The Importance of Forage Legume Inclusion in Agricultural Swards to Enhance Earthworm Activity and Water Infiltration Rates

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Abstract
Increased grassland productivity in temperate regions has largely been achieved through perennial ryegrass *Lolium perenne* (PRG), coupled with large quantities of nitrogen fertiliser. However, concern is growing regarding the negative implications of excessive dependence on nitrogen fertilisers. Research has demonstrated the benefits of legume inclusion on primary productivity, however, their potential to influence other processes is less well established.

Sampling was undertaken in autumn 2017 on twenty randomised plots representing five sward types, replicated four times. These had been established and managed by cutting since 2013. Sward types included: **1)** PRG (250kg N ha^{-1} yr^{-1}); **2)** PRG; **3)** PRG and white clover *Trifolium repens*; **4)** 6 species mix comprised of PRG, timothy *Phleum pratense*, cocksfoot *Dactylis glomerata*, white clover, red clover *Trifolium pratensis* and greater birdsfoot trefoil *Lotus pedunculatus*; **5)** species included in mix 4 with the addition of ribwort plantain *Plantago lanceolate*, chicory *Cichorium intybus* and yarrow *Achillea millefolium*. Mixes 2-5 inclusive received 90kg N ha^{-1} yr^{-1}. Measurements included: soil bulk density, water infiltration rates, and estimated earthworm activity via surface cast counts.

Soil bulk density did not differ in response to sward type. However, highest infiltration rates were recorded within the PRG and white clover swards, with an average of 29.7 (±3.5) mm hr^{-1}, while lowest rates were recorded from the two PRG monocultures (2.43 (±0.5) and 4.2 (±1.2) mm hr^{-1} for the 90 and 250 kg N ha^{-1} yr^{-1} swards respectively). Surface cast numbers differed significantly between sward types ($P< 0.001$). Numbers ranged from 127 (±7) casts m^{-2} for PRG & white clover, to 48 (±5) casts m^{-2} for the PRG monocultures.

Our findings indicate the importance of legume inclusion within agricultural grasslands managed under reduced nitrogen fertiliser inputs for wider ecosystem service provision.

Introduction
Current reseeding advice and subsequent sward management and productivity in the Republic of Ireland, almost entirely revolves around perennial ryegrass *Lolium perenne* (PRG). This species currently accounts for 95% of forage grass seed sales per year (DAFM, 2018). PRG has many positive traits including the ability to yield high levels of good quality forage and to recover quickly following defoliation. However, it is also a nitrogen (N) demanding grass that quickly disappears from the sward when N levels become limiting (Sheridan et al., 2008). N inputs represent a significant direct cost to farmers and also contributes to wider environmental problems (Stark and Richards, 2008). In addition, there is increasing concern regarding the loss of biodiversity in these simplified swards on wider ecosystem functioning. The role of *Trifolium repens* and *T. pratense* when grown with grass alone, or with grass and forage herbs, in promoting sward productivity, while reducing reliance on fertiliser nitrogen is well established (Grace et al., 2019; Moloney et al., 2020). This, together with their potential to address a range of other agronomically important challenges (see Lüscher et al., 2014) make their inclusion in agricultural grasslands key to enhancing resource use efficiency and addressing environmental concerns associated with fertiliser N dependence. However, their potential to facilitate the provision of a wider range of ecosystem services which may be equally important within agricultural grasslands, has received less attention. The aim of this study was to investigate whether increasing plant species diversity within grassland swards to include legumes +/- forage herbs influenced earthworm activity, soil bulk density and water infiltration rates.

Methods and Study Site

This plot (1.95 x 10m) experiment consisting of 5 sward types replicated 4 times was established at UCD Lyons Farm (53°18´ N, 6°32´W) in 2013. Sward types were 1) PRG (250kg N ha^{-1} yr^{-1}); 2) PRG; 3) PRG and white clover *Trifolium repens*; 4) a 6 species mix comprised of PRG, timothy *Phleum pratense*, cocksfoot *Dactylis glomerata*, white clover, red clover *Trifolium pratensis* and greater birdsfoot trefoil *Lotus pedunculatus*; 5) a 9 species mix included the six species included in mix 4 with the addition of ribwort plantain *Plantago lanceolate*, chicory *Cichorium intybus* and yarrow *Achillea millefolium*. Mixes 2-5 inclusive received 90kg N ha^{-1} yr^{-1}. Sward types are referred to hereinafter as 1) PRG250; 2) PRG90; 3) PRGWC, 4) Simple; 5) Complex.

A full description of plot establishment, site description and management details to 2016 are available in Grace et al. (2019). The data presented in this current paper were collected in 2017. Plots received no fertiliser in 2017 and they were maintained by cutting with a mulch mower on a weekly basis from April to June 2017 and subsequently on an approximately 28 day basis until the end of the growing season. The botanical composition of the plots in 2016, prior to the commencement of the current study is presented in Table 1.

