? *Frontiers in Plant Science*, *7*, 1397.

Moon, C. D., Tapper, B. A., & Scott, B. (1999). Identification of *Epichloë* endophytes in planta by a microsatellite-based PCR fingerprinting assay with automated analysis. *Applied and Environmental Microbiology*, *65*, 1268–1279.

Mullen, B., & Porteous, A. (2013). *2012-13 drought: A summary*. National Institute of Water and Atmospheric Research, New Zealand. https://www.niwa.co.nz/sites/niwa.co.nz/files/2012-2013%20drought%20-%20a%20summary.pdf

Nie, Z. N., Chapman, D. F., Tharmaraj, J., & Clements, R. (2004). Effects of pasture species mixture, management, and environment on the productivity and persistence of dairy pastures in south-west Victoria. 2. Plant population density and persistence. *Australian Journal of Agricultural Research*, *55*, 637–643.

Parsons, A. J., Edwards, G. R., Newton, P. C. D., Chapman, D. F., Caradus, J. R., Rasmussen, S., & Rowarth, J. S. (2011). Past lessons and future prospects: Plant breeding for yield and persistence in cool-temperate pastures. *Grass and Forage Science*, *66*, 153–172.

Peakall, R., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*, 288–295.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics*, *28*, 2537–2539.

Perry, R. N., Moens, M., & Starr, J. L. (Eds.) (2009). *Root-knot nematodes* (p. 488). Wallingford, UK: CAB International.

R Core Team (2014). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rawnsley, R., Donaghy, D., & Stevens, D. (2007). What is limiting production and consumption of perennial ryegrass in temperate dairy regions of Australia and New Zealand. Proceedings of the Australasian Dairy Science Symposium: Meeting the Challenges for Pasture-Based Dairying, pp. 256–276.

Rijswijk, K., & Brazendale, R. (2016). Pasture renewal practices, experiences and attitudes: A comparison over time. *Journal of New Zealand Grasslands*, *78*, 51–56.

Sartie, A., Matthew, C., Easton, H., & Faville, M. (2011). Phenotypic and QTL analyses of herbage production-related traits in perennial ryegrass (*Lolium perenne*, L.). *Euphytica*, *182*, 295–315.

SAS (2011). *SAS for Windows version 9.3*. Cary, NC: SAS Institute Inc.

Stewart, A., Kerr, G., Lissaman, W., & Rowarth, J. (2014). *Pasture and forage plants for New Zealand. Grassland research and practice series no. 8*. Dunedin, New Zealand: New Zealand Grassland Association.

Tait, A., Henderson, R., Turner, R., & Zheng, X. (2006). Thin plate smoothing spline interpolation of daily rainfall for New Zealand using a climatological rainfall surface. *International Journal of Climatology*, *26*, 2097–2115.

Tait, A., & Woods, R. (2007). Spatial interpolation of daily potential evapotranspiration for New Zealand using a spline model. *Journal of Hydrometeorology*, *8*, 430–438.

TEAGASC (2014). *Moorepark dairy levy research update: Pocket manual for reseeding*. Fermoy, Co Cork, Ireland: Moorepark Animal and Grassland Research and Innovation Centre.

Thom, E. R., Popay, A. J., Hume, D. E., & Fletcher, L. R. (2012). Evaluating the performance of endophytes in farm systems to improve farmer outcomes – a review. *Crop and Pasture Science*, *63*, 927–943.

Tozer, K. N., Bourdot, G. W., & Edwards, G. R. (2011). What factors lead to poor pasture persistence and weed ingress? In Mercer, C. F. (Ed.), *Pasture persistence symposium. Grasslands research and practice series no. 15* (pp. 129–138). Hamilton, New Zealand: New Zealand Grassland Association.

Tozer, K. N., Chapman, D. F., Bell, N. L., Crush, J. R., King, W. M., Rennie, G. M., . . . Aalders, L. T. (2014). Botanical survey of perennial ryegrass-based dairy pastures in three regions of New Zealand: Implications for ryegrass persistence. *New Zealand Journal of Agricultural Research*, *57*, 14–29.

Venuto, B. C., Redfearn, D. D., Pitman, W. D., & Alison, M. W. (2004). Impact of seeding rate on annual ryegrass performance. *Grass and Forage Science*, *59*, 8–14.

VSN International Ltd. (2011). *GenStat for Windows*, 14th ed. Hemel Hempstead, UK: VSN International Ltd.

Watson, R. N., & Marsden, R. S. (1982). Effects of black beetle (Coleoptera: *Scarabaeidae*) feeding on two grass species. In K. E. Lee (Ed.), *Proceedings of the 3rd Australasian conference on grassland invertebrate ecology* (pp. 107–115). Australasian Conference on Invertebrate Ecology: Adelaide, South Australia.

Wright, S. (1969). *Evolution and the genetics of populations*, Vol. *2*. Chicago, IL: University of Chicago Press.

Zydenbos, S. M., Barratt, B. I. P., Bell, N. L., Ferguson, C. M., Gerard, P. J., Mcneill, M. R., . . . Jackson, T. A. (2011). *The impact of invertebrate pests on pasture persistence and their interrelationship with biotic and abiotic factors*. In C. F. Mercer (Ed.), *Pasture persistence symposium. Grassland research and practice series no. 15* (pp. 109–117). Hamilton, New Zealand: New Zealand Grassland Association.

SUPPORTING INFORMATION

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