Table 1 Actual species composition of sward types measured in 2016.

Sward types	Species percentages in 2016							
	ryegrass	timothy	cocksfoot	white clover	red clover	plantain	chicory	yarrow
PRG90	72.60% (±4.31%)	0.1% (±0.07%)	0.87% (±0.46%)	24.65% (±5.21%)	0%	0.02% (±0.02%)	0%	0%
PRG250	97.90% (±0.45%)	0.25% (±0.15%)	0.02% (±0.02%)	1.62% (±0.60%)	0%	0%	0.1% (±0.1%)	0%
PRGWC	53.80% (±0.65%)	0.02% (±0.02%)	0.32% (±0.32%)	45.7% (±0.76%)	0%	0%	0%	0%
Simple	20.65% (±0.79%)	3.02% (±0.34%)	32.20% (±1.49%)	29.07% (±2.43%)	14.90% (±1.43%)	0%	0%	0%
Complex	18.30% (±0.73%)	2.72% (±0.63%)	31.40% (±1.98%)	29.15% (±3.36%)	16.45% (±2.26%)	0.02% (±0.02%)	1.62% (±0.83)	0%

Three soil cores to a dept of 30cm were taken from each plot using a split corer with and inner diameter of 5cm (Eijkelkamp Agrisearch) in Feb-March (winter sample) and in June-July (summer sample) 2017. Sampling was carried out when the soil moisture deficit for poorly drained soils was above -4 mm. Cores were located a minimum of 1m from each other and a minimum of 25cm from the plot edge. Cores were divided at 10 cm depth increments and each 10 cm section was placed in a labelled aluminium tray (that was weighed beforehand) and fragmented to small pieces and dried at 105^{o} C for 20 hours. Before weighing the soil, stones were removed by hand, their volume was recorded by immersing in water and measuring the change in volume. Stone volume was then subtracted from the total volume of the sample. Bulk density was calculated by dividing the dry soil mass by sample volume minus stone volume.

Water infiltration rates was measured across all plots in September 2017 using the double ring infiltrometer method to measure the near-saturated hydraulic conductivity in the field (Bharati et al., 2002). Earthworm activity was accessed in the different sward types in October 2017. Ten days after the plots were cut, surface casts were counted using a 2500 cm^2 quadrat. Every cast in the quadrat was flagged to prevent double counts and to ensure that all casts were counted. Casts with a diameter smaller than one cm were moved aside and only counted if an entrance hole to a burrow was seen. Counts from two randomly placed quadrats per plot were taken. Patches of white clover that had invaded the PRG plots (90kg N ha^{-1} yr^{-1}) where avoided when all sampling was undertaken.

All data was analysed using ANOVA in R version 3.3.3 (R Core Team, 2017). Where more than one sample per plot was taken a nested ANOVA was used to prevent pseudo replication, Tukey HSD test were performed to check for significance between treatments. Significance was interpreted at the level of $p<0.05$. Generalized linear models (GLM) were selected to investigate the relationship between infiltration and the number of earthworm casts (log transformed).

Results:

Average bulk density ranged from 1.09 (±0.014) to 1.05 (±0.011) g cm^{-3} in the top 10 cm, and from 1.22 (±0.021) to 1.19 (±0.015) g cm^{-3} at a depth of 20 to 30 cm. In general, bulk density increased with depth ($F_{2,238}$= 91.26, P< 0.001).

Water infiltration rates differed significantly between sward types ($F_{4,15}$= 20.56, P< 0.001). Highest infiltration was recorded within PRGWC plots with an average of 29.7 (±3.5) mm hr^{-1}, followed by the complex and simple mixtures with 15.7 (±2.2) and 10.5 (±3.1) mm hr^{-1} respectively, PRG90 and PRG250 had the lowest infiltration rates with 2.43 (±0.5) and 4.2 (±1.2) mm hr^{-1} respectively, although these did not differ significantly from the simple mixture (Fig 1). It is interesting to note, that when sward types were grouped according to functional groups, i.e. grass (G); grasses and legumes (GL); and grasses, legumes and herbs (GLH), sward types containing legumes didn't significantly differ in their infiltration rates and had significantly higher infiltration rates than grass only swards (Fig. 1).

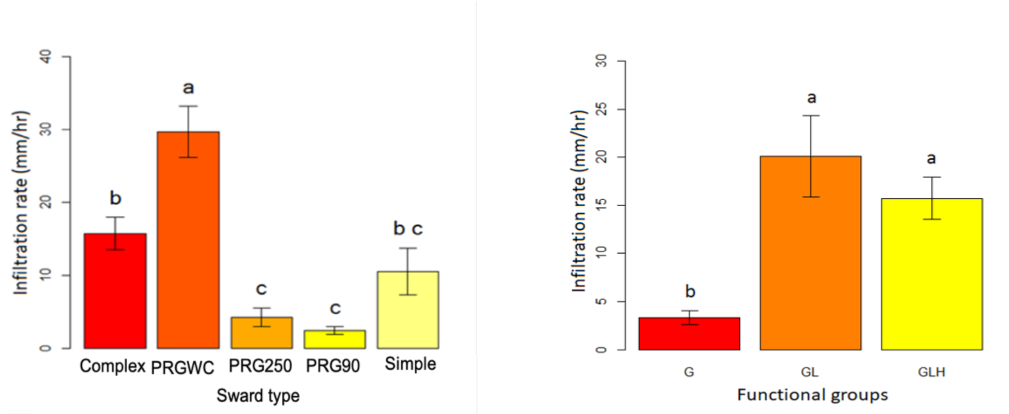

***Fig 1**: Variation in infiltration rates at near saturation hydraulic conductivity between sward types (left) and sward types grouped according to functional groups (right). Letters represent significant differences between treatments, P < 0.05 (left) and P < 0.01 (right) by Tukey's post hoc testing. G= grass, GL = grass and legume, GLH = grass, legume and herb.*

Surface cast numbers, used as a proxy for earthworm activity, differed significantly between sward types ($F_{4,15}$= 42.86, P< 0.001). Numbers ranged from 127 (±7) casts m^{-2} for PRGWC to 48 (±5) casts m^{-2} for PRG90 and PRG250, which in turn had significantly lower numbers of casts present compared to the three other sward types. The simple mixture didn't differ significantly from either PRGWC or complex sward types (Fig. 2). Similarly to the infiltration results, when sward types were grouped by plant functional group, swards containing legumes had significantly higher numbers of surface casts compared to grass only treatments (Fig. 2). Regression analysis revealed a significant positive effect of earthworm activity on infiltration rates (P<0.001). At the plot level, surface cast numbers were found to explain 59.2% of the variation in infiltration rates (Fig 2).

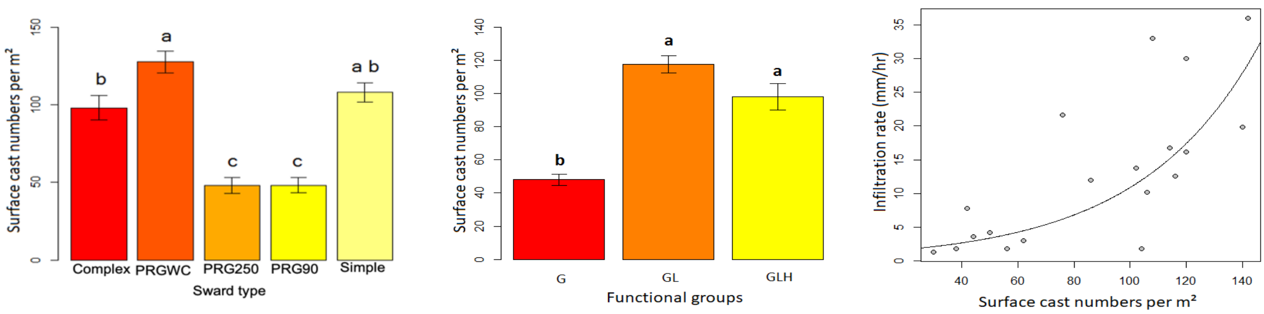

Fig. 2: Variation in earthworm activity measured by surface cast numbers among sward types (left) and sward types grouped according to functional groups (middle). Letters represent significant differences between treatments, P < 0.05 (left) and P < 0.01 (centre) by Tukey's post hoc testing. Error bars represent one standard error of the mean; and relationship between Infiltration and earthworm activity (right). G = grass, GL = grass and legume, GLH = grass, legume and herb.

Discussion

Within this study, moderately diverse sward types had significantly higher infiltration rates and surface casts numbers, compared to the perennial ryegrass monocultures. It is likely that the inclusion of legumes indirectly increased infiltration rates (Fischer et al., 2014) by increasing earthworm activity (Schmidt et al., 2003; Fischler et al., 2014) thus increasing the proportion of macropores (Chan, 2004) open to preferential water flow during intense rainfall events. Increased water infiltration can translate into decreased surface runoff (Edwards et al., 1992) and can have a positive effect on the water holding capacity of a soil. Poorly drained, saturated soils will limit livestock and machinery trafficability on farm, as they can cause severe damage to soil structure and increase compaction (Schulte et al., 2012), negatively affecting plant growth, by reducing photosynthetic rates when soil conditions are anoxic (Laidlaw, 2009) and interfering with root growth (Tracy et al., 2012). Thus, in high rainfall areas (mean annual rainfall>1000 mm yr^{-1}), such as Ireland, increased infiltration rates could potentially translate to an increase in the number of available grazing days per season (Fitzgerald et al., 2008) and thus improved herbage production by allowing soils to dry faster. This could be particularly beneficial where soils have a high clay content.

